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ORIGINAL PAPER

INFLUENCE OF BIOSTIMULANTS ON THE CONTENT OF MACRO- AND MICRONUTRIENTS IN BROCCOLI PLANTS EXPOSED TO DROUGHT STRESS[*]

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Abstract

The use of biostimulants is a method of limiting the negative effect of stress on crops. The aim of the study was to determine the influence of amino acids and a combination of *Ascophyllum nodosum filtrate* and amino acids on the content of macro- and micronutrients in two broccoli cultivars exposed to drought stress. The experiment was conducted in vegetation chambers in two cycles in 2015. The plants were watered with *Ascophyllum nodosum* filtrate three days before planting and sprayed three times with amino acids two, four and six weeks after planting. The control plants were not treated with biostimulants. The water content in the substrate was determined with moisture sensors 5TE and Em50 data logger (Decagon Devices, Inc., USA). The water capacity of the soil was 40% v/v. During the 12 days of cultivation, drought stress was repeated three times. Each time it lasted until the substrate reached 15% v/v. Next, the plants were watered to 40% v/v and the stress started again. The content of macro- and micronutrients in broccoli leaves was determined when the experiment finished. The level of plant stress caused by the factors under study was determined by measuring chlorophyll fluorescence parameters. The maximum photochemical efficiency of PSII and the quantum yield of electron transport were calculated. The biostimulants significantly affected the content of nitrogen, phosphorus, potassium, magnesium, copper and manganese in broccoli leaves. The content of magnesium and sodium depended on a cultivar. The research revealed a highly significant linear dependence between the content of nitrogen, phosphorus, potassium, magnesium and sodium and the value of quantum yield of electron transport in the plants treated with the biostimulants.

Keywords: *Brassica oleracea* var. *italica*, *Ascophyllum nodosum*, nutrients, amino acids, water deficit.

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INTRODUCTION

Abiotic stresses, including drought stress, cause morphological, physiological, biochemical and molecular changes in plants, thus affecting their growth and productivity (Wang et al. 2001). The earliest response to drought stress is the closure of stomata so as to limit transpiration (Galmes et al. 2007). It is accompanied by limited CO_2 absorption, which reduces the photosynthetic activity (Nayyar, Gupta 2006). Drought stress causes changes in the chlorophyll fluorescence parameters, including the photochemical efficiency of PSII and the quantum yield of electron transport (Efeoglu et al. 2009).

As a result of stomatal closure, limited transpiration reduces the uptake of nutrients due to disordered active transport and destabilisation of cell membranes (Tanguilig et al. 1987).

Plants' resistance to stress can be enhanced by applying biostimulants (Calvo 2014). Protein hydrolysates containing a marine algae extract and humus compounds are the most popular biostimulants in crop cultivation (Ertani et al. 2009). According to Xu and Lescovar (2015), the application of a biostimulant containing an *Ascophyllum nodosum* extract to plants exposed to drought stress improved the leaf water relations, reduced the stomatal closure and, in consequence, increased the rate of photosynthesis. Biostimulants also increased the content of antioxidants in plants, thus strengthening their resistance to stress (Ertani et al. 2011). According to Zodape et al. (2011), the application of a 5% seaweed extract positively affected the uptake of nutrients from the substrate. According to the authors, in comparison with the control sample, the content of nitrogen in tomato shoots increased by nearly 48%, phosphorus – by 59% and potassium – by 62%. As far as micronutrients are concerned, the content of iron and zinc increased significantly. Szczepanek, Grzybowski (2016) reported a positive effect of the Kelpak biostimulant on the content of nitrogen, phosphorus and potassium in wheat grains. According to El-Attar, Ashour (2016), biostimulants also positively affected the content of nitrogen, phosphorus and potassium in camomile plants exposed to drought stress. The findings of the research conducted by Norrie et al. (2002) and Khan et al. (2012) were opposite. They did not observe any changes in the content of nutrients in grapevine leaves after the application of an *Ascophylum nodosum* extract. Apart from that, Khan et al. (2012) did not observe in grapes any influence after the application of a mixture of *Ascophylum nodosum* extract and amino acids.

To the best of our knowledge, there are no studies on the content of nutrients in broccoli plants treated with biostimulants, especially under stress conditions. The focus of our study was to determine the influence of amino acids and *Ascophyllum nodosum* filtrate on the content of macro- and micronutrients in two broccoli cultivars exposed to drought stress.

MATERIAL AND METHODS

Plant material and growth conditions

The experiment was conducted in two cycles, using two broccoli cultivars Monaco and Parthenon grown in a growing chamber in 2015. The temp. was 18/16°C (day/night), photoperiod – 16 h, relative humidity – 90%, photosynthetic photon flux density – 150 µmol m^{-2} s^{-1}. Broccoli seedlings were produced in 0.09 dm^3 pots filled with peat substrate for growing cruciferous vegetables (Kronen-Klasmann, Klasmann-Deilman Polska Sp. z o.o., Poland). The seedlings with three-four leaves were transplanted to larger containers (5 dm^3). Before planting, the nutrients were supplemented to the maximum optimum level (mg dm^{-3} of substrate): $N\text{-}NO_3$ – 250; P – 200; K – 600; Ca – 1600; Mg – 160 + microelements (Fe – 10; Mn – 3; Cu – 12; B – 3; Zn – 1; Mo – 1). Additionally, during the growing period, the plants were fed with 0.5% Kristalon™ blue (Yara Poland Sp. z o.o.)

The plants were watered with *Ascophyllum nodosum* filtrate three days before planting and sprayed three times with amino acids two, four and six weeks after planting. The control plants were not treated with biostimulants. The water capacity of the soil was 40% (v/v). Drought stress was induced by abandoning the watering of plants after 2-3 weeks following the last treatment with amino acids. During the 12 days of cultivation, drought stress was repeated three times. Each time it lasted until the water capacity in the substrate was 15% v/v. Then, the plants were watered up to 40% v/v and the stress was repeated. The water content in the substrate was determined with moisture sensors 5TE and Em50 data logger (Decagon Devices, Inc., USA).

Chlorophyll fluorescence measurements

The level of plant stress caused by the factors under study was determined by measuring chlorophyll fluorescence (OS1-FL Fluorometer, OptiSciences Inc., USA). Fluorescence parameters: $F_{m,}$ F_v were determined after eight hours of darkness using photosynthetic photon flux density (PPFD) <0.15 µmol $m^{-2}s^{-1}$. F_m was measured after 0.8 s saturating white light pulse (>15 000 µmol $m^{-2}s^{-1}$ PPFD). F_v/F_m (the maximum photochemical efficiency of PSII) was calculated. The Y value (the quantum yield of electron transport) was calculated according to Genty et al. (1989).

Analysis of macro- and micronutrients in the plant material

After the experiment was terminated, the broccoli heads were dried in an exhaust dryer at a temp. of 105°C for 48 hours. The plant material was ground and 1.0 g of each sample was wet-mineralised in concentrated H_2SO_4 (pure for analysis), where 30% H_2O_2 was used as an oxidant, and transferred to flasks of 250 cm^3. After the mineralisation, the colouri-metric method was applied to measure the phosphorus content by means of a SPEKOL 210 apparatus with ammonium molybdate. The content of potassium, calcium and sodium was measured by means of flame photometry with a Zeiss AAS 3 apparatus, whereas the content of magnesium was measured by means of flame atomic absorption spectroscopy (FAAS) with a Zeiss AAS 3 apparatus (Kozik, Golcz 2011). In order to measure the content of general forms of nitrogen, the plant material was subjected to mineralisation in sulphosalicylic acid, where sodium thiosulphate was applied as a reducer and a selenium mixture as a catalyser. Next, it was measured in a Parnas and Wagner apparatus by distillation according to the Kjeldahl method (Kozik, Golcz 2011). The same plant material was ground and 2.5 g of each sample was dry-mineralised at a temperature of 450°C, in a Linn Elektro Therm furnace. The content of copper, zinc, manganese and iron in the plant material was measured by means of flame atomic absorption spectroscopy (FAAS) with an AAS-3 spectrophotometer (Zeiss). The accuracy and precision of analytic measurements were checked by analysing *Rye Grass* ERM®-CD281 reference material (certified by European Commission, Joint Research Centre, Institute for Reference Materials and Measurements / /IRMM, Geel BE/). Certified content/content after mineralization (mg kg^{-1}): Fe 180/174.13; Cu 10.2/9.11;Zn 30.5/ 28.78.

Statistical analysis

The experiment was established in a two-factor design with four replicates (one plant in each). The significance of the impact of the biostimulants on the content of macronutrients and micronutrients as well as fluorescence parameters was determined with ANOVA.

Atatistical analysis for a three-factorial experiment was carried out to determine the influence of the cultivation cycle. Differences between the means were estimated with the Duncan test at a significance level of $P = 0.95$.

RESULTS AND DISCUSSION

The influence of biostimulants on the content of macroelements and sodium

The analysis of variance (Table 1) revealed that the biostimulants significantly influenced the content of nitrogen, phosphorus, potassium and magnesium in both cultivation cycles and the content of calcium and sodium in the first cycle. In both cycles, the cultivar had significant influence only on the content of magnesium and sodium.

The content of nitrogen was higher than in the control sample when amino acids as well as a combination of amino acids and *Ascophyllum nodosum* filtrate were applied to the Monaco cultivar in the second cycle. As far as the Parthenon cultivar is concerned, the content of nitrogen was higher in the first cycle when both biostimulants were applied (Table 2). The applica-

Table 1

ANOVA *F*-values for the N, P, K, Ca, Mg and Na content

Cycle	Treatment	N	P	K	Ca	Mg	Na
I	cultivar	**	ns	**	*	**	**
	treatment	**	*	**	**	**	**
	cultivar x treatment	**	**	**	ns	**	**
II	cultivar	ns	**	ns	ns	**	**
	treatment	*	**	*	ns	**	ns
	cultivar x treatment	**	*	*	ns	ns	ns

* significance level *P* = 0.90, ** significance level *P* = 0.95, ns – not significant at *P* = 0.95

Table 2

The content of macronutrients and sodium in leaves of two broccoli cultivars (g kg^{-1} d.w.)

Ingredients	Cycle	Monaco			Parthenon		
		C$^{\#}$	A	A+AN	C	A	A+AN
N	I	34.1*a**	28.1*bc*	29.3*c*	27.1*c*	22.6*d*	32.2*a*
	II	32.9*b*	39.7*a*	38.2*a*	39.3*a*	38.2*a*	33.4*b*
P	I	3.5*a*	3.0*b*	2.9*b*	3.0*b*	3.0*b*	3.4*a*
	II	3.9*bc*	3.9*bc*	3.6*ab*	4.5*a*	4.2*b*	3.5*d*
K	I	26.0*a*	20.4*c*	23.4*b*	20.4*c*	21.6*c*	23.4*b*
	II	30.5*ab*	30.0*ab*	30.4*ab*	31.8*a*	27.9*bc*	26.2*c*
Ca	I	18.0*c*	21.5*b*	23.7*a*	17.8*c*	21.2*b*	22.1*b*
	II	22.6*a*	22.3*a*	21.9*a*	23.4*a*	21.4*a*	22.2*a*
Mg	I	2.8*c*	2.7*c*	2.6*c*	3.2*b*	2.8*c*	3.4*a*
	II	3.7*a*	3.6*a*	3.8*a*	3.6*a*	3.1*b*	3.6*a*
Na	I	6.5*a*	5.5*b*	4.7*c*	3.3*d*	2.4*e*	5.1*bc*
	II	6.6*ab*	6.7*ab*	7.3*a*	5.1*c*	5.0*c*	5.7*bc*

$^{\#}$ C – control, A – amino acids, AN – *Ascophyllum nodosum* filtrate;
* Values marked with the same letter in each row do not differ significantly at *P* = 0.95.

tion of biostimulants did not increase the content of phosphorus in the Monaco cultivar in either cultivation cycle. The content of phosphorus in the Parthenon cultivar increased significantly only in the first cycle when a combination of amino acids and *Ascophyllum nodosum* filtrate was applied. However, it dropped in the second cycle. When a combination of amino acids and *Ascophyllum nodosum* filtrate was applied, the content of potassium in the Parthenon cultivar increased only in the first cycle, but it decreased in the second cycle. Mancuso et al. (2006) conducted a study on *Vitis vinifera* and observed that an extract of marine algae had a positive effect on the uptake of nitrogen, phosphorus and potassium by plants exposed to drought stress. According to the authors, marine algae extracts increase plants' toleration towards water stress, maintain higher water potential and stomatal conductance during the stress and accelerate plants' regeneration after the stress. Hammad, Ali (2014) and Pranckietienė et al. (2015) also reported a positive effect of amino acids on the uptake of nitrogen, phosphorus and potassium by plants exposed to drought stress. This observation was not confirmed in our study, where the application of amino acids to both cultivars did not increase the content of potassium and phosphorus in them. On the contrary, the content of these nutrients even dropped. These results are in agreement with those reported by Gajc-Wolska et al. (2012), who showed that the content of nitrogen, phosphorus, potassium and calcium did not increase in leaves of endive after a treatment with amino acids and *Ascophyllum nodosum*. Szczepanek et al. (2008) also observed that an Asashi biostimulant treatment did not affect the phosphorus, calcium and magnesium content in carrot.

In our study, the content of nitrogen in both cultivars decreased after the application of amino acids in the first cycle. There were no significant differences between the Parthenon cultivar and the control sample in the second cycle.

In our study, after an application of amino acids and a combination of amino acids and *Ascophyllum nodosum* filtrate, the content of calcium increased in both cultivars only in the first cycle (Table 2). The biostimulants did not affect the content of calcium in the second cycle. In our study, the content of magnesium increased only in the Parthenon cultivar in the first cycle when amino acids and a combination of amino acids and *Ascophyllum nodosum* filtrate were applied. We did not observe this dependence in the second cycle. In neither cycle did the biostimulants affect the content of magnesium in the leaves of the Monaco cultivar. Mancuso et al. (2006) also did not observe any positive effect of the marine algae biostimulant on the content of calcium. However, the authors found that when the biostimulant was applied, the content of magnesium increased significantly.

In our study, when a combination of amino acids and *Ascophyllum nodosum* filtrate was applied, the content of sodium increased in the Parthenon cultivar in both cycles, but in the Monaco cultivar it increased only in the second cycle. When amino acids were applied to both cultivars, the content of sodium was comparable to the control sample or it was significantly lower. Szczepanek et al. (2015) found a higher content of sodium in carrot roots when they applied a biostimulant made from marine algae (*Ecklonia maxima*).

The influence of biostimulants on the content of microelements

The biostimulants significantly influenced the content of copper and manganese in both cultivation cycles. They affected the content of zinc in the first cycle and the content of iron in the second cycle (Table 3). On the cont-

Table 3

ANOVA *F*-values for the Fe, Cu, Zn and Mn content

Cycle	Object	Fe	Cu	Zn	Mn
I	cultivar	ns	ns	**	**
	treatment	ns	**	*	**
	cultivar x treatment	**	**	ns	ns
II	cultivar	**	ns	ns	ns
	treatment	**	**	ns	**
	cultivar x treatment	ns	**	**	**

* significance level $P = 0.90$, ** significance level $P = 0.95$, ns – not significant at $P = 0.95$

rary, Mancuso et al. (2006) found that biostimulants had no effect on the content of iron, manganese or zinc in *Vitis vinifera* plants.

An interaction between the biostimulants and the cultivars was observed for the content of iron, copper in both cycles and for the content of zinc and manganese in the second cycle. The influence of the cultivar was significant for the content of zinc and manganese in the first cycle and for the content of iron in the second cycle.

The content of iron increased significantly in both cycles only when a combination of amino acids and *Ascophyllum nodosum* filtrate was applied to the Parthenon cultivar. However, no change was observed in the Monaco cultivar (Table 4). The content of copper in this cultivar increased only in the first cycle when a combination of amino acids and *Ascophyllum nodosum* filtrate was applied, whereas the content of manganese increased in the second cycle after a treatment with Aminoplant alone. The biostimulants did not affect the increase of copper or manganese in the Parthenon cultivar.

Table 4

The content of selected micronutrients in leaves of two broccoli cultivars (mg kg^{-1} d.w.)

Ingredients	Cycle	Monaco			Parthenon		
		C[#]	A	A+AN	C	A	A+AN
Fe	I	32.05*bc**	32.66*b*	29.95*c*	31.21*bc*	29.91*c*	35.26*a*
	II	30.88*c*	32.44*bc*	32.27*bc*	33.26*bc*	34.91*b*	38.34*a*
Cu	I	4.22*cd*	4.57*bc*	5.54*a*	5.19*a*	4.02*d*	4.75*b*
	II	5.32*a*	5.49*a*	3.96*b*	5.07*a*	5.03*a*	5.16*a*
Zn	I	53.77*ab*	55.72*a*	54.28*ab*	49.96*bc*	54.44*ab*	47.69*c*
	II	60.77*a*	54.62*bc*	50.83*cd*	48.13*d*	59.25*ab*	54.98*bc*
Mn	I	16.64*a*	15.72*b*	15.39*b*	14.11*c*	13.37*d*	13.81*cd*
	II	14.62*c*	20.95*a*	15.35*c*	19.71*ab*	18.53*b*	14.28*c*

[#] C – control, A – amino acids, AN – *Ascophyllum nodosum* filtrate;

* Values marked with the same letter in each row do not differ significantly at $P = 0.95$.

The content of zinc increased only in the Parthenon cultivar when amino acids were applied in both cultivation cycles and when a combination of amino acids and *Ascophyllum nodosum* filtrate was applied in the second cycle. Godlewska, Ciepiela (2016) observed that a seaweed extract (Kelpak) significantly influenced the content of zinc, copper, iron and manganese in two species of grass.

Chlorophyll fluorescence

Plants' physiological response to drought stress is shown by means of two chlorophyll fluorescence parameters, i.e. the maximum photochemical efficiency of PSII (F_v/F_m) and the quantum yield of electron transport (Y) – Table 5. The studies conducted by Hu et al. (2010) and Rahbarian et al (2011) confirmed that drought stress significantly reduced the F_v/F_m value.

Our study revealed that the cultivation cycle had a highly significant influence (Table 6) both on the F_v/F_m value and the Y parameter. Reduced

Table 5

Chlorophyll fluorescence in two broccoli cultivars treated with biostimulants and exposed to drought stress

Cultivar	Treatment	F_v/F_m		Y	
		cycle 1	cycle 2	cycle 1	cycle 2
Monaco	C#	0.81*a**	0.79*de*	0.58	0.46*c-e*
	A	0.80*a-c*	0.80*a-d*	0.55	0.43*de*
	A+AN	0.81*ab*	0.80*a-c*	0.55	0.42*e*
Parthenon	C	0.79*c-e*	0.78*e*	0.55	0.51*ab*
	A	0.79*a-c*	0.80*b-d*	0.56	0.48*cd*
	A+AN	0.80*b-d*	0.79*de*	0.56	0.45*c-e*

C – control, A – amino acids, AN – *Ascophyllum nodosum* filtrate;
* Values marked with the same letter in each row do not differ significantly at $P = 0.95$.

Table 6

ANOVA *F*-values for F_v/F_m and Y

Object	F_v/F_m	Y
Cultivar	**	ns
Treatment	ns	ns
Cultivar x treatment	ns	ns
Cycle	**	**
Cultivar x cycle	ns	*
Treatment x cycle	*	ns
Cultivar x treatment x cycle	ns	ns

* significance level $P = 0.90$, ** significance level $P = 0.95$, ns – not significant at $P = 0.95$

values of both parameters in the second cycle (Table 5) indicate that drought stress was more severe than in the first cycle. According to EFEOGLU et al. (2009), drought stress reduces the Y value due to the reduced transport of electrons in the photosystems. It is caused by noticeable stomatal closure and reduced CO_2 assimilation efficiency. The difference in the stress level may account for differences between the cycles in the content of nutrients in the plant material under study. This fact is confirmed by the study conducted on wheat by ABDALLA (2014).

The analysis of variance revealed that the F_v/F_m value depended on a cultivar and the interaction between the method of cultivating the plants (with or without using biostimulants) and the cultivation cycle (Table 6). The Y parameter value depended on an interaction between a cultivar and a cultivation cycle.

The analysis revealed a highly significant linear dependence between the content of nitrogen, phosphorus, potassium, magnesium and sodium and the quantum yield of electron transport value (Y) in plants treated with the biostimulants (Table 7). When the Y value was lower, i.e. under heavier stress, the content of these elements was higher. This observation is in agreement with the findings of the study conducted by HAMMAD, ALI (2014), who noted that the content of nitrogen, phosphorus and potassium in biostimulated plants was higher under moderate water stress than when the water content in soil was optimal. According to these authors, the content of these macronutrients decreased under severe water stress. HAMMAD (2008) also reported that biostimulants positively affected the content of nitrogen, phosphorus and potassium in pea leaves exposed to drought stress.

Table 7

The dependence between the content of macro- and micronutrients (y) and the quantum yield of electron transport (x) in plants treated with biostimulants

Ingredient	Regression equation	Correlation coefficient (R)
N	$y = -0.0862x + 0.7819$	0.8301**
P	$y = -0.9408x + 0.8234$	0.7089**
K	$y = -0.1503x + 0.8819$	0.9271***
Ca	$y = -0.7857x + 2.2057$	0.5419 ^{ns}
Mg	$y = -1.0733x + 0.8435$	0.8091**
Na	$y = -0.3174x + 0.6682$	0.7581**
Fe, Cu, Zn, Mn		<0.5356^{ns}

*** significance level $P = 0.99$, ** significance level $P = 0.95$, ns – not significant at $P = 0.95$, $n = 12$, (y) – dependent variable, (x) – independent variable

In our study, the dependence between the Y value and the content of calcium, iron, zinc, copper and manganese was not significant.

CONCLUSIONS

1. The application of biostimulants significantly influenced the content of nitrogen, phosphorus, potassium, magnesium, copper and manganese in broccoli leaves exposed to drought stress. The impact of biostimulants depended on the level of stress.

2. The study revealed that the cultivars differed significantly in the content of magnesium and sodium in broccoli leaves exposed to drought stress.

3. The quantum yield of electron transport was significantly correlated with the content of nitrogen, phosphorus, potassium, magnesium and sodium.

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