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INTERACTIVE EFFECTS OF SALINITY STRESS WITH OR WITHOUT NICOTINAMIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF TOMATO SEEDLING

INTERAKCJA ODDZIAŁYWANIA STRESU SOLNEGO W POŁĄCZENIU Z AMIDEM KWASU NIKOTYNOWEGO LUB BEZ NIEGO NA PARAMETRY FIZJOLOGICZNE I BIOCHEMICZNE SIEWEK POMIDORA

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Streszczenie. Celem pracy było określenie wpływu soli NaCl i KCl osobno lub w połączeniu z amidem kwasu nikotynowego na wzrost i niektóre parametry biochemiczne pomidora odmiany Vilma, w warunkach laboratoryjnych i szklarniowych. Zastosowane kombinacje roztworów soli miały negatywny wpływ na zdolność kiełkowania nasion i cechy morfologiczne 14-dniowych siewek pomidora. Dodatek soli NaCl miał pozytywny wpływ na zawartość *Chl a* i *Car*, a roztwór soli KCl obniżał zawartość barwników fotosyntetycznych i niefotosyntetycznych w siewkach pomidora. W warunkach polowych zastosowane roztwory soli NaCl (3 i 6 g) powodowały wzrost stężenia *Chl a*, *Chl b* i *Car* oraz proliny. Ponadto liście roślin stanowiących kontrolę były ciemniejsze od liści roślin z pozostałych kombinacji doświadczenia. Zaobserwowano, że dodatek amidu kwasu nikotynowego do pożywki nie miał ochronnego wpływu na rośliny rosnące w warunkach zasolenia, poza niewielkim zwiększeniem stężenia proliny.

Key words: *Lycopersicon esculentum*, nicotinamide, salinity, proline, malondialdehyde (MDA), chlorophyll.
Słowa kluczowe: *Lycopersicon esculentum*, amid kwasu nikotynowego, zasolenie, prolina, dialdechyd malonowy (MDA), chlorofil.

INTRODUCTION

Salinity is one of the most commonly occurring stress factor that severely limits growth and yield of economically important crops (Sadak et al. 2010). Deleterious effect of salinity on plant growth is associated with low osmotic potential of soil solution, nutritional and hormonal imbalance, specific ion effect, and induction of oxidative stress, or a combination of these factors (Abdelhamid et al. 2013). In addition, large amounts of salt can accumulate in chloroplasts and exert direct toxic effects on photosynthesis via destabilization of protein

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complexes destroying photosynthetic pigments. A possible survival strategy for plants under saline conditions is to use some compounds that could alleviate the salt stress effect. The use of vitamins as antioxidants mediated salt tolerance as a selection factor as well as a driving force for improving resistance and adaptation to salt stress (Azooz et al. 2013). Vitamins are required in trace amount to maintain normal growth and proper development of all organisms. In addition, vitamins are cofactors of many metabolic reactions (Abdelhamid et al. 2013). Vitamin supplements are known to enhance the plant activities and did not have toxic or mutagenic action (Hassanein et al. 2009; Azooz et al. 2013). Nicotinamide is a water–soluble vitamin and is part of the vitamin B group. It is a stress–associated compound that induces and regulates secondary metabolic accumulation and/or the manifestation of defense metabolism in plants (Azooz et al. 2013). The role of vitamins in modifying the salt stress induced changes in osmoprotectant contents and was also investigated by Bassuony et al. (2008), Sadak et al. (2010), Abdelhamid et al. (2013) and Krupa-Małkiewicz et al. (2015).

The major objective of this study was to investigate the effects of nicotinamide on number of physiological aspects of tomato (*Lycopersicon esculentum*) cultivar Vilma under saline condition.

MATERIAL AND METHODS

Petri dishes test. Seeds of *Lycopersicon esculentum* cv. Vilma were surface–sterilized with 70% (v/v) ethanol solution for 30 s and then thoroughly rinsed with sterile water. After the preliminary disinfection, the seeds soaked for 15 minutes in 10% (v/v) solution of sodium hypochlorite (NaOCl), after rinsing three times in sterile water. Next, seeds were placed on Petri dishes lined with filter paper and moistened with 15.0 cm^3 of salt solution. To obtain a salinity effect, eight combinations of two salt solutions (NaCl and KCl) were used: 100 mM NaCl, 100 mM NaCl + 1 mM nicotinamide, 200 mM NaCl, 200 mM NaCl + 1 mM nicotinamide, 100 mM KCl, 100 mM KCl + 1 mM nicotinamide, 200 mM KCl, 200 mM KCl + 1 mM nicotinamide, with a pH of 6.0. Sterile water was used as the control. 30 seeds were used per treatment, including control. The experiments were repeated 3 times. The Petri dishes with the seeds were stored in a growth chamber at a constant temperature of 25ºC in white light (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 16 h and 8 h dark per a day. After 14 days, plants were removed and washed with deionized distilled water. Biometric measurements were performed, in which the following parameters were determined: the number of germinating seeds, root and shoot length (cm) and chlorophylls *a* and *b* (*Chl a, Chl b*) and carotenoid (*Car*) concentrations ($mg \cdot g^{-1}fw$) in tomato seedlings.

Greenhouse test. Seeds of cultivar Vilma were disinfected with 10% solution of sodium hypochlorite (NaOCl) for 15 minutes. Then, were washed three times with sterile water and seeded in pots (diameter 10 cm, high 15 cm). Pots were moved to a heated greenhouse and placed on cultivation tables. After the development of the root system of a plant (about 3 weeks after planting of seeds), watering using NaCl solution in four combinations was initiated: 3.0 $g \cdot dm^{-3}$ NaCl, 3.0 $g \cdot dm^{-3}$ NaCl + 1 mM nicotinamide, 6.0 $g \cdot dm^{-3}$ NaCl, 6.0 $g \cdot dm^{-3}$ NaCl + 1 mM nicotinamide. Salination was carried out for six weeks, by applying

once a week 150 cm^3 of stock solution per pot. The control group involved plants watered with distilled water at the same doses. The experiment was established in three replications, with 10 plants in a replications. At the end of the experimental period (45 days) plant samples were collected for determination of growth parameters, length of the shoots and roots (cm), number of shoots and roots, the levels of *Chl a, Chl b* and *Car* (mg · g^{-1}fw), concentrations of proline (µmol · g^{-1}fm) and MDA (nmol · g^{-1}fm) in leaves were measured.

Determination of proline and MDA content. The proline (Pro) accumulation were determined according to Bates (1973). Content of the malondialdehyde (MDA) in plant tissue was determined by the method described by Sudhakar et al. (2001).

Determination of pigments. The extraction of leaf pigments was performed with 80% (v/v) acetone. Chlorophyll *a*, *b* and *Car* content was determined spectrophotometrically at 663, 645 and 440 nm, according to Arnon et al. (1956) in modification to Lichtenthaler and Wellburn (1983).

Leaf pigmentation. The leaf pigmentation measurement was carried out using spectrophotometer CM-700d (Konica Minolta, Japan), in glass cuvettes of 1 mm optical length. Measurements were made in CIE Lab system, in which L* stands for white (100) and black color (0), a* – green (–100) and red color (+100), b* – blue (–100) and yellow color (+100). The 10º observer type and D65 illuminant was applied. Color was measured in triplicates for each experimental combination.

Statistical analysis The significance of differences was determined by means of variance analysis and Tukey's test, at the level of significance of $\alpha = 0.05$. Homogenous groups between analysed combinations were labeled with successive letters of alphabet.

RESULTS AND DISCUSSION

The effect of salinity and nicotinamide treatment on growth parameters of *L. esculentum* cv. Vilma showed that salinity stress caused significant reduction in growth parameters (Table 1). The highest germination of seeds (90%) occurred in the control medium without the addition of salt. The addition of nicotinamide and 100 mM of NaCl solution caused an increase in the number of germinated seeds in comparison to seeds germinating under salitnity conditions (Table 1). However, in terms of 100 mM of KCl salt solution, addition of nicotinamide had no positive effect on the number of germinated tomato seeds cv. Vilma. The 200 mM NaCl and KCl solution inhibited seed germination. The obtained results are consistent with those observed by Jones (1986), who found that increased salt concentration in the medium that is above 100 mM has an inhibitory effect on seeds' germination and plant growth. The addition of NaCl and KCl to the medium exhibited inhibitory effect on the length of roots and shoots of tomato seedlings. The longest roots (11.03 cm) and shoots (11.23 cm) were observed in control plants (Table 1). For other combinations in the experiment, root and shoot length ranged from 5.30 cm up to 6.47 cm and from 4.67 cm up to 6.57 cm, respectively. According to many authors (Cuartero and Fernandez-Muñoz 1999; Smolik et al. 2011; Krupa-Małkiewicz et al. 2015) young seedlings are the most sensitive to the effect of salt solution. In our study, it was observed that the addition of 100 mM NaCl to the medium has a positive impact on *Chl a* and *Car* content (44.82 and 20.43 mg · g^{-1}fw, respectively) for which the value exceeded the one observed in control (Table 1).

Table 1. Seed germination, seedling growth and *Chl a*, *Chl b*, *Car* concentrations of tomato cv. Villma at different salt treatment
Tabela 1. Zdolność kiełkowania, parametry wzrostu i stężenie *Chl a*, *Chl b* oraz *Car* siewek pomidora odmiany Vilma w warunkach stresu solnego

Treatment Roztwór soli	Germination Zdolność kiełkowania [%]	Root length Długość korzeni [cm]	Shoot length Długość pędu [cm]	Pigments contents Zawartość barwników		
				Chl a	*Chl b*	*Car*
				mg · g^{-1}fm		
Control – Kontrola	90.0	11.03 ± 8.26 a	11.23 ± 5.72 a	36.90 ± 3.95 ab	13.42 ± 2.15 a	17.46 ± 2.43 a
100 mM NaCl	46.6	5.30 ± 3.77 a	4.67 ± 3.84 a	44.82 ± 6.82 a	12.84 ± 2.44 a	20.43 ± 2.97 a
100 mM NaCL + 1 mM nicotinamide – amid kwasu nikotynowego	66.6	6.47 ± 4.65 a	6.23 ± 4.37 a	22.48 ± 2.44 b	7.19 ± 0.75 c	10.38 ± 0.77 b
100 mM KCl	50.0	5.83 ± 3.84 a	6.57 ± 4.19 a	26.36 ± 6.13 b	8.69 ± 2.37 bc	11.92 ± 2.60 b
100 mM KCL + 1 mM nicotinamide – amid kwasu nikotynowego	43.3	5.30 ± 4.87 a	6.53 ± 4.3 a	38.73 ± 0.45 a	12.31 ± 0.81 ab	16.50 ± 0.36 a
$LSD_{0.05}$ $NIR_{0,05}$		11.21	10.92	8.92	3.62	4.09

Means in the same column followed by the same letter are not significantly different at $\alpha < 0.05$ according to Tukey test – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się według testu Tukeya na poziomie istotności $\alpha < 0,05$; ± SD – standard deviation – odchylenie standardowe.

Table 2. The influence of nicotinamide on alleviating salt stress of tomato cv. Vilma under field condition
Tabela 2. Wpływ amidu kwasu nikotynowego na łagodzenie skutków stresu solnego pomidora odmiany Vilma w warunkach polowych

Treatment Roztwór soli	Shoot length Długość pędu [cm]	Number of shoots per plant Liczba pędów na roślinie	Root length Długość korzeni [cm]	Number of root Liczba korzeni
Control – Kontrola	27.58 ± 9.13 a	5.75 ± 2.17 a	6.30 ± 2.93 a	30.60 ± 5.97 a
3 g · dm^{-3} NaCl	26.0 ± 6.71 a	5.92 ± 1.61 a	8.43 ± 2.10 a	24.63 ± 4.80 ab
3 g · dm^{-3} NaCl + 1mM nicotinamide – amid kwasu nikotynowego	25.17 ± 5.32 a	6.25 ± 1.86 a	8.90 ± 1.26 a	20.51 ± 3.21 b
6 g · dm^{-3} NaCl	24.17 ± 4.8 a	5.42 ± 1.44 a	7.64 ± 2.96 a	22.88 ± 2.61 b
6 g · dm^{-3} NaCl + 1mM nicotinamide – amid kwasu nikotynowego	23.42 ± 3.28 a	6.42 ± 1.78 a	7.33 ± 1.71 a	19.34 ± 3.12 b
$LSD_{0.05}$ $NIR_{0.05}$	10.33	2.99	3.83	7.21

Means in the same column followed by the same letter are not significantly different at $\alpha < 0.05$ according to Tukey test – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się według testu Tukeya na poziomie istotności $\alpha < 0,05$; ± SD – standard deviation – odchylenie standardowe.

Table 3. Concentration of *Chl a, Chl b, Car*, MDA and Pro in leaves of tomato cv. Vilma depending on different salt treatment
Tabela 3. Stężenie *Chl a, Chl b, Car*, MDA oraz Pro w liściach pomidora odmiany Vilma w zależności od zastosowanego roztworu soli

Treatment Roztwór soli	*Chl a* [mg · g^{-1}fm]	*Chl b* [mg · g^{-1}fm]	*Car* [mg · g^{-1}fm]	Pro [µmol · g^{-1}fm]	MDA [nmol · g^{-1}fm]
Control – Kontrola	94.10 ± 0.26 b	61.54 ± 41.13 a	66.37 ± 45.84 a	3.80 ± 0.23 e	26.18 ± 1.21 ab
3 g · dm^{-3} NaCl	204.77 ± 8.43 a	73.08 ± 2.46 a	83.07±1.37 a	17.13 ± 0.41 d	25.97 ± 0.27 ab
3 g · dm^{-3} NaCl + 1 mM nicotinamide – amid kwasu nikotynowego	236.25 ± 32.65 a	81.64 ± 17.99 a	92.31 ± 15.77 a	23.22 ± 0.97 b	24.73 ± 0.45 b
6 g · dm^{-3} NaCl	212.12 ± 58.0 a	80.15 ± 25.60 a	86.77 ± 24.43 a	19.54 ± 0.71 c	26.83 ± 0.82 a
6 g · dm^{-3} NaCl + 1 mM nicotinamide – amid kwasu nikotynowego	244.75 ± 83.10 a	91.36 ± 33.88 a	101.40 ± 34.59 a	30.76 ± 1.94 a	25.43 ± 0.83 ab
$LSD_{0.05}$ $NIR_{0,05}$	92.50	53.56	55.76	1.99	1.53

Means in the same column followed by the same letter are not significantly different at α<0.05 according to Tukey test – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się według testu Tukeya na poziomie istotności α <0,05; ± SD – standard deviation – odchylenie standardowe.

Moreover, addition of nicotinamide to the medium caused a decrease in the values of biochemical parameters studied by half. In contrast, the use of KCl salt solution led to a significant decrease of chlorophyll (*Chl a, Chl b*) and carotenoid contents (Table 1). Seedlings of such combination of media were characterized by decreased content of *Chl a*, *Chl b,* and *Car* compared to the control (26.36, 8.69, 11.92 $mg \cdot g^{-1}$fm, respectively). In this case, the addition of nicotinamide to the medium positively affected biochemical parameters tested, the values of which were similar to that observed in the control group. Ashraf et al (2002), Bybordi (2012), Azooz et al. (2013) and Saeidi-Sar et al. (2013) suggested that it is probably due to the inhibitory effect of the accumulated ions of various salts on chlorophyll biosynthesis or increase of its degradation by chlorophyllase, which is more active under salinity stress.

Based on the laboratory tests' results, field experiment was conducted. The effect of salinity and nicotinamide treatment on growth parameters of tomato cv. Vilma showed that these plants exhibited reduction in their growth and number of root per plant compared to the control plants (Table 2). It was observed that the addition of nicotinamide regardless of NaCl concentration did not show protective effect on plants grown under salinity. Roots length of tomato cv. Vilma was slightly increased compared the control plants (6.30 cm). The reduction of plants growth under salt stress conditions were also observed by Hassanein et al. (2009) in *Zea mays*, Sadak et al. (2010) in two varieties of sunflower (hysun336 and Euroflor), and Azooz et al. (2013) and Abdelhamid et al. (2013) in *Vicia faba* L. According to Hassanein et al. (2009) salt stress causes an increase in the concentration of ABA (Abscisic acid), which exhibits inhibitory effect on cell division and/or expansion.

According to Abdelhamid et al. (2013) vitamins might act as activators of protein synthesis through modulating the activity of enzymes involved in the metabolism of proteins. MDA is one of the end products that are produced as a result of lipid peroxidation damage by free radical. Increase in MDA concentration after the application of 100 mM NaCl was described by Khattab (2007) in canola, Sadak et al. (2010) and Abdelhamid et al. (2013) in faba bean. Moreover, they suggested that application of nicotinamide could attenuate the effect of salinity, by decreasing MDA level, compared to the corresponding salinity level. In our study, it was observed that tomato plants treated with 6 $g \cdot dm^{-3}$ NaCl had the highest level of MDA in leaves (26.83 $nmol \cdot g^{-1}$ fm) in comparison to the control or those with other treatment (Table 3). According to Smolik et al. (2013) higher concentration of proline and MDA in plant tissue may suggested that plant were affected by salt stress. The contents of proline was significantly increase (30.76 $\mu mol \cdot g^{-1}$ fm) when 6 $g \cdot dm^{-3}$ NaCl with combination of 1mM nicotinamide were used in comparison to the control (3.80 $\mu mol \cdot g^{-1}$ fm). According to Azooz et al. (2013) most vitamins tend to increase the proline content.

Furthermore, the addition of nicotinamide to the medium regardless of NaCl concentration slightly increased the content of *Chl a* in tomato leaves investigated in comparison to the control (Table 3). However the addition of nicotinamide to the salt solution had no significant effect on *Chl b* and *Car* contents in tomato leaves. Similar results were obtained by Hassaein et al. (2009) in *Zea mays*, Sadak et al. (2010) in sunflowers and Azooz et al. (2013) in *Vicia*

faba L., who also found that the chlorophyll content in the tested plants increases after the application of vitamin C and/or nicotinamide. Contents of photosynthetic pigments in tomato cv. Vilma leaves is closely correlated with their color. Although leaves of tomato from the control group were darker compared to other plants. It is evidenced by the value of L* parameter, which was 30.79. At the same time, it was observed that with increasing NaCl concentration in the medium, the value of L* parameter was higher in comparison to control by 14–18% (Fig. 1).

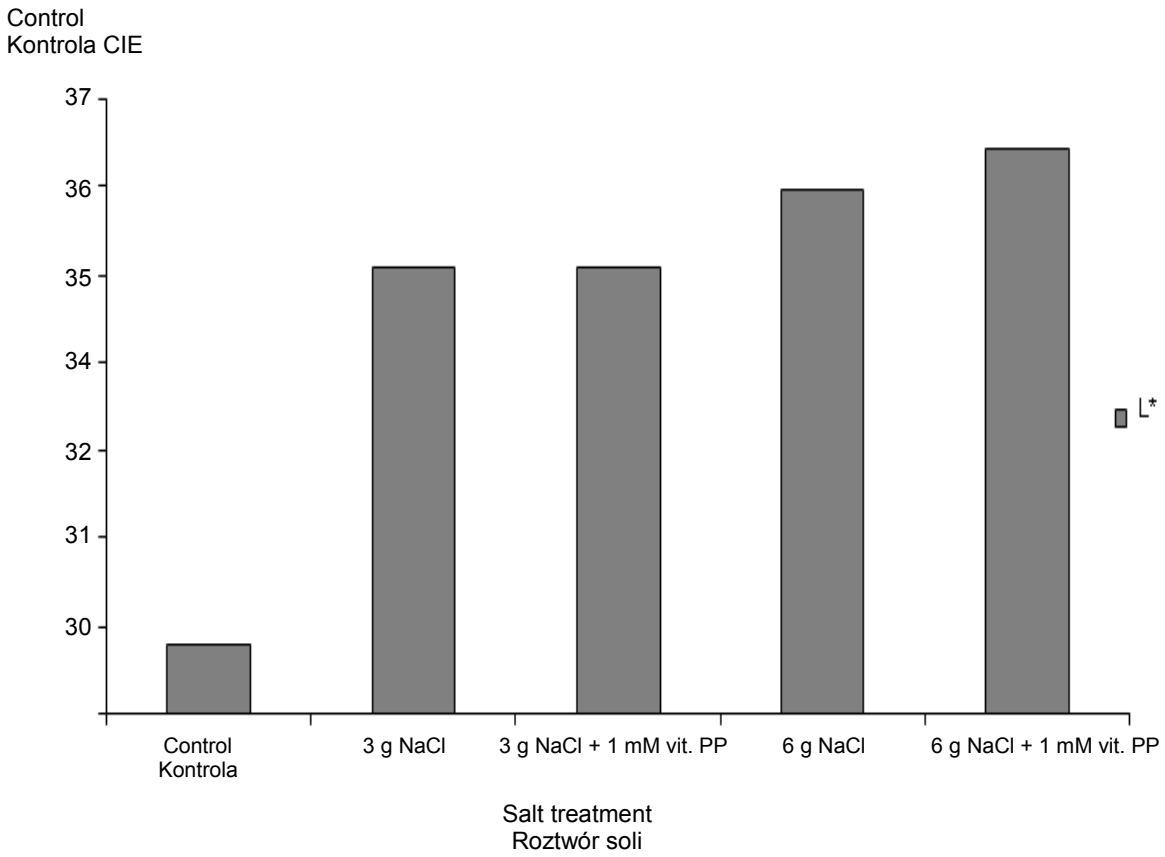

Fig. 1. Effects of 1mM nicotinamide (vit. PP) application on L* parameter – black (0) or white (100) color on tomato cv. Vilma leaves under non-saline and saline conditions
Ryc. 1. Wpływ dodatku 1mM amidu kwasu nikotynowego (vit PP) na parametr L* – barwa czarna (0) lub biała (100) w liściach pomidora odmiany Vilma w warunkach kontrolnych i zasolenia

Adding 1mM nicotinamide to the medium under saline conditions for tomato cv. Vilma had no effect on intensity of green color determined by parameter a^* (Fig. 2). In contrast, after stress factor activation it was observed that tomato leaves were more yellow as compared to the control as evidences by b^*parameter (Fig. 2).

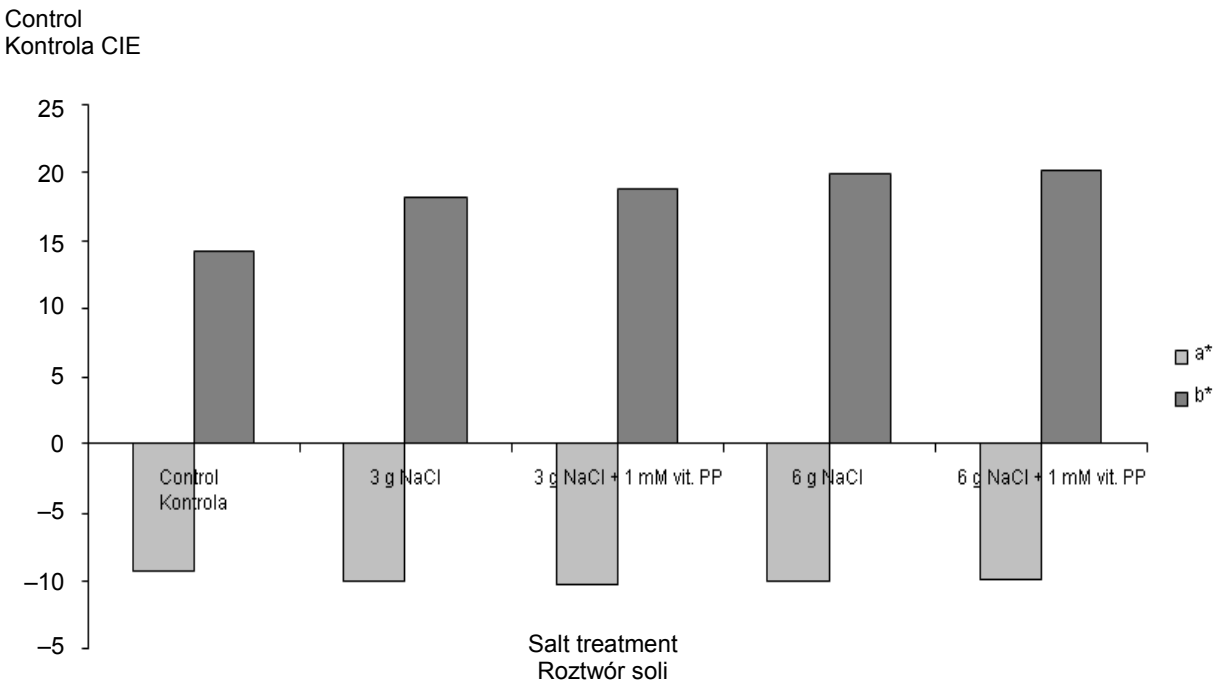

Fig. 2. Effects of 1mM nicotinamide (vit PP) application on a^* – green (–100) or red (+100) color ai b^* – blue (–100) or yellow (+100) color parameters on tomato cv. Vilma leaves under non-saline ai saline conditions

Ryc. 2. Wpływ dodatku 1mM roztworu amidu kwasu nikotynowego (vit. PP) na parametr a^* – barwa zielona (–100) lub czerwona (+100)] oraz b^* – barwa niebieska (–100) lub żółta (+100) w liściach pomidora odmiany Vilma w warunkach kontrolnych i zasolenia

CONCLUSION

The study shows that salinity stress had a negative influence on the growth and development, as well as biochemical parameters of Vilma tomato cultivar shoots grown under *in vitro* and *in vivo* conditions. However, in order to better recognize and understand how vitamin PP might be related with salt tolerance, additional and more precise research should be carried out.

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Abstract. The aim of this study is to determine the effect of both NaCl and KCl salt alone or in combination with nicotinamide, on growth and some biochemical parameters of Vilma cultivar of tomato under laboratory and field conditions. The combinations of salt solutions used had a negative impact on the ability of seed germination and morphological characteristics of 14-day-old tomato seedlings. The addition of NaCl salt had a positive impact on the content of *Chl a* and *Car* in contrast KCl salt solution led to the decrease of photosynthetic and non--photosynthetic pigments. Under field conditions, NaCl salt solution exhibited inhibitory effect on plant growth, concentration of *Chl a, Chl b,* and *Car,* simultaneously increasing oxidative stress parameters (proline and malondialdehyde – MDA). Moreover, leaves of tomato from the control group were darker in comparison to the remaining plants. It was observed that the addition of nicotinamide to the solution did not show protective effect on plants grown under salinity, except in a small increase of the concentration of proline.