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Assessment of 1-Triacontanol treatment of sweet corn (*Zea mays* L. convar. *saccharata*) aimed at the improvement of salt tolerance based on a pot experiment

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Abstract: In our research, the salt stress of two sweet corn hybrids (Tyson and Sweetstar) was induced by irrigation with saline water in a pot experiment. We studied the possibility of the decrease of the negative effects of salt stress by applying *1-Triacontanol* (TRIA) in various (3, 6, and 9 µM) doses. Plant height, raw biomass, root biomass, parameters characterizing the photosynthetic activity (SPAD, NDVI, chlorophyll content), and proline content were determined in order to quantify the effect of the TRIA treatment on salt tolerance. We concluded that triacontanol treatments had a positive effect on the studied parameters of both hybrids.

Keywords: salt stress, Sweetstar, photosynthetic activity, chlorophyll content

1. Introduction

The European Union and the USA are the most significant sweet corn growers worldwide. Preceding France, Hungary is the leading sweet corn producer in the European Union [1]. In Europe, the area utilized with sweet corn is the largest in Hungary, which provides 6 percent of the world's production.

Hungary is a net exporter regarding the most important vegetable products (green pepper, watermelon, green peas, sweet corn). The export of sweet corn products is 22 times higher than the import [2]. In the case of this vegetable, profitability has increased by 64% in the past four years, so farmers producing sweet corn should reckon with a strong market position [3]. The agroecological (climatic and soil) conditions of Hungary are favourable for sweet corn production, which is the most successful vegetable crop in Hungary.

As the water demand of sweet corn is high, safe production can only be ensured with irrigation. Increasing the proportion of irrigated areas is particularly important in horticultural crop production [4], making cultivation safer with higher yields. Nevertheless, irrigation could be a limiting factor in agriculture, as the salinity of irrigation water (and in soils) affects at least 800 million hectares throughout the world, which is more than 6% in terms of the total land area [5, 6].

Unfortunately, the salinity of waters used for irrigation is also high in some regions of Hungary [7], which causes difficulties in vegetable production, as a number of literature data have proven that most vegetable species are sensitive to high salinity [8]. It is an existing problem especially in the region of Great Cumania, as the water used for irrigation is saline in several cases. In their research, Zsembeli et al. [9] proved that in Karcag the water coming from the underground is not suitable for irrigation. According to García et al. [10], half of all irrigated soils are affected by soil salinity in that region.

Salinity is a major environmental challenge in agricultural production, as the salts transported to and accumulated in the root zone could affect crop yield and quality negatively as they are generating an osmotic pressure [11, 12].

The growing population of the world poses a major challenge to agricultural production. Based on several research studies, the population of the world will grow significantly over the next 50 years, reaching more than 9-10 billion people. The growing population requires more intensive food production, so it is necessary to increase the amount of corn products as well [13].

As the population grows very rapidly, land and water resources are used in a volume that can lead to the exploitation of fertile agricultural land and freshwater resources. As sweet corn is a high-value vegetable, it is definitely recommended in the long term to expand the cultivation areas, which calls for the inclusion of less favourable but still high-quality land and water resources in production [14].

Under abiotic stresses, exogenously applied plant hormones, such as *Triacontanol* (TRIA), which is a long-chain primary alcohol, can increase the growth, photosynthetic pigments, and yields of several crop species (rice, wheat, maize, tomato) [15, 16].

Numerous researchers have reported that TRIA has a positive effect on several plant physiological processes such as carbon dioxide and nitrogen fixation, protein synthesis, uptake of water and nutrients; furthermore, it can increase free amino acid content and enzyme activities in maize (*Zea mays* L.) [17, 18].

The aim of our experiment was to investigate and quantify the effect of three different doses of TRIA, applied as a foliar fertilizer, on the salt tolerance of two sweet corn hybrids. In our research, salt stress was induced by irrigation with saline water in a pot experiment, as we examined the possibility of utilizing agricultural areas that are suitable for sweet corn production yet characterized with unfavourable agro-ecological conditions (saline irrigation water, risk of secondary salinization). Given that the population demand for food resources is estimated to increase [19], the examination of the possibilities of growing crops in less favourable areas is becoming more and more important in order to promote sustainable agriculture.

2. Material and methods

In our study, the farming practice of Great Cumania was represented, which is a typical region with salt-affected soils in Hungary. We induced salt stress by using irrigation water characterized by high (> 500 mg L^{-1}) salt content. The aim of the experiment was to increase the salt tolerance of sweet corn and to quantify the difference in salt tolerance between the two hybrids under study.

The pot experiment

In our pot experiment (*Fig. 1*), we tested two sweet corn hybrids, namely Sweetstar and Tyson (Syngenta®), which are proven to be salt-tolerant on the basis of the results of a preliminary experiment.

Figure 1. Sweet corn hybrids in the pot experiment

The experiment was set up on 17/09/2020 in plastic pots filled with 5 kg of calcareous chernozem soil. The pots were placed in a heated greenhouse, where we could maintain air temperature values (22 ± 7ºC) optimal for the development of sweet corn. The main properties of the soil filled into the pots are summarized in *Table 1*.

Table 1. Some parameters of the 0–20 cm layer of the soil used in the pot experiment

Parameter	**$PH_{(KCl)}$**	**Electronic conductivity (EC)**	**Humus content**	**NO_3-N**	**P_2O_5**	**K_2O**	**Na**	**$CaCO_3$**
Unit		mS cm^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	7.8	0.44	1.2	18.4	722	334	58	2.49

The soil can be characterized by weakly alkaline pH and 0.44 mS cm^{-1}, which means low salinity. The soil used in the experiment is poor in humus (1.2%). The soil of the pots is very well supplied with phosphorus and potassium and has a medium sodium content. In terms of calcium carbonate content, this calcareous chernozem soil can be considered weakly calcareous.

Induction of salt stress

Besides the beneficial effects of irrigation, we must also pay attention to its unfavourable effects reducing soil fertility and yields. If the irrigation water applied is inadequate, improper use can result in secondary salinization. The process of salinization is a change in the adsorption conditions in which the amount and proportion of sodium ions among the cations bound on the surface of the soil colloids increase, resulting in unfavourable soil physical properties. Secondary salinization occurs when the negative effects are caused by the high salinity of the irrigation water. As the total dissolved salt content of the water used for irrigation is also very high in Karcag and in its surrounding area, we used irrigation water originating from the drinking water network of Karcag to induce salt stress in our experiment. The main parameters of the irrigation water applied are summarized in *Table 2*.

Table 2. Some parameters of the irrigation water in Karcag

pH	EC	Dry matter content	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	NO_2^{-} $+NO_3^{-}$
	mS cm^{-1}	g l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
7.5	1.4	0.8	4.2	2.5	9.0	0.1	0	800	88.8	0.00	5.1

As according to the Hungarian standards water is not suitable for irrigation (total soluble salt content is above 500 mg L^{-1}), its use carries the risk of secondary salinization and can cause salt stress in cultivated plants.

Stressing the plants with salty water was initiated in the same time as sowing and was continued until the end of the experiment (the beginning of male flowering). The irrigation protocol, the electrical conductivity and salt concentration of the irrigation water, and the salt loads by irrigation are summarized in *Table 3*.

Table 3. The irrigation protocol

Date of irrigation	**Dosage of irrigation**	**EC**	**Salt concentration of the irrigation water**	**Salt input**
	L per pot	*mS cm^{-1}*	*mg L^{-1}*	*mg per pot*
09/09/2020	0.5	1.41	902.4	451.2
14/09/2020	0.5	1.41	902.4	451.2
18/09/2020	1	1.41	902.4	902.4
21/09/2020	0.3	1.41	902.4	270.7
06/10/2020	0.2	1.41	902.4	180.5
09/10/2020	0.3	1.41	902.4	270.7
16/10/2020	0.3	1.6	1024	307.2
27/10/2020	0.3	1.6	1024	307.2
30/10/2020	0.3	1.6	1024	307.2
04/11/2020	0.3	1.6	1024	307.2
09/11/2020	0.3	1.6	1024	307.2
Total	4.3			4,063

In total, 4.3 litres of saline water containing 4,063 mg of salts was irrigated on each pot in order to satisfy the water needs adapted to the growth of the plants until the beginning of flowering.

The indicator crops were fertilized four times with Ferticare IV (YARA) water-soluble fertilizer and two times with Mono Zn (Natur Agro) foliar fertilizer.

Triacontanol treatments

The plants exposed to salt stress were treated with foliar fertilizer made from TRIA. It is known to affect metabolism and regulate a number of physiological and biochemical processes.

During our study, we treated the plants with three different doses: 3, 6, and 9 micromolar (µM) of TRIA. In each case, these solutions were prepared from 1 litre of deionized water and the amount of TRIA (molar mass: 438.8 g MOL^{-1}) corresponding to the doses used, to which 1 ml of TWEEN 20 (E432) adhesive was added in order to make the applied solution adhere to the foliage as much as possible.

As control, only deionized water was sprayed on the plants. TRIA foliar treatment was performed a total of 7 times, each time 2 ml being applied onto the foliage of each plant.

As per to the main goal of the experiment, we wanted to investigate the possibility of using TRIA treatment to reduce the negative plant physiological effects on sweet corn suffering from salt stress.

Examined parameters

The indicator crops were harvested at the time of flowering. Because of the different lengths of the vegetation period of the hybrids, Sweetstar was harvested at 75 days of age and Tyson at 87 days of age. During the research, SPAD (Minolta SPAD 502, Japan), NDVI values (Trimble® GreenSeeker® crop sensor), and the total chlorophyll content (mg per 100 g fresh weight) of the plants were measured based on the "AOAC 942.04 (1995)" method, these values characterizing the photosynthetic activity of the plants. Plant height (cm), the raw biomass of the young plant (g), root weight (g), and number of leaves (per plant) were also examined to monitor the growth and development of the plants. Furthermore, proline content (100 μM per 100 g of fresh weight) as a plant stress hormone was also determined based on the method of Bates et al. [20].

Statistical data analysis

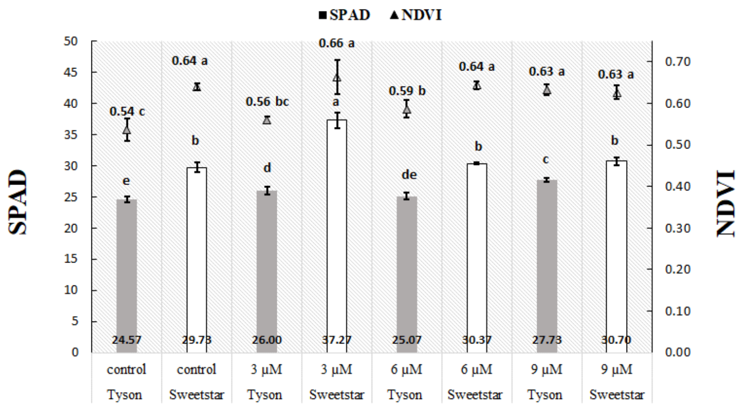
The statistical reliability of our results was tested with the SPSS 25.0 software package. One-way analysis of variance ($p < 0.05$) was used, as we were primarily interested in the effects of the different doses of the foliar fertilizer. Duncan's test ($p < 0.05$) was used to determine the statistical reliability of the differences between the means. One treatment was performed in 5 replicates.

3. Results and discussion

Effect of the treatments on the parameters characterizing the photosynthetic activity of sweet corn

We considered important to examine the effect of the treatments on the photosynthetic activity of sweet corn, as this parameter can be easily modified by environmental and technological conditions as well as by various stress factors.

Figure 2 shows the effect of the treatments on the SPAD and NDVI values of plants. The SPAD value gives us information about the relative chlorophyll content of the leaves while the NDVI about the relative chlorophyll content of the foliage.



Note: Means signed with the same letter are not significantly different at the 5% significance level.

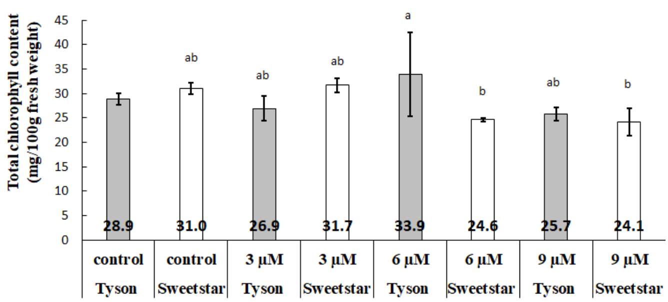
Figure 2. Effect of the treatments on the SPAD and NDVI values of sweet corn

Based on our results, it can be established that in the case of the hybrid Tyson, the different doses of TRIA foliar treatment slightly improved the SPAD and NDVI values compared to the control of the same hybrid, and these differences were statistically proven. The best results were measured in the case of the dose of the 9 µM treatment (SPAD: 27.7 NDVI: 0.63).

For the hybrid Sweetstar, the different doses of TRIA treatments improved the SPAD values in all cases compared to the control, while the NDVI values were only improved by the 3 µM TRIA treatment. The 3 micromolar treatment was proven to be the best for both parameters (SPAD: 37.2, NDVI: 0.66) for that hybrid.

The SPAD and NDVI values measured for Sweetstar were significantly more favourable than those for Tyson.

Chlorophylls are photosynthetic pigments that fundamentally determine the photosynthetic activity, and therefore the effect of the treatments on the total chlorophyll content of the plants was also examined. The relevant results are shown in *Figure 3*.



Note: Means signed with the same letter are not significantly different at the 5% significance level.

Figure 3. Effect of the treatments on the total chlorophyll content of sweet corn

In the case of the hybrid Tyson, the dose of 6 µM of the TRIA treatment (33.9 mg per 100 g of fresh weight) while in the case of hybrid Sweetstar the effects of the 3 µM dose (31.7 mg per 100 g of fresh weight) were the highest in terms of the total chlorophyll content of the plant leaves. Examining the total chlorophyll content of Sweetstar, we found that the use of TRIA treatment in doses higher than 3 µM had a negative effect on this parameter. However, these results could not be verified at a significance level of 5%.

Effect of the treatments on some morphological parameters of sweet corn

Since abiotic stress factors also affect the vegetative plant development, we considered to determine the effect of the treatments on the parameters of plant height, total aboveground (raw) biomass, root mass, and total number of leaves as important in our experiment. The results of these measurements are summarized in *Table 4*.

Table 4. The effect of different doses of TRIA treatments on some morphological parameters of sweet corn

Hybrid	Treatment	Plant height	Total aboveground biomass	Root mass	Total number of leaves
		cm	g per plant	g per plant	leaves per plant
Tyson	control	56.0cd	43.5c	1.6d	11.0ab
Sweetstar	control	76.2b	56.8ab	3.1bc	10.5b
Tyson	3 µM	57.3c	51.4b	1.8d	10.7b
Sweetstar	3 µM	86.0a	61.8a	4.0a	9.7c
Tyson	6 µM	62.0c	51.6b	1.8d	11.0ab
Sweetstar	6 µM	48.1c	32.6d	3.5ab	9.7c
Tyson	9 µM	64.3c	52.9b	1.8d	11.7a
Sweetstar	9 µM	71.3c	38.2cd	2.9c	9.7c

Note: Means followed by the same letter within the column are not significantly different at the 5% significance level.

Based on the morphological parameters, it can be concluded that for Tyson the TRIA treatments resulted in greater plant height and total aboveground (raw) biomass in all cases compared to the untreated individuals. The highest plant height and total aboveground biomass values of Tyson were measured for the 9 µM TRIA treatment (64.3 cm and 52.9 g) – the differences compared to the control were statistically proven.

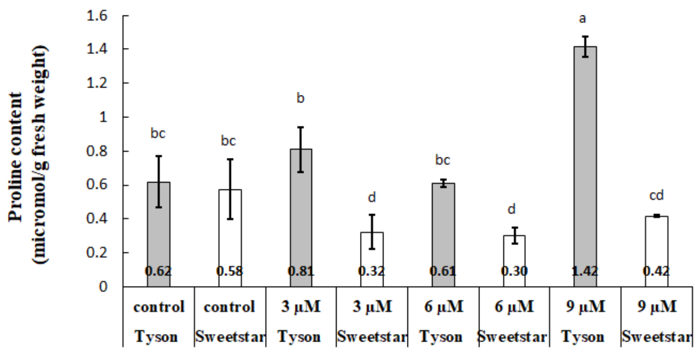
In the case of Sweetstar, both plant height and total aboveground biomass were improved by the 3 µM treatment compared to the control (86.0 cm and 61.8 g); however, the higher doses negatively affected these parameters, just like in the case of the total chlorophyll content.

Based on the examination of the root mass of the sweet corn hybrids we used, it can be established that in the case of Tyson, the different doses of TRIA improved the root weight equally (1.8 g), while in the case of Sweetstar the TRIA treatment of 3 µM dose was the most favourable, resulting in a 4.0 g increase, which was statistically significant.

The salt stress induced in our study did not cause such a stress effect on sweet corn that would have seriously affected the leaf number of the plants and thus the size of the assimilation surface. In the case of Tyson, the 9 µM TRIA treatment, which had a positive effect on several parameters, minimally increased the average number of leaves per stem (11.7). However, for Sweetstar, the TRIA treatments had no positive effect on the average leaf number.

Effect of the treatments on the proline content of the two sweet corn hybrids

Against salinity and osmotic stress, plants accumulate proline in their organisms in order to deal with environmental stresses [21]. Numerous researchers have reported that the accumulation of proline in sweet corn is a natural response to salt stress [22]. Based on Celik and Atak [23], proline accumulation can be a stress effect (a response to stress factors) or can be the cause of stress tolerance (a kind of protector agent).



Note: Means signed with the same letter are not significantly different at the 5% significance level.

Figure 4. Effect of the treatments on the proline content of sweet corn

Based on the results gained from the measurement of proline content, it can be established that regardless of the treatment, the hybrid Sweetstar produced less proline than the hybrid Tyson, suggesting that Sweetstar can be considered more salt tolerant than Tyson. This fact was also proven by the research of Karimi et al. [24], as they found that the amount of the accumulated proline was significantly higher in the more salt-sensitive maize genotypes than in the more salt-tolerant ones.

As Sweetstar is proven to be more salt tolerant, it accumulated significantly less proline than Tyson. Since the lowest proline (0.32 and 0.30 µM per 100 g of fresh weight) was measured in Sweetstar after the 3 and 6 micromolar treatments, we can conclude that the less proline accumulates in a more salt-tolerant plant, the less stress the latter experiences under the given environmental conditions.

In the case of Tyson, which was proven to be more sensitive to salt stress, proline accumulation increased as a result of the TRIA treatments. Proline production was the highest as a result of the 9 µM dose treatment (1.42 µM per 100 g of fresh weight), which was found to be favourable for other parameters too, which indicates that the plants tried to protect themselves against the negative effects of salt stress.

4. Conclusions

Based on our results, we found that the hybrid Sweetstar was more salt tolerant than the hybrid Tyson in all tested parameters. In the case of Sweetstar, the 3 µM TRIA treatment improved the parameters characterizing the photosynthetic activity of the plant, as well as plant height, raw biomass, root weight, and proline content. However, the higher doses negatively affected several parameters of Sweetstar (total chlorophyll content, plant height, raw biomass, leaf number). For the less salt-tolerant Tyson, higher doses of TRIA treatments were proved to be more effective for most of the parameters.

We concluded that TRIA as a foliar fertilizer is suitable for improving the salt tolerance of sweet corn exposed to salt stress. Based on the examined parameters, it can be stated that different doses of TRIA can be recommended for treating the hybrids with different degrees of salt tolerance. We suggest a lower dose (3µM) of TRIA application for tolerant hybrids and a higher dose (9µM) for salt-sensitive ones.

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References

[1] Felföldi, J., Kerekes, V. (2016), Economic analysis of sweet corn production through the example of an integrator in Hungary. In: *Abstract: Applied studies in agribusiness and commerce*. 1–12.

[2] Gergely, G. N. T. (2019), Kertészeti zöldségtermesztés vizsgálata Magyarországon és az EU-ban. *Acta Agronomica Óváriensis* 60(1).

[3] van Landbouw, M. (2021), Fruit and vegetable production in Hungary. *Nieuwsbericht – Agroberichten Buitenland*. https://www.agroberichtenbuitenland.nl/actueel/nieuws/2021/02/22/hungary-tldr-horti-fruit-veg (downloaded on: 06/06/2021).

[4] Helyes, L. (2005), Az öntözés szerepe, jelentősége. Gazdálkodás. *Scientific Journal on Agricultural Economics* 49(5), 63–69.

[5] Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., Hernandez, J. A. (2017), Plant responses to salt stress: Adaptive mechanisms. *Agronomy* 7(1), 18.

[6] Munns, R., Tester, M. (2008), Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.

[7] Zsembeli, J., Kovács, G., Mándoki, A. (2011), Water use efficiency of maize and different sorghum hybrids under lysimeter conditions. In: *14. Gumpensteiner Lysimetertagung*. 227–229.

[8] Slezák, K. A. (2001), Fehér termésű paprika sótűrése. Doctoral dissertation. Szent István University, Gödöllő.

[9] Zsembeli, J., Kovács, Gy., Szűcs, L., Tóth, J. (2013), Examination of secondary salinization in simple drainage lysimeters. In: *15. Gumpensteiner Lysimetertagung*. 153–156.

[10] Garcia, A. R., Tuba, G., Kovács, G., Sinka, L., Zsembeli, J. (2021), Methodology adaptation and development to assess salt content dynamics and salt balance of soils under secondary salinization. *Acta Agraria Debreceniensis* (1), 199–206.

[11] Zsembeli, J., Sinka, L., Rivera-García, A., Czellér, K., Tuba, G., Krištof, K., Findura, P. (2019), Effect of soil conditioning on the moisture content and the salt profile of the soil under irrigation with saline water. *Agriculture* 65(2), 77–87.

[12] Ashraf, M., Harris, J. C. (2004), Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166, 3–16.

[13] Abdul, R., Mahmood, K. (2012), Rehabilitation of saline ecosystems through cultivation of salt tolerant plants. *Pakistan Journal of Botany* 44, 69–75.

[14] Futó, Z., Bencze, G. (2017), Új lehetőségek a kukorica (*Zea mays* L.) öntözésében. *Jelenkori Társadalmi és Gazdasági Folyamatok* 12(3), 67–79.

[15] Perveen, S., Shahbaz, M., Ashraf, M. (2012), Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress? *Journal of Applied Botany and Food Quality* 85(1), 41.

[16] Perveen, S., Iqbal, M., Parveen, A., Akram, M. S., Shahbaz, M., Akber, S., Mehboob, A. (2017), Exogenous triacontanol-mediated increase in phenolics, proline, activity of nitrate reductase, and shoot k+ confers salt tolerance in maize *(Zea mays* L.). *Brazilian Journal of Botany* 40(1), 1–11.

[17] Naeem, M., Khan, M. M. A., Moinuddin. (2012), Triacontanol: A potent plant growth regulator in agriculture. *Journal of Plant Interactions* 7(2), 129–142.

[18] Harrabi, S., Boukhchina, S., Mayer, P. M., Kallel, H. (2009), Policosanol distribution and accumulation in developing corn kernels. *Food Chemistry* 115(3), 918–923.

[19] Corona-López, E., Román-Gutiérrez, A. D., Otazo-Sánchez, E. M., Guzmán-Ortiz, F. A., Acevedo-Sandoval, O. A. (2021), Water–food nexus assessment in agriculture: A systematic review. *International Journal of Environmental Research and Public Health* 18(9), 4983.

[20] Bates L. S., Waldren R. P., Teare I. D. (1973), Rapid determination of free proline for water stress studies. *Plant Soil* 39, 205–207.

[21] Anjum, M. A. (2008), Effect of NaCl concentrations in irrigation water on growth and polyamine metabolism in two citrus rootstocks with different levels of salinity tolerance. *Acta Physiologiae Plantarum* 30(1), 43–52. DOI: 10.1007/s11738-007-0089-3.

[22] Cha-Um S., Kirdmanee, C. (2009), Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pakistan Journal of Botany* 41(1), 87–98.

[23] Çelik, Ö., Atak, C. (2012), The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. *Turkish Journal of Biology* 36(3), 339–356.

[24] Karimi, S., Eshghi, S., Karimi, S., Hasan-Nezhadian, S. (2017), Inducing salt tolerance in sweet corn by magnetic priming. *Acta Agriculturae Slovenica* 109(1), 89–102.