

Inhibition of deoxyhypusine synthase by GC7 induces the modification of fruit setting and polyamine catabolism in tomato plants exposed to salt stress

ÁGNES SZEPESI, LILLA SÍPOS, RÉKA SZÓLLÓSI, PÉTER PÁLFI and LÁSZLÓ BAKACSY

Department of Plant Biology, Institute of Biology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Hungary

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Abstract. Tomato is one of the primary agricultural plants, grown in various areas affected by salt stress. Protein translation is a crucial process in the stress tolerance of crop plants. There is evidence to indicate that hypusination, which is a polyamine-dependent metabolic post-translational modification of eukaryotic translation factor 5A, may play a crucial role in tolerance to salt stress. In the present study, in order to clarify the importance of the first step of hypusination, the deoxyhypusine synthase (DHS) pharmacological inhibitor, GC7, was applied. A pot experiment was undertaken to examine the effects of the inhibition of DHS by GC7 on tomato (*Solanum lycopersicum*) flower and fruit parameters under conditions of salt stress. The results revealed that GC7 promoted the formation of more flowers and fruits in tomato plants subjected to salt stress, and effectively increased the bioactive lycopene content during conditions of salt stress. On the whole, the findings of the present study demonstrate that the modulation of hypusination may be a promising breeding target with which to enhance the tolerance of tomato plants to salt stress.

Introduction

Salinity is one of the most threatening abiotic stress factors affecting not only agriculture but also our food safety. The size of salt-affected areas is >833 million hectares, and changing climate conditions are responsible for its increasing incidence worldwide (1). Irrigation with brackish water or ground water

contaminated with salty water also threatens the yield of salt-sensitive crop plants (2).

Tomato (*Solanum lycopersicum* L.), one of the primary agricultural crop plants worldwide, is a moderately salt-tolerant species. As it widely produced in areas affected by salt stress, it is crucial to develop methods to improve the salt tolerance of this species. The effect of salinity is pleiotropic; salinity not only affects growth and development, but also the reproductive system (3).

In order to enhance the salt stress tolerance of tomatoes, the exogenous addition of polyamines (PAs) is a widely used technique in agriculture (4). The significance of these essential PAs is diverse; for example, the triamine, spermidine (Spd), may be a substrate for hypusination (5). Hypusination is the essential metabolic post-translational modification of eukaryotic translation factor 5A (eIF5A), which is dependent on the level of Spd (6). The biosynthesis of hypusine, a rare amino acid essential for the activation of eIF5A, requires two enzymatic reactions mediated by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), respectively. This Spd-dependent eIF5A hypusination is involved in plant growth and development, such as in flowering and fruit development (7). However, there is a lack of evidence regarding the role of hypusination during salt stress in tomatoes, since only plants grown under optimal conditions have been examined thus far (8).

It was previously found (9) that GC7 (N1-guanyl-1,7-diaminoheptane), an inhibitor of exogenously applied DHS, was effective in alleviating salt stress in *Arabidopsis thaliana* seedlings; however, the importance of the first step of hypusination for the reproductive system of tomatoes during salt stress is not yet known. The present study thus aimed to confirm that the exogenously applied DHS inhibitor, GC7, could improve fruit production by modulating hypusination during salt stress in tomatoes, affecting PA catabolism. The results obtained in the present study demonstrate that the modulation of hypusination may be a promising strategy with which to improve the reproductive system of tomato plants during conditions of salt stress.

Materials and methods

Plant growth conditions and treatments. The determinate *Solanum lycopersicum* cv Manó from Rédei Kertmag

Correspondence to: Dr Ágnes Szepesi, Department of Plant Biology, Institute of Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary
E-mail: szepesia@bio.u-szeged.hu

Abbreviations: eIF5A, eukaryotic translation factor 5A; DHS, deoxyhypusine synthase; DOHH, deoxyhypusine hydroxylase; PAs, polyamines; Put, putrescine; Spd, spermidine; DAO, diamine oxidase; PAO, polyamine oxidase

Key words: hypusination, tomato, fruit set, polyamines, GC7, salt stress

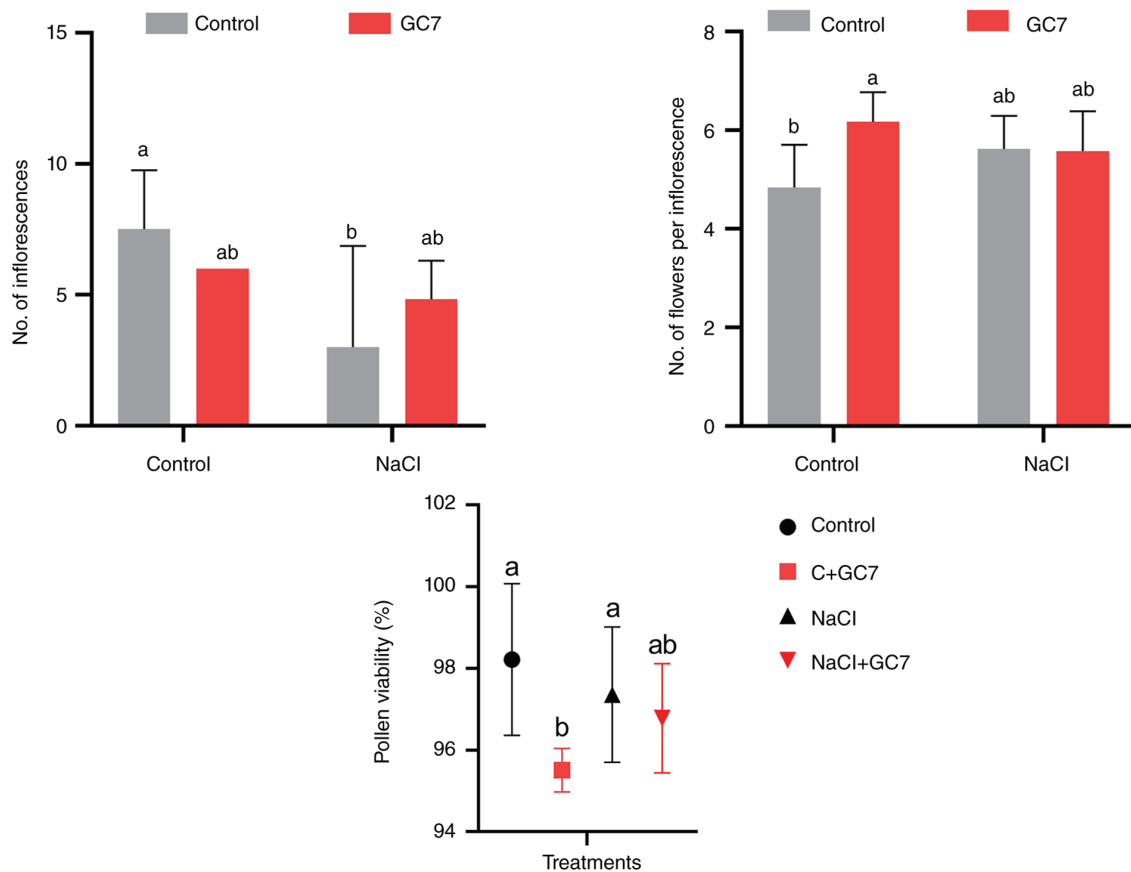


Figure 1. Effects of GC7 on the flowering parameters of tomatoes (*Solanum lycopersicum* cv Manó) during conditions of salt stress. Grey columns represent the values of plants prior to treatment with GC7, whereas red columns represent the values of GC7-treated plants. GC7 is the spermidine analogue, N1-guanyl-1,7-diaminoheptane. Values are the mean \pm SD; the means within each column followed by different letters indicate significant differences ($P < 0.05$).

(Budapest, Hungary) was used as a model plant for plant material. Plants were grown in pots filled with sand:perlite (ratio, 1:3) in the greenhouse of the Department of Plant Biology, University of Szeged (Szeged, Hungary). Nutrients were supplied by irrigation with nutrient solution as previously described (10). The plants were divided into four groups as follows: i) The control group without any treatment; ii) the control + GC7 group which was treated with GC7, but without NaCl; iii) the NaCl group, which was exposed to salt stress without GC7; and iv) the NaCl + GC7 group, which was exposed to salt stress (NaCl) and treated with GC7. The concentration of GC7 was 1 mM, and moderate NaCl stress was induced by 100 mM NaCl (Reanal Finechemical Co.) based on the findings of a previous study by the authors (9). The application of GC7 (MilliporeSigma) was conducted by leaf cover with a brush in the third week of the vegetative period of the tomato. NaCl treatment was applied during the entire growing period.

Parameters of fruit production and PA catabolism. Some parameters associated with fruit set were analyzed, such as the mean fruit weight, Brix index and lycopene content (11). Pollen viability was determined as previously described (12). Enzyme activities involved in PA catabolism, i.e., diamine oxidase (DAO) and PA oxidase (PAO) were measured using a spectrophotometer (KONTRON), as previously described (13).

Statistical analysis. The data were analyzed using GraphPad software Prism version 8.0.1.244 or Windows (Dotmatics). In the graphs, different letters on the bars indicate significant differences, based on one-way ANOVA followed by Tukey's multiple range test. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

GC7 was applied during the vegetative period on the tomato leaves. As regards reproductive growth, GC7 treatment produced more inflorescences in the plants exposed to salt stress (Fig. 1). Despite the decreased number of inflorescences in the GC7-treated plants, the flower numbers were higher compared with the control. When analyzing pollen viability in the present study samples (Fig. 1), it was found that GC7 was effective in increasing pollen viability in the salt-stressed plants, but not in the control plants. It is thus suggested that precise hypusination is critical for maintaining the viability of pollen; however, in the case of salt stress, low hypusination may result in higher viability (Fig. 1).

The investigation of tomato fruit production in the treated plants revealed that GC7 treatment increased the fruit number (Fig. 2). In addition, the fruit fresh weight was higher compared with the untreated plants treated with or without NaCl (Fig. 3). The Brix index decreased in the GC7-treated fruits,

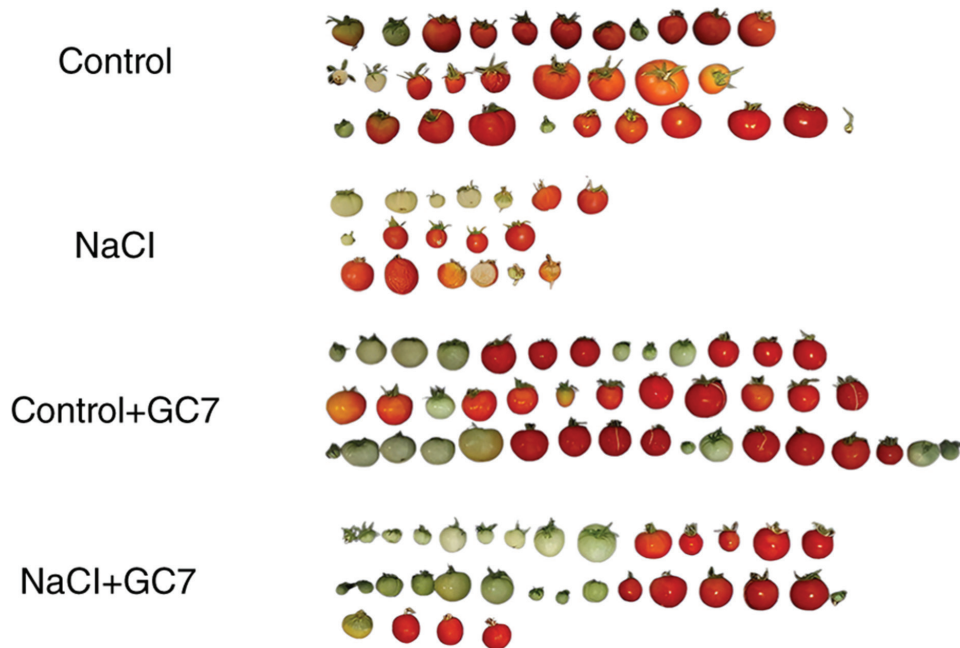


Figure 2. Effects of GC7 on the fruit number of tomatoes (*Solanum lycopersicum* cv Manó) during conditions of salt stress. GC7 is the spermidine analogue, NI-guanyl-1,7-diaminoheptane.

demonstrating that the possible inhibition of DHS caused the modification of sugar metabolism. In the NaCl-treated plants, a significantly higher lycopene content was observed following the application of GC7, suggesting that reduced hypusination may be a target for inducing lycopene synthesis during conditions of salt stress (Fig. 3).

In order to explore potential Spd accumulation in fruits, the present study investigated PA-catabolic enzyme activities. GC7 decreased the activation of both enzymes (Fig. 4); however, in the fruits exposed to salt stress, only the PAO activity decreased, suggesting that more Spd could accumulate in these samples.

Discussion

The modulation of hypusination, a metabolite-dependent post-translational modification of eIF5A, can result in an enhanced tolerance of plants to abiotic stress (14,15). The role of the first enzyme, DHS, in hypusination and its effect on tomato growth and development has been previously investigated (8); it was found that the antisense suppression of DHS in tomato delays fruit softening, and alters growth and development. The present study aimed to elucidate its involvement in the reproductive system of tomato during salt stress. In order to better elucidate this, a determinate tomato cultivar was used in conjunction with a pharmacological approach using GC7, a DHS inhibitor. Using GC7 as an inhibitor of hypusination by reducing DHS activity is a widely used method in human and animal experiments. The application of GC7 in plants is very rare; to the best of our knowledge, the present study is the first to investigate the inhibition of DHS during conditions of salt stress in tomatoes. The hypothesis was that GC7 could inhibit the first step of hypusination in tomato, resulting in higher PA levels which can contribute to improved fruit production and

quality in plants exposed to salt stress. Salt stress decreased the number of inflorescences and the number of fruits, resulting in a lower yield (4). This finding is in accordance with the study of Ghanem *et al* (16), who demonstrated that salt stress reduced the pollen viability of tomato plants. PAs are essential for the optimal developmental regulation of pollen production and viability (17). It has been proven that Spd synthase down-regulation is detrimental to pollen development (18), suggesting that a high Spd level is essential for optimal pollen viability. Furthermore, Song and Tachibana (19) provided evidence that the reduction in the viability of tomato pollen during long-term dry storage in a freezer involves a decline in the capacity to enhance gene translation for PA biosynthetic enzymes upon rehydration. In the present study, GC7 treatment produced more inflorescences with higher pollen viability and the fruit set was higher, which suggests that the modulation of the hypusination process by altering eIF5A isoforms resulting in a higher Spd level may be advantageous for breeding and enhanced stress tolerance. Fruit production is strongly associated with PA metabolism; thus, hypusination, which is a Spd-related metabolic post-translational modification of eIF5A plays a crucial role in fruit production (6). In order to enhance the current knowledge of hypusination-mediated fruit production in tomato plants, further studies are required. For example, further studies are warranted to perform metabolomic assays to elucidate the role of hypusination in the regulation of nutrient values of tomato fruits and gene expression studies to decipher the role of this process in plants.

Another key fruit quality parameter is the Brix index, which represents the total soluble solids content of the fruit. The Brix index increases with the higher electric conductivity of the nutrient solution (20). The Brix index and lycopene content may be higher during conditions of salt stress and following GC7 treatment, as demonstrated in the present study. Mehta *et al* (21)

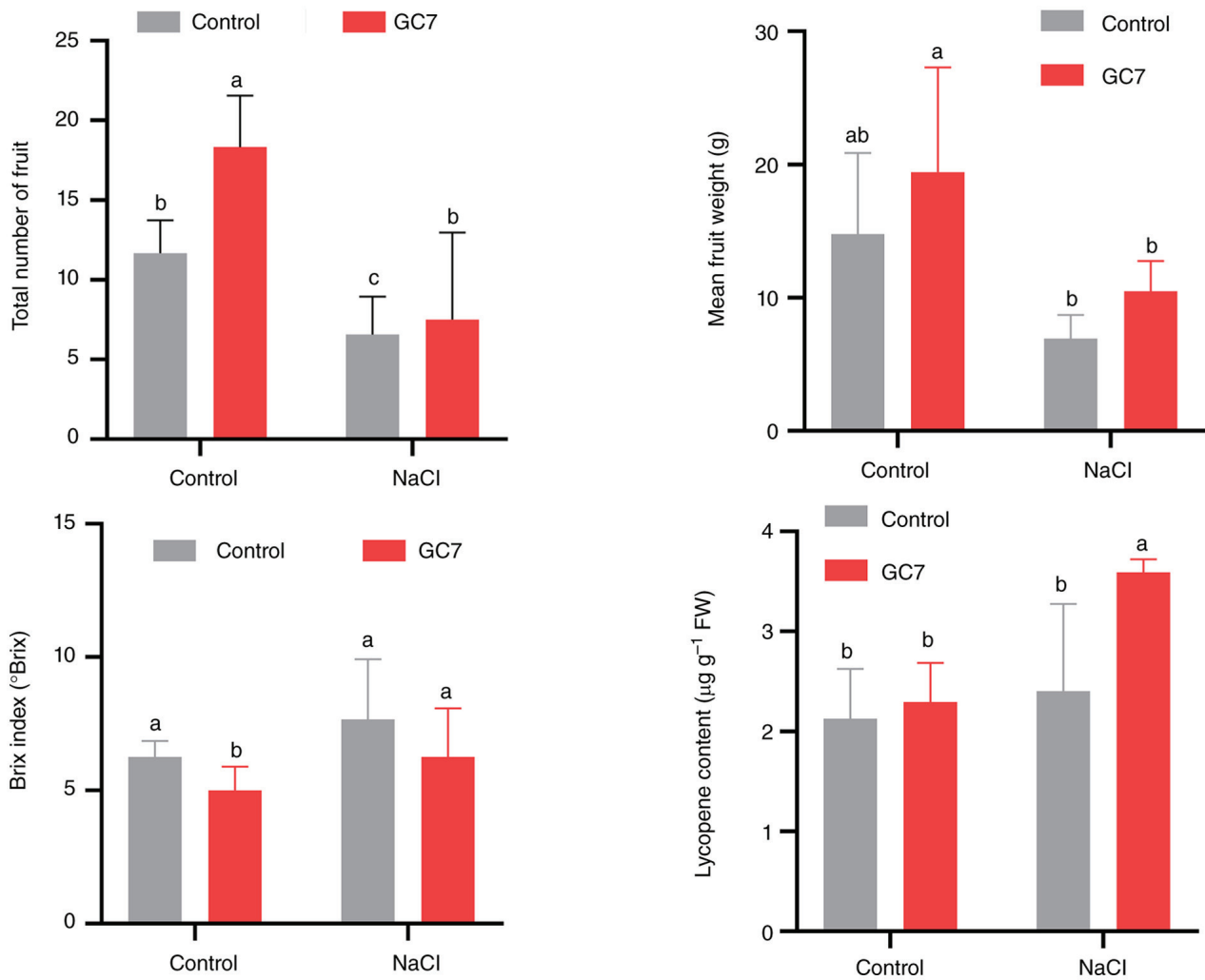


Figure 3. Effects of GC7 on the fruit set parameters of *Solanum lycopersicum* cv Manó during conditions of salt stress. Grey columns represent the values of plants prior to treatment with GC7, whereas red columns represent the values of GC7-treated plants. GC7 is the spermidine analogue, N1-guanyl-1,7-diaminoheptane. Values are mean \pm SD; means within each column followed by different letters indicate significant differences ($P < 0.05$).

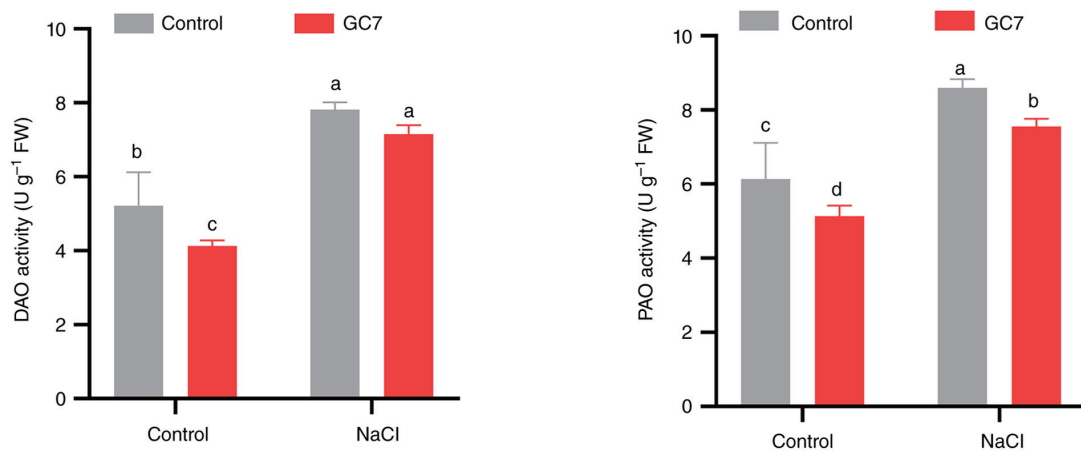


Figure 4. DAO and PAO activities following the application of GC7. Grey columns represent the values of plants prior to treatment with GC7, whereas red columns represent the values of GC7-treated plants. GC7 is the spermidine analogue, N1-guanyl-1,7-diaminoheptane. Values are the mean \pm SD; the means within each column followed by different letters indicate significant differences ($P < 0.05$). DAO, diamine oxidase; PAO, polyamine oxidase; FW, fresh weight.

provided evidence that transgenic tomato plants with higher PA levels enhanced the nutrient value and juice quality. In addition,

Handa and Mattoo (22) demonstrated that lycopene levels were positively associated with the Spd and spermine contents in

tomatoes. Based on the findings of the present study, GC7 affected fruit quality, improving the Brix index and the lycopene content of tomato plants exposed to salt stress. However, further research is required to determine the role of hypusination in fruit sugar metabolism. It is suggested that via the inhibition of hypusination and DHS activity, GC7 may contribute to the increased level of Spd, which could improve the nutrient value of tomato fruits.

The level of PAs, particularly that of Spd, is crucial for efficient hypusination (6). PA catabolism is one of the main regulatory processes which can affect the optimal PA level (5). During the oxidation of PAs, hydrogen peroxide can be generated as a secondary product, inducing oxidative stress or the antioxidant defense system, depending on its concentration. PA catabolism can occur by terminal oxidation, breaking down the PAs or back-conversion to other PAs. If the activities of enzymes involved in PA catabolism, namely the DAO and PAO could be reduced, then these may contribute to the enhanced level of PAs, such as Spd. The results of the present study demonstrate that PA catabolism was also reduced following GC7 treatment in the controls and fruits exposed to salt stress, resulting in higher Spd contents and reduced oxidative stress, by decreased hydrogen peroxide levels. However, further studies are required to decipher the eIF5A-dependent effects and the antioxidant effects of GC7 in plants. Based on animal and human-related studies, it cannot be disclosed that GC7 may have effects independent from eIF5A (23); however, in plants, further experiments are required in the future. A recent study suggested that GC7 could disrupt the energy metabolism mediated by mitochondria in cancer cells (24); therefore, further research is required to reveal the precise mechanisms of GC7 action in plants. Furthermore, the GC7-induced inhibition of DHS could affect the translation of certain key proteins involved in tomato fruit setting (6,25).

In conclusion, the present study demonstrates that reduced DHS activities resulted in higher PA contents and maintained fruit production, contributing to an improved reproductive system in tomatoes exposed to salt stress. The inhibition of hypusination modulated the tomato reproductive system during conditions of salt stress. GC7, a pharmacological inhibitor of DHS, promoted flower and fruit production. The application of GC7 was efficient to increase the lycopene content of tomato fruits. Furthermore, alleviated polyamine catabolism could enhance the PA level during conditions of salt stress, resulting in improved salt tolerance. However, further studies are required to elucidate the role of the salt-induced hypusination process for breeding salt stress-resistant tomatoes.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ÁS and LB conceived the study. LS and PP performed the plant growth experiments. ÁS, LS, PP and LB were involved in the acquisition of data, in the design of the study, and in the writing of the manuscript. RS and PP performed the microscopic analyses. ÁS and LB performed the statistical analyses. ÁS and LB confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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