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MKS: designed the experiment, supervised the research, and prepared the manuscript; NM: carried out the experiment with greenhouse and lab activities

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ORIGINAL RESEARCH PAPER

Effects of different levels of glycine in the nutrient solution on the growth, nutrient composition, and antioxidant activity of coriander (*Coriandrum sativum* L.)

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Abstract

A nutrient solution experiment was performed using sand culture to evaluate the effects of different glycine levels on the growth and nutrient uptake of coriander (*Coriandrum sativum* L.). Different glycine concentrations of 0, 5, 10, 20, or 40 mg L^{-1} were applied to plants via Hoagland's nutrient solution in a completely randomized design with four replications and under greenhouse conditions. The results showed that leaf SPAD (soil and plant analysis development; an indicator of leaf greenness) value, stem diameter, and fresh and dry weights of shoots and roots were significantly increased by 10 mg L^{-1} glycine in comparison to the control plants. Application of glycine at 40 mg L^{-1} reduced many plant growth parameters, whereas leaf proline concentration was increased. All glycine levels except for 40 mg L^{-1} increased root fresh weight. Leaf protein content was increased by glycine applied at 10 or 20 mg L^{-1}, whereas leaf antioxidant activity was increased at all glycine levels. Application of glycine increased leaf concentrations of nitrogen and potassium (at 10 mg L^{-1}), magnesium (at 5 mg L^{-1}), and zinc (at all glycine levels) compared to the control plants. The results indicate that moderate level of glycine (10 mg L^{-1}) in the nutrient solution can improve the growth and nutritional quality of coriander.

Keywords

amino acid; biofortification; biostimulation; fertilization; proline; crop quality

Introduction

Application of chemical fertilizers has an inevitable role in agricultural food production. However, no or reduced fertilizer approaches are more important in sustainable agriculture to increase plant growth and nutrient uptake. This can help to reduce the amount of fertilizers that are applied for achieving higher yields or quality [1–3]. For many decades, increasing nutrient use efficiency has been a matter of interest for many scientists and farmers. From economic and environmental points of view, it is more desirable to improve plant nutrient uptake and use efficiency of fertilizers in all plant–soil–fertilizer systems [4,5]. Recently, application of amino acids to plants, particularly under adverse environmental conditions, has been a focus in agricultural science [1,6–8]. Plants can directly absorb a wide range of nitrogenous compounds including various amino acids [4]. Amino acids are rapidly taken up by plant roots from the medium as intact molecules [9,10]. This uptake is faster than the uptake of other forms of nitrogen, probably due to specific transporters that are involved in translocation of amino acids to plant organs [11,12]. Foliar or root (via nutrient solution) application of amino acids has been shown to improve plant growth, yield, and nutrient uptake [6,8,13–16]. Amino acids are zwitterion molecules that can act either as an acid or as a base, depending on the pH of the medium, and therefore they are effective in balancing deficiencies [5].

Application of chemical fertilizers and pesticides has raised serious doubt on the safety of vegetable product consumption in many parts of the world, especially where regulatory control is limited or not fully enforced. Leafy vegetable crops are mainly consumed fresh and can have significant effects on human health conditions. The application of low or "organic" fertilizer approaches to improve plant yield and quality as well as the nutritional value of crops is necessary for the production of "safe" nutritious foods in horticulture and agriculture. Coriander (*Coriandrum sativum* L., Apiaceae) is a valuable leafy vegetable crop with a high consumption in the Middle East, including Iran. It is a versatile vegetable that can be cultivated under various seasonal and climatic conditions. Safe production of leafy vegetable crops such as coriander with enhanced nutritional value is thus very important. Amino acids such as glycine have been shown to have beneficial effects on the yield and quality of leafy vegetable crops [5,16]. The present study was therefore designed to investigate the effects of different levels of glycine added to a hydroponic culture system on selected growth and physiological parameters of coriander.

Material and methods

This study was performed in hydroponic sand culture during the spring of 2017 at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Four-liter black plastic pots were filled with fine sand and about 60 seeds of coriander obtained from a local population were sown at 1 cm depth and the pot surface wetted with distilled water using a portable sprayer and continued until germination. Two weeks after emergence, seedlings were reduced to 20 per pot. Plants were supplied with Hoagland's nutrient solution for the first 10 days after emergence. Thereafter, glycine treatments were applied to plants via the nutrient solution. Different concentrations of glycine, namely 0, 5, 10, 20, or 40 mg L^{-1}, were applied to plants during the 5-week growth period. Standard Hoagland's nutrient solution (without glycine) was used as a control treatment. Plants were fed daily with 200–400 mL of the nutrient solution per pot (with or without glycine), depending on the plant growth stage and plant size. Every 4 days, pots were washed with additional tap water to prevent salt accumulation in the root medium. Plants were grown under standard greenhouse conditions where the temperature was 24 ±7°C, with 75–80% humidity, and an average photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1}. All plants were harvested about 7 weeks after germination (of which for 5 weeks plants were treated with different glycine concentrations). Plant heights (cm) were determined using a ruler, and leaf SPAD (soil and plant analysis development; an indicator of leaf greenness) values recorded using a portable SPAD meter (model 502 Plus; IL, USA) just before the final harvest. The average of 30 readings of plant leaves per pot was recorded as average leaf SPAD value. Plants shoots were cut at the soil surface and roots were carefully separated from any adhering sand particles using tap water pressure. Shoot and root fresh weights were measured by a digital balance after cleaning, washing, and drying with tissue paper. All samples were placed in an oven at 60°C for 48 h before determination of shoot and root dry weights.

Leaf protein concentrations were determined following the Bradford method [17]. The antioxidant activity of leaves was determined using the free radical scavenging activity (FRSA) of methanolic leaf extracts against DPPH (1,1-diphenyl-2-picryl hydrazyl) solution following the method of Brand-Williams et al. [18]. For this purpose, 2 g fresh leaves were ground in liquid nitrogen and extracted overnight using 75% methanol. The reaction mixture consisted of 500 μL of sample, 3 mL ethanol 95%, and 300 μL methanolic DPPH radical solution (10 mg L^{-1}). The changes in color from dark violet to light yellow were due to the reduction of DPPH by hydrogen donation of antioxidant compounds. The changes in color were recorded at 517 nm using a spectrophotometer (Shimadzu, model UV-160; Japan) after 10 min of reaction. A mixture of ethanol (3 mL) and a sample (0.5 mL) was used as a blank. The percentage of radical scavenging was calculated as follow: $DPPH_{\text{inhibition}}$ (%) = $100 \times [(Abs_{\text{sample+DPPH}}) - (Abs_{\text{sample blank}})] / [(Abs_{\text{DPPH}}) - (Abs_{\text{solvent}})]$

Free proline concentrations in leaves were determined using 2 mL of the leaf alcoholic extract, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid. The UV absorption of samples was then measured against standard proline concentrations at 520 nm. Leaf nutrient concentrations were determined by different methods: total nitrogen concentrations in leaves were determined by the Kjeldahl method, potassium (K) by flame photometry, and magnesium (Mg), iron (Fe), and zinc (Zn) by atomic absorption spectrophotometry (Shimadzu, model AA-7000).

Excel software (Microsoft, WA, USA) was used for calculation of means, standard deviations, and drawing of figures. Data were analyzed by SPSS software and comparison of means was performed at the 5% probability level by Duncan's multiple range test.

Results

The results showed that application of different concentrations of glycine differently affected the growth of coriander. Application of glycine at 40 mg L^{-1} significantly reduced plant height and stem diameter, whereas the other concentrations of glycine showed no difference compared to the control plants (Tab. 1). Application of glycine at 10 mg L^{-1} significantly increased leaf SPAD value (Tab. 1) and plant shoot fresh and dry weights (Fig. 1), whereas application of glycine at 40 mg L^{-1} reduced these in comparison to the control. Application of glycine at 5 or 20 mg L^{-1} showed no influence on the leaf SPAD value and plant shoot fresh and dry weights (Tab. 1 and Fig. 1). However, root fresh and dry weights (Fig. 2) showed a different pattern. The application of glycine at 5, 10, and particularly 20 mg L^{-1} increased root fresh and dry weights compared to the

Tab. 1 Selected growth parameters and leaf SPAD value at different concentrations of glycine in the nutrient solution.

Glycine concentrations (mg L^{-1})	Plant height (cm)	Stem diameter (mm)	Leaf SPAD
0 (control)	15.1 ±1.3 ^{a}	1.14 ±0.08 ^{a}	17.2 ±2.3 ^{b}
5	15.5 ±1.2 ^{a}	1.17 ±0.19 ^{a}	16.8 ±0.7 ^{b}
10	16.9 ±1.3 ^{a}	1.27 ±0.16 ^{a}	21.4 ±3.0 ^{a}
20	16.0 ±0.9 ^{a}	1.18 ±0.17 ^{a}	14.1 ±2.2 ^{bc}
40	11.3 ±0.5 ^{b}	0.81 ±0.13 ^{b}	12.4 ±1.5 ^{c}

Plants were fed with glycine for 5 weeks. Means ±*SD*; $N = 4$. Values in each column followed by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

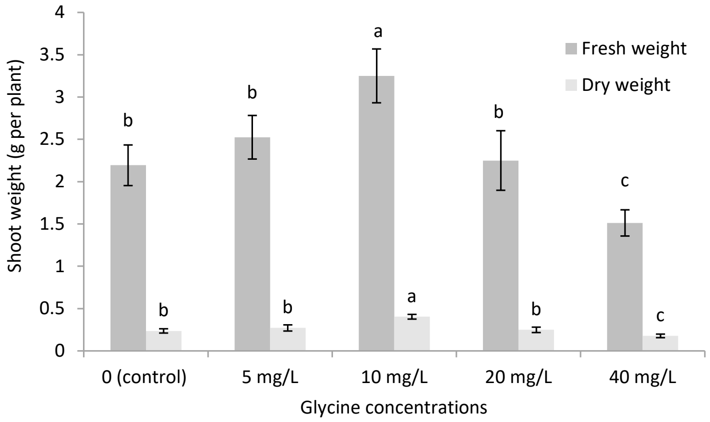

Fig. 1 Fresh and dry weights of coriander shoots at different concentrations of glycine in the nutrient solution. Means ±*SD*; $N = 4$. Values for each parameter marked with different letters on top of the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

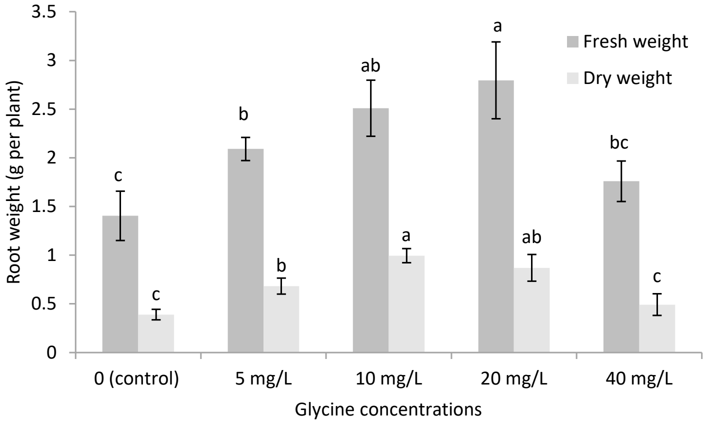

Fig. 2 Fresh and dry weights of coriander roots at different concentrations of glycine in the nutrient solution. Means ±*SD*; *N* = 4. Values for each parameter marked with different letters on top of the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Tab. 2 Concentration of selected nutrients in leaves of coriander grown at different concentrations of glycine after 5 weeks in the nutrient solution.

Glycine concentrations (mg L^{-1})	N (%)	K (%)	Mg (%)	Fe (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)
0 (control)	2.6 ±0.4 ^{b}	2.0 ±0.24 ^{b}	0.42 ±0.07 ^{b}	59 ±7 ^{ab}	33 ±3 ^{b}
5	2.7 ±0.3 ^{b}	2.1 ±0.26 ^{b}	0.56 ±0.08 ^{a}	66 ±10 ^{a}	45 ±5 ^{a}
10	3.3 ±0.2 ^{a}	2.6 ±0.22 ^{a}	0.49 ±0.09 ^{ab}	71 ±12 ^{a}	47 ±3 ^{a}
20	2.7 ±0.3 ^{b}	2.0 ±0.31 ^{b}	0.44 ±0.06 ^{ab}	57 ±5 ^{ab}	44 ±5 ^{a}
40	3.0 ±0.5 ^{ab}	1.7 ±0.21 ^{c}	0.37 ±0.05 ^{b}	49 ±10 ^{b}	43 ±4 ^{a}

Means ±*SD*; *N* = 4. Values in the each column followed by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

control plants, and the greatest root fresh and dry weights were found for 20 mg L^{-1} glycine. There was no difference in root fresh and dry weights of plants treated with glycine at 40 mg L^{-1} in comparison to the control plants. Root dry weight was highest in plants treated with glycine at 10 mg L^{-1} and showed no significant difference with plants treated with glycine at 20 mg L^{-1}.

Determination of leaf nutrient contents (Tab. 2) revealed that an application of glycine at 10 mg L^{-1} increased leaf N concentrations. A similar response was observed for leaf K concentrations. The application of glycine at 10 and 40 mg L^{-1} increased and decreased leaf K concentrations in comparison to the control plants, respectively (Tab. 2). Leaf Mg concentrations were increased by glycine at 5 mg L^{-1}, whereas other glycine concentrations were ineffective. Leaf Fe concentrations were not affected by glycine treatments. However, application of glycine at 5 or 10 mg L^{-1} resulted in higher leaf Fe concentrations compared to plants treated with glycine at 40 mg L^{-1}. All glycine concentrations increased leaf Zn concentrations by comparison with the control plants (Tab. 2).

Application of glycine at 10 or 20 mg L^{-1} caused an increase in leaf protein concentration (Fig. 3), whereas glycine concentrations of 5 or 40 mg L^{-1} showed no influence compared to the control plants. Antioxidant activity of the leaf extract increased at all glycine concentrations (Fig. 4) and the highest leaf activity was noted in plants treated at 10 mg L^{-1}. Leaf free proline accumulation increased in plants treated with glycine at 20 or 40 mg L^{-1} (Fig. 5). The application of glycine at 40 mg L^{-1} resulted in significantly higher amounts of leaf proline concentrations compared to plants treated with glycine at 20 mg L^{-1}.

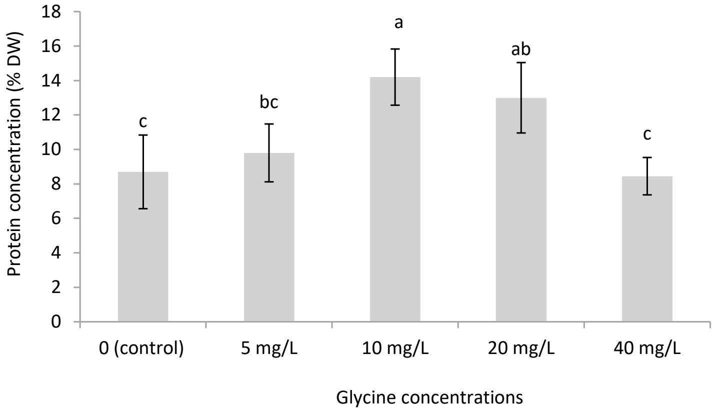

Fig. 3 Leaf protein concentration of coriander grown at different concentrations of glycine in the nutrient solution. Means ±*SD*; *N* = 4. Values with different letters on top of the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

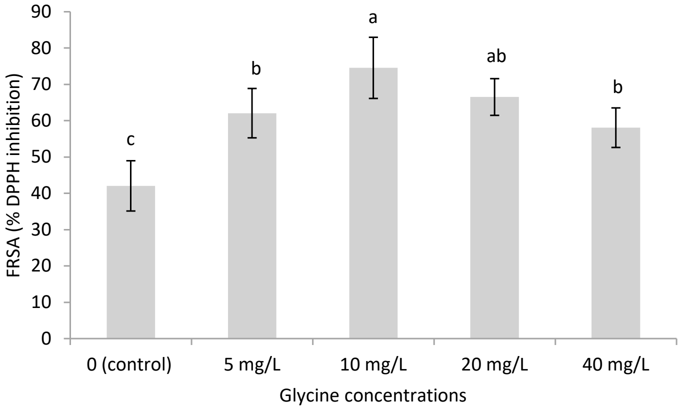

Fig. 4 Antioxidant activity of leaf extract of coriander grown at different concentrations of glycine in the nutrient solution. Means ±*SD*; *N* = 4. Values with different letters on top of the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

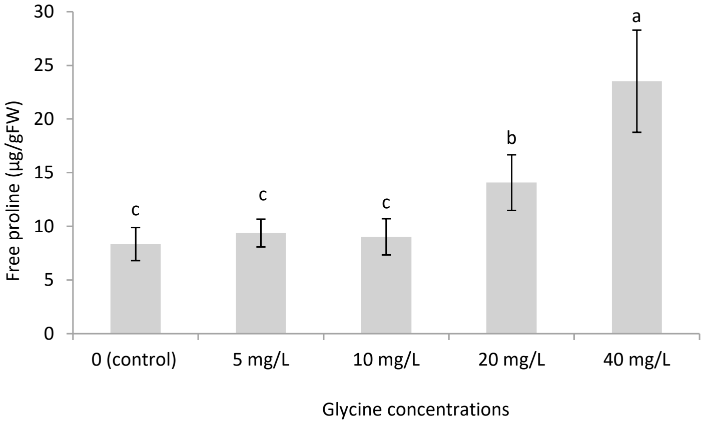

Fig. 5 Leaf proline concentration of coriander plants at different concentrations of glycine in the nutrient solution. Means ±*SD*; *N* = 4). Values with different letters on top of the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Discussion

In the present study, the increase in selected growth parameters under the influence of moderate glycine concentrations can be due to a stimulatory effect and the different roles of this amino acid in plant metabolism [4,5,19]. This effect of amino acids on plant growth has been also reported in other studies [1,6,13,20]. Amino acids are key elements in plant metabolism, as many physiological processes are directly and indirectly associated with these metabolites. However, our results showed that the highest concentration of glycine (40 mg L^{-1}) was toxic to plants and caused a negative effect on coriander growth. Glycine is one of the main amino acids and precursors that are essential for protein biosynthesis in plant cells [4,8,20]. Plant growth is broadly related to leaf protein biosynthesis and protein content [4,5]. In this study, moderate concentrations of glycine (10 or 20 mg L^{-1}) increased leaf protein concentrations, probably due to higher protein biosynthesis or a decrease in protein degradation [4]. It is also possible that glycine acted as a stress signal resulting in higher leaf protein biosynthesis [5]. Foliar application of amino acids has been shown to increase the cytokinin-like compounds in plant tissues that can enhance protein biosynthesis [4,5].

Exogenous application of amino acids can increase chlorophyll biosynthesis and photosynthetic rates resulting in improved plant growth particularly under adverse climatic conditions [6,19,21]. Plants supplied with amino acids generally have higher contents of sugars, proteins, and other nutritional elements, indicating their superior nutritional quality. This quality also helps to provide a better protection of plants against salinity, drought, or temperature stresses [2,5,15]. Nevertheless, the beneficial influence of low to moderate concentrations of glycine on coriander growth supports the stimulatory effects of glycine on plant growth rather than its sole effect as a nitrogen source. Application of amino acids can also enhance the control of stomata and gene expression toward better plant growth [5,10].

Our results showed that the positive effect of glycine application on nutrient uptake by coriander was concentration-dependent. The increase in leaf nutrient concentrations by foliar or root application of amino acids has been also reported in other works [1,6,7,15,16,22]. In biological systems, it has been shown that amino acids have a chelating effect on metal ions and so a protective role [7]. In fact, amino acids can act as ligands for metals with low solubility such as Fe or Zn, or they may be used either for nutrient mobilization from the soil or for their translocation/retranslocation within the plant [5,23]. This generally results in better availability of nutrients for plant roots or leaf tissues. In addition, higher activity of plant roots induced by glycine may contribute in higher nutrient uptake and concentrations in coriander leaves [6,22,23]. Furthermore, as mentioned earlier, higher leaf nutrient status can enhance photosynthesis and so greater assimilate production and better plant growth and yield [7,8,14–16,24]. In the present study, a stronger stimulatory effect of glycine was observed in relation to root fresh than dry weights. This could be due to a greater water content of root tissues. Stress signaling induced by glycine is probably involved in higher root biomass production. Improvement in root growth and biomass induced by glycine signals, or by restricted nitrate uptake may occur under glycine application [4,5]. Changes in plant phytohormone levels may also be a secondary reason behind the observed growth-promoting effect of glycine [4,5].

Similarly, increase in leaf antioxidant activity induced by glycine could be due to biostimulation or/and stress signaling effects. Free amino acids such as proline or glycine betaine and many others can act as osmoregulators and antioxidants in plant tissues [4,5]. Antioxidant mechanisms are common strategies to enhance salt tolerance by plants [4,18,19,25]. Various components may be involved in antioxidant activity of leaf extracts including enzymes such as superoxide dismutase, ascorbate peroxidase, catalase, and antioxidant molecules such as ascorbic acid, α-tocopherol, different phenolic compounds, carotenoids, and reduced glutathione [25].

The high proline concentrations in plants treated with glycine at 20 and particularly at 40 mg L^{-1} can be due to induced stressful conditions. Glycine is a reduced form of nitrogen that in high concentrations can induce phytotoxicity, similarly to those effects of ammonium overfertilization in many plant species [4,26]. Proton release and associated damage during assimilation of glycine [27], and/or chelating micronutrients that makes them unavailable to plants [5], are probably the main mechanisms related to toxicity of high glycine concentrations in the present study. This may also be due to decrease in proline oxidase activity, and/or increase in γ-glutamyl kinase activity under glycine application [4]. Synthesis and accumulation of free proline as a compatible osmolyte is an active metabolic adjustment of cell components (particularly proteins) under adverse climatic conditions. It has been shown in barley that pretreatment with proline rather than glycine betaine was more effective in reducing ion leakage damage induced by NaCl salinity [28].

Application of amino acids in the form of aminochelate fertilizers that contain single or multiple nutrients is widespread nowadays in agriculture. In spite of improvement in the nutritional status of plants, metal ligands such as amino acids can have intracellular roles as chelators for sequestering metal ions in the cytosol or in subcellular compartments. In addition, amino acids can act as osmolites and protect plants from adverse environmental conditions [2,4,15]. Their role in detoxification of toxins and trace metals in plants have been well documented [5,23]. Derivatives of glycine, cysteine, and glutamine are involved in plant protection and tolerance under stress conditions probably by a phytochelatin-like action [4]. The biostimulatory effect of amino acids has been a matter of interest in recent years and their protective role against stress conditions could be due to their various effects, mainly their hormone-like activity and acting in signal transduction [5,10]. Application of amino acids can result in restricted nitrate uptake and nitrate accumulation in plant tissues, a quality that is very important in leafy vegetable crops [14,21]. In suspension cell cultures of maize, adequate amino acid levels in cells significantly reduce nitrate uptake [21,29].

The uptake of amino acids such as glycine by roots is very rapid, but the fate of absorbed glycine might be quite different. Glycine may enter and be involved in the metabolic pathway and can be used for protein biosynthesis or synthesis of other amino acids. Moreover, it may actively contribute to xylem and/or phloem transport of nutrients. It is also possible that in plant tissues, glycine is inactivated by oxidation reactions or it may change to glycine derivatives like trimethylglycine (glycine betaine), or by root exudation enter the rhizosphere [30].

Leafy vegetable crops have a unique role in human diets. They contain various health-promoting factors including vitamins, minerals, proteins, fiber, and many antioxidant molecules with an inevitable role in human health. As the results of the present study showed in coriander, it is possible to increase these quality parameters by application of low to moderate levels of glycine (5–10 mg L^{-1}) in the nutrient solution. Healthy and nutritive production of leafy vegetables, such as coriander, garden cress, spinach, and lettuce due to their fresh consumption of leaves, is surely important.

Conclusion

In the present study, application of moderate glycine concentrations (e.g., 10 mg L^{-1}) together with the nutrient solution increased coriander growth. On the other hand, the reduction of plant growth and high leaf proline concentrations in the highest concentration of glycine employed (40 mg L^{-1}) indicates sensitivity and stressful conditions induced by this concentration. Application of glycine increased the leaf antioxidant activity and to some extent leaf protein content at moderate levels. These results indicate that moderate concentrations of glycine significantly stimulated growth of coriander, its yield, and nutritional value. Therefore, its use could reduce the pressure on fertilizer application to soil and so can result in better use of soil nutrients. However, further studies are needed particularly to discriminate the stress signaling effects from the biostimulatory effects of glycine on plant growth.

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Wpływ zróżnicowanego poziomu glicyny w roztworze odżywczym na wzrost, skład mineralny oraz aktywność antyoksydacyjną kolendry (*Coriandrum sativum* L.).

Streszczenie

W celu oceny wpływu zróżnicowanego poziomu glicyny na wzrost oraz pobieranie składników mineralnych przez rośliny kolendry (*Coriandrum sativum* L.) przeprowadzono eksperyment z zastosowaniem kultur piaskowych oraz roztworu odżywczego. Różne stężenia glicyny 0, 5, 10, 20 lub 40 mg L^{-1} aplikowano roślinom w warunkach szklarniowych za pośrednictwem pożywki Hoaglanda w układzie kompletnie zrandomizowanym w czterech powtórzeniach. Uzyskane wyniki wskazują, że wartość SPAD liścia, średnica łodygi, świeża i sucha masa pędów i korzeni były istotnie większe po zastosowaniu 10 mg L^{-1} glicyny w porównaniu z roślinami kontrolnymi. Aplikacja 40 mg L^{-1} glicyny spowodowała zmniejszenie wielu parametrów wzrostu roślin, podczas gdy stężenie proliny w liściach uległo podwyższeniu. Wszystkie zastosowane poziomy glicyny, z wyjątkiem 40 mg L^{-1}, wpływały na zwiększenie świeżej masy korzeni. Zawartość białka w liściach wzrastała po aplikacji glicyny w stężeniach 10 lub 20 mg L^{-1}, podczas gdy aktywność przeciwutleniająca liści uległa podwyższeniu po zastosowaniu wszystkich stężeń glicyny. W porównaniu z roślinami kontrolnymi aplikacja glicyny powodowała w liściach wzrost stężenia azotu i potasu (przy 10 mg L^{-1}), magnezu (przy 5 mg L^{-1}) i cynku (przy wszystkich zastosowanych poziomach glicyny). Wyniki wskazują, że umiarkowany poziom glicyny (10 mg L^{-1}) w roztworze odżywczym może wpływać korzystnie na wzrost i wartość odżywczą kolendry.