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Growth, Physiological and Metabolic Responses of Gerbera (*Gerbera jamesonii* L.) to Various Combinations of Calcium and Humic Acid Levels

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ABSTRACT

Gerbera is one of the significant cut flower crops worldwide suffering from loss of flower quality induced mainly by Calcium (Ca) deficiency. In this research, the influence of Humic Acid (HA) and Ca in nutrient solution was studied on the growth parameters of gerbera. A completely randomized hydroponic experiment was designed by adding HA (0, 100, 500, and 1,000 mg L^{-1}) and Ca (3.5 and 7 meq L^{-1} nutrient solution) to the nutrient solution of gerbera, with three replications. The effects of the treatments were evaluated on the growth, protein content, proline content, transpiration, CO_2 assimilation, photosynthesis, SPAD value, number of harvested flower, and antioxidant activity in gerbera cv. Malibu. Results showed that decreasing Ca level to 3.5 meq L^{-1} decreased Superoxide Dismutase (SOD), Peroxidase (POD), and CO_2 assimilation. However, this treatment caused an increase in Malondialdehyde (MDA), protein content, proline content, chlorophyll, and photosynthesis. Transpiration and number of harvested flowers were not affected by Ca concentration significantly. The highest level of HA (1,000 mg L^{-1}) increased POD and transpiration (30 and 11%, respectively). However, SOD and protein content increased at 500 and 1,000 mg L^{-1} HA levels. When HA was accompanied with Ca, SPAD value, transpiration, and CO_2 assimilation were improved, especially at high levels of HA (500 and 1,000 mg L^{-1}) and higher level of Ca (7 meq L^{-1} Ca). The results suggested that HA could increase the number of harvested flowers and improve plant health by enhancing the plant enzymatic antioxidant defense system.

Keywords: Malondialdehyde, Nutrient deficiency, Peroxidase, Stomatal conductance, Superoxide dismutase, Transpiration.

INTRODUCTION

Gerbera (Asteraceae) is indigenous to South Africa and Asia as a perennial herb, mostly inhabiting temperate and mountainous regions. Gerbera hybrida (*G. jamesonii*×*G. viridifolia*) (Bremer, 1994; Hansen, 1999), commonly called gerbera daisies, are grown and sold as bedding, potted plants, and cut flowers. Because of their worldwide popularity, clonally propagated gerbera daisies are propagated by in vitro culture (Sujatha *et al*., 2002). Humic Acid (HA) is a main part of soil organic matter, which is beneficial to plants through improving plant growth, respiration, photosynthesis, nutrient uptake, and hormonal status (Jindo *et al*., 2020). HA makes complex compounds with many metals like micronutrients (Varanini and Pinton, 2001) and increases their solubility by forming aqueous complexes (Lobartini *et al*., 1997). Our previous report with gerbera plants showed that HA not only increased the number of harvested flowers per plant, but also extended the vase life of harvested flowers (Nikbakht *et al*., 2008). They suggested that

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these effects are mainly related to Ca accumulation in scapes and hormone like activity of HA. Haghighi *et al*. (2013) showed that HA increased macro and micronutrient uptake in different nutrient solutions in gerbera hydroponic production. These investigators (Haghighi *et al*., 2013) showed that HA application compensated N, Mg, and Fe deficiency in the half-Nutrient Solution (NS) concentration and improved acquisition and utilization efficiency of nutrients. Although the literature provides some information on nutrient uptake affected by HA, there is limited information specifically on the effect of HA on Ca absorption, which is the key nutrient affecting vase life of cut flowers, including gerbera and its growth and physiological responses.

Ca plays a regulatory role in physiology, cell wall stability, and major physiological processes and enzyme activity (Hyacinthe *et al*., 2015). Ca is a secondary messenger to hormonal and environmental signals (Carafoli and Klee, 1999; Roberts and Harmon, 1992) and regulates the stomata closing, which, in turn, affects the pattern of gas exchange (Atkinson *et al*., 1992; Ruiz *et al*., 1992).

The effects of different Ca levels have been studied by several investigators. For instance, Albino-Garduño *et al*. (2008) used three levels of Ca in the nutrient solution, including 6, 9, and 12 meq L^{-1} Ca for two gerbera varieties, namely, 'Amaretto' and 'Darling'. These investigators found that Ca concentration in the leaves was dependent on Ca content of the nutrient solution. They suggested that the best calcium concentration in nutrient solution for 'Amaretto' was 12 meq L^{-1}, and for 'Darling' it was 9 meq L^{-1}.

Although there are several studies concerning the effect of HA on nutrient uptake by plants, no specific work is dedicated to the simultaneous effect of Ca and HA on gerbera growth, photosynthesis, and antioxidant activity. The aim of this experiment was to study these gaps in hydroponic system considering that gerbera is presently a major greenhouse crop for cut flowers in the world and often suffers from short vase life and bent neck incidence (Mencarelli *et al*., 1995; Zheng *et al*., 2011), which mainly stems from insufficient Ca uptake (Nikbakht *et al*., 2008).

MATERIALS AND METHODS

Plant Material and Culture Conditions

Gerbera plant *Gerbera jamesonii* cv. Malibu was grown in a greenhouse at Zhejiang University, Hangzhou (30° 15' N 120° 10' E), China. The 3-month old plants with 4 leaves had been derived through micro-propagation. Plants were grown in 4-L pots containing a mixture of perlite with a particle size of 2-5 mm and peat moss (Fafard Co., Canada) (1:1). A completely randomized design was conducted with three replicates. HA at four levels (0, 100, 500, and 1,000 mg L^{-1}) and Ca at two levels (3.5 and 7 meq L^{-1}) were applied. The 7 meq L^{-1} is equal to the nutrient solution and was used as a control. HA prepared from leonardite (containing C, 61.2%, N, 3.13 g kg^{-1} dry mater and P, 2.89 g kg^{-1} dry base) was purchased from a Chinese company (Dalian Yano Agriculture Co.) and applied to the nutrient solution. Each experimental plot included 10 gerbera plants. The pots were irrigated from the top with nutrient solution (Savvas and Gizas, 2002). The nutrient solution was prepared with distilled water and the concentrations of nutrients in all treatments were as follows: (macronutrients in meq, micronutrients in µM): K (5.84), Ca (7), Mg (2.2), NH_4 (1.1), NO_3 (11.2), SO_4 (2.54), and P (1.2) and Fe (35), Mn (5), Zn (4), Cu (0.75), B (30) and P (1.2). Fe was added as Fe-EDDHA. The Electrical Conductivity (EC) of the nutrient solution was 1.8–1.9 dS m^{-1} and the pH of the supplied nutrient solution was set at 5.6 in all treatments. Each fertilizer was added from a separate stock solution tank.

Each plant received precisely 250 mL (February to April) or 500 mL (May to

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September) nutrient solution daily. The plants were spaced 35 cm apart. Immediately after planting, each treatment was supplied with the corresponding nutrient solution. Mean air temperatures in the greenhouse were 29±2/24±3°C (day/night) and relative humidity varied between 75.5±5.2% and 50±8.5%. Average daily PAR during irrigation treatments, measured in the greenhouse (data logger system), was 420±150 to 140.5±60 w m^{-2}.

Plant Antioxidant Activities

At the end of experiment, to determine the Superoxide Dismutase (SOD), Peroxidase (POD) activities, and the Malondialdehyde (MDA) content, the leaves were prepared according to Haghighi *et al*. (2010). Briefly, after washing the leaves thoroughly with deionized water, they were homogenized with a mortar and pestle under chilled condition in 50 mM phosphate buffer. The homogenate was centrifuged at 12,000 rpm for 10 minutes at 4°C, and the supernatants were used for the enzyme assays.

The SOD activity was assayed according to Beauchamp and Fridovich (1971) with some modifications. Briefly, the assay mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 µM NBT, 0.025% (w/v) Triton X-100 and 0.0044 (w/v) riboflavin. The photo-reduction of NBT (the formation of purple formazan) was measured at 560 nm. One unit of SOD activity was defined as the volume of extract that caused the inhibition of the photo-reduction of NBT by 50%.

The POD activity was measured using the method of Chandlee and Scandalios (1984) with some modifications. Briefly, the reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.1), 1% guaiacol (w/v), 0.4% H_2O_2 (v/v) and enzyme extract. The increase in absorbance due to the oxidation of guaiacol at 470 nm was measured. The enzyme activity was calculated as the µM of guaiacol oxidized min^{-1} g FW^{-1} at 25±2°C.

MDA was assayed by the method described by Dhindsa *et al*. (1981) with some modifications. Briefly, the extracted supernatant (2 mL) was mixed with 2 mL thiobarbituric acid (0.6%) and heated at 100°C for 10 minutes. The mixture was subsequently cooled and centrifuged at 10,000×g for 10 minutes, and the absorbance of the supernatant was measured at 532, 600, and 450 nm. The MDA concentration was calculated according to the following formula:

MDA concentration= $6.45\times(A_{532} - A_{600})-0.56\times A_{450}$

Growth and Physiological Parameters

From the flower production, which started approximately 40 days after potting, data were taken in the following 6-month period. Thus, the number and initial fresh weight of flowers, head diameter, and length of scapes were recorded. Protein content was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

SPAD Value and Photosynthetic Attributes

SPAD value was measured using a nondestructive dual-wavelength chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA). Five measurements were taken per replicate.

Photosynthetic rate (Pn) (µmol CO_2 m^{-2} s^{-1}) and CO_2 assimilation (µmol CO_2 m^{-2} s^{-1}) and transpiration (mmol H_2O m^{-2} s^{-1}) were determined with a portable unit (Li-Cor, Li-3000, USA). They were determined on three fully expanded leaves.

The shoots were separated from the roots and after recording the fresh weight, they were oven-dried at 70°C to constant weight.

Data Analysis

The factorial experiment had a Completely Randomized Design (CRD). Data were analyzed statically using two–way analysis

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of variance, followed by the LSD comparisons of the means using procedures described in the Statestix 8.

RESULTS

Decreasing Ca level to 3.5 meq L^{-1} decreased SOD, POD, and CO_2 assimilation and did not affect MDA, protein content, proline, SPAD value, photosynthesis, transpiration, and the number of harvested flowers significantly (Table 1).

High level of HA (1,000 mg L^{-1}) increased POD and transpiration. However, with increasing HA level, MDA content decreased. SOD and protein increased at the 500 and 1,000 mg L^{-1} HA levels. Proline content decreased with increasing HA, and the lowest content was found at the 1,000 mg L^{-1} HA level. CO_2 assimilation, number of harvested flowers and photosynthesis were not affected significantly by HA addition to the culture solution (Table 2).

HA and Ca did not change the fresh and dry shoot/root ratio significantly (data not shown). The lowest number of harvested flowers (Mean= 5) of gerbera was observed in 7 meq L^{-1} Ca without HA incorporation (although it was not significantly different from 100 and 1,000 mg L^{-1} HA) and the highest number (Mean= 8) of the harvested flowers was in 500 mg L^{-1} HA and 7 meq L^{-1} Ca. There were no significant differences between other treatments (Figure 1).

The highest protein content was found in plants receiving 1,000 mg L^{-1} HA and 3.5 meq L^{-1} Ca, although it did not significantly change with 7 meq L^{-1} Ca without HA addition, 500 mg L^{-1} HA and either of the two Ca levels (Figure 2).

With increasing HA level, proline content decreased in 7 meq L^{-1} Ca, although the difference was not significant between the 500 and 1,000 mg L^{-1} HA levels. There were no significant changes in proline content between HA levels in 3.5 meq L^{-1} Ca (Figure 3).

SPAD value increased with increasing HA level and there was no significant difference between two levels of Ca when each HA concentration was applied (Figure 4). The highest SPAD value was observed in 1,000 mg L^{-1} HA and 7 meq L^{-1} Ca, while the lowest was in 3.5 meq L^{-1} Ca without HA addition (Figure 4).

The interaction effect of HA and Ca did not show a significant difference between treatments on photosynthesis (data not shown). There were no significant differences between the two levels of Ca on transpiration. HA at 1,000 mg L^{-1} increased transpiration sharply by 100 and 65% in, respectively, 3.5 and 7 meq L^{-1} of Ca (Figure 5).

CO_2 assimilation significantly increased in 7 meq L^{-1} Ca in 100 and 500 mg L^{-1} HA treatments and the highest CO_2 assimilation was in 7 meq L^{-1} Ca in 100 and 500 mg L^{-1} HA, while the lowest was in 3.5 meq L^{-1} Ca in 100 and 500 mg L^{-1} HA treatment (Figure 6).

With increasing HA level, the MDA content slightly decreased and reached the lowest level in 1,000 mg L^{-1} at both Ca levels. There were no significant differences between the two levels of Ca in each HA concentration for MDA. The lowest MDA content was recorded in the 1,000 mg L^{-1} HA and 7 meq L^{-1} Ca treatment, and the highest amount was observed in the 3.5 meq L^{-1} Ca without HA addition (Figure 7).

POD activity increased in the 100 and 500 mg L^{-1} HA when 7 meq L^{-1} Ca was applied compared with 3.5 meq L^{-1} Ca**.** The highest POD activity was found in the 1,000 mg L^{-1} HA at both Ca levels, and in 100 and 500 mg L^{-1} HA with 7 meq L^{-1} Ca application (Figure 8).

SOD activity increased with increasing HA level and reached the highest level in the 1,000 mg L^{-1} treatment. There were no significant differences between the 3.5 meq L^{-1} and 7 meq L^{-1} Ca on SOD activity at each level of HA (Figure 9). The main effect of Ca and HA showed that each of them separately increased SOD and POD activities (Tables 1 and 2).

The interaction effect showed that with increasing HA level (1,000 mg L^{-1}) the SOD

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Table 1. Effect of Ca on protein, yield, antioxidant and photosynthesis changes of gerbera.[a]

Ca (meq L^{-1})	MDA content (nmol g^{-1})	POD activity (U g^{-1} FW min^{-1})	SOD activity (U mg^{-1} FW)	Protein conc (µg g^{-1})	Proline (g FW^{-1})	SPAD value	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	CO_2 assimilation (µmol mol^{-1})	Number of harvested flowers
3.5	4.28 [a]	0.03 [b]	45.46 [b]	4.13 [a]	2.61 [a]	51.78 [a]	10.24 [a]	0.11 [a]	379.71 [b]	6.00 [a]
7 (Control)	3.76 [a]	0.04 [a]	49.10 [a]	4.40 [a]	2.50 [a]	53.37 [a]	10.63 [a]	0.11 [a]	380.15 [a]	6.44 [a]

[a] Within a column, means followed by the same letter are not significantly different at P< 0.05 according to the LSD test.

Table 2. Effect of HA on protein, yield, antioxidant and photosynthesis changes of gerbera.

HA (mg L^{-1})	MDA content (nmol g^{-1})	POD activity (U g^{-1} FW min^{-1})	SOD activity (U mg^{-1} FW)	Protein concentration (µg g^{-1})	Proline (g FW^{-1})	SPAD value	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration (mmol $m^{-2}s^{-1}$)	CO_2 assimilation (µmol mol^{-1})	Number of harvested flowers
0	5.85 [a]	0.03 [b]	39.13[c]	3.99 [b]	2.69 [a]	49.61 [b]	9.44 [a]	0.09 [b]	379.99 [a]	5.55 [a]
100	4.46 [ab]	0.03 [b]	46.41 [b]	3.63 [b]	2.60 [ab]	51.5 [ab]	10.03 [a]	0.09 [b]	379.94 [a]	6.44 [a]
500	3.78 [bc]	0.03 [b]	50.38 [a]	4.72 [a]	2.51 [ab]	54.37 [a]	11.16 [a]	0.10 [b]	379.96 [a]	6.77 [a]
1000	2.01 [c]	0.04 [a]	53.21 [a]	4.71 [a]	2.42 [b]	54.76 [a]	11.11 [a]	0.1 [a]	379.83 [a]	6.11 [a]

[a] Within a column, means followed by the same letter are not significantly different at P< 0.05 according to the LSD test.

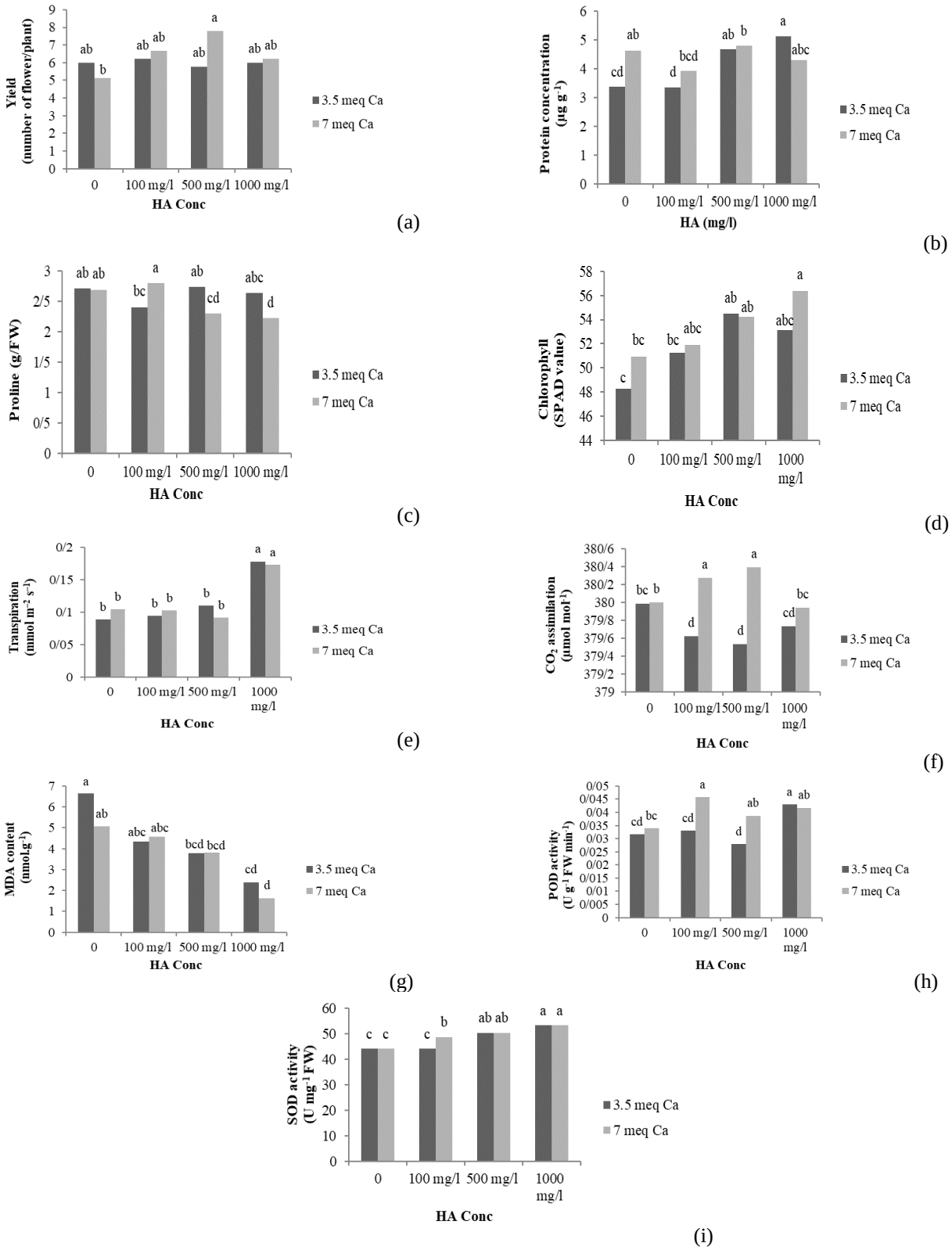

Figure 2. The interaction effect of Ca and HA on the (a) the yield of gerbera, (b) protein concentration of gerbera, (c) proline content of gerbera, (d) chlorophyll content of gerbera, (e) transpiration of gerbera, (f) CO_2 assimilation of gerbera, (g) MDA content of gerbera, (h) POD activity of gerbera and (i) on SOD activity.

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and POD activities increased at both levels of Ca, although it was the same in 500 mg L^{-1} HA treatment for SOD. At the lowest HA level (100 mg L^{-1}), SOD and POD activities increased at the higher level of Ca. In treatments without HA, the SOD and POD activities did not change significantly with Ca level (Figures 8 and 9).

DISCUSSION

The improving effect of HA on SOD and POD of lettuce (Haghighi, 2012), tomato, and celery (unpublished data) was previously investigated. Ca did not change the MDA content, but HA decreased it (Tables 1 and 2): Ca×HA interaction showed that, with increasing HA level, MDA content decreased and Ca level did not change it significantly.

Considering the previous research works, this is the first report that shows the improving effect of Ca on SOD and POD, and the positive effect of HA on SOD of gerbera as well as the increasing effect of HA on MDA. Ca did not affect protein content, but HA increased it at the higher levels (500 and 1,000 mg L^{-1}) (Tables 1 and 2). Our results on the interaction effect of HA and Ca are inconclusive as the previous reports on lettuce (Haghighi, 2012) as well as an adverse effect that showed a decrease in the protein content of *Pinus laricio* leaves and increase in the protein content of *Pinus pinaster* leaves (Panuccio *et al*., 2001). These effects can be related to the plant species and the origin of the HA used (Haghighi, 2012).

There are numerous works showing the improving effects of HA on SPAD value, like *Agrostis stolonifera* (Chen *et al*., 1999), *Asparagus officinalis* L., (Tejada and Gonzalez, 2003), grape (Ferrara *et al*., 2007), and *Lolium perenne* (Cheng *et al*., 2007). The improvement effect of HA on SPAD value can be due to some reasons like the effect of HA on plant growth, which is partially attributed to the enhanced SPAD value in the leaves (Chen and Aviad, 1990). HA increased the Fe and Zn uptake (Chen *et al*., 1999) as well as improving effect of HA on macro and micronutrients uptake, including Fe, N, Zn, and other elements (Haghighi *et al*., 2013).

HA and Ca did not affect photosynthesis, so, did not result in any changes in the number of harvested flowers (Tables 1 and 2). The interaction effect of Ca and HA did not affect photosynthesis either, but total harvested flower increased in 500 mg L^{-1} HA and 7 meq L^{-1} Ca treatment (Figure 1). These results are in contrast with some previous works that showed the improving effects of HA on photosynthesis and yield (Liu *et al*., 1998; Yang *et al*., 2004; Nardi *et al*., 2002). The possible explanation can be related to plant species and HA origin as well as the decrease in growth at higher concentrations of HA (Chen and Aviad, 1990). Ca at high levels (9 and 12 meq L^{-1}) influenced the yield of 'Darling' and 'Amaretto', respectively, and decreased yield at the lower level (6 meq L^{-1}) (Albino-Garduño *et al*., 2008). Albino-Garduño *et al*. (2008) predicted that Ca may affect the yield of 'Malibu' at higher concentration. Any change in photosynthesis results in changes in yield, and higher growth is one of the results of increased photosynthesis.

Our results showed that CO_2 assimilation increased following Ca elevation in the nutrient solution (Table 1) in Malibu cultivar. In the same way, the interaction effect of Ca and HA (Figure 6) showed that Ca at all levels of HA increased CO_2 assimilation, and this increase was more in the 100 and 500 mg L^{-1} HA treatments. However, it is reported that Ca did not affect CO_2 assimilation in 'Darling', while decreased it in 'Amaretto' (Albino-Garduño *et al*., 2008).

These adverse results can be related to the different tendencies observed in different cultivars and the diminution of stomatal conductance at the highest tested Ca levels (Albino-Garduño *et al*., 2008). On the other hand, the role of Ca on stomatal movement was proved, and increase in Ca level in apoplast of guard cells resulted in stomata

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closing (Mansfield *et al*., 1990). Albino-Garduño *et al*. (2008) showed this adverse effect at high Ca levels (9 and 12 meq L^{-1}). This can be a possible answer for the different results found in this study, where lower Ca concentration was used (3.5 and 7 meq L^{-1}).

Ca did not affect transpiration, but HA increased it at the highest level (1,000 mg L^{-1}). The highest transpiration in Malibu cultivar was recorded in 1,000 mg L^{-1} HA treatment with both Ca levels (Figure 5) and transpiration was reduced significantly in 'Amaretto' and 'Darling' with 6 meq L^{-1} Ca (Albino-Garduño *et al*., 2008). It should be noted that different genotypes may respond differently to different levels of Ca regarding their photosynthetic attribute. For instance, Ca levels in 'Darling' and 'Amaretto' treated with 9 and 12 meq L^{-1} Ca (Albino-Garduño *et al*., 2008) were higher than that in Malibu cultivar and almost the same as 'Cyprus' and 'Heart Breaker' treated with 6.49 meq L^{-1} Ca (D'Agliano *et al*., 1994; Issa *et al*., 2001). Although it is predicted that due to changes in stomatal conductance, Ca affected transpiration (Salisbury and Ross, 1994), in the study of Albino-Garduño *et al*. (2008), who worked with Malibu cultivar as well as 'Amaretto' and 'Darling', there was no relation between Ca concentration of nutrient solution and transpiration (Haghighi *et al*., 2013).

Increased HA concentration in the nutrient solution was associated with a parallel increase in SOD content ($r= 0.64$, $P< 0.01$) and a parallel decrease in MDA content ($r= -0.73$, $P< 0.01$). This clearly demonstrates the improving effect of HA on enzymatic antioxidant defense system of gerbera plant. More interestingly, we found that SOD activity was significantly related to the protein content of the leaves ($r= 0.64$, $P< 0.01$), which reveals a protective effect of HA on cell membrane and total proteins of cells, leading to more integrity of cell. On the other hand, this improvement resulted in more flower production by plants. general plant health enhance by lower MDA content of leaves were negatively correlated with the number of harvested flowers ($r= -0.60$, $p< 0.01$). The antioxidative defense system is vital for limiting oxidative damages to the plant cells (Haghighi *et al*., 2010; Haghighi *et al*., 2012).

Thus, it seems that using HA can improve qualitative and quantitative characteristics of gerbera, especially in higher levels of Ca concentration in the nutrient solution.

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رشد، پاسخهای فیزیولوژیکی و متابولیکی ژربرا (*Gerbera jamesoni* L.) به ترکیبات مختلف کلسیم و اسید هیومیک

م. حقیقی، و ع. نیک بخت

چکیده

ژربرا یکی از مهمترین محصولات گل شاخه بریده در سراسر جهان است که ازمشکل ریزش گل ناشی از کمبود کلسیم (کلسیم) برخوردار است. تأثیر اسید هیومیک (HA) و کلسیم در محلول غذایی بر روی پارامترهای رشد ژربرا مورد مطالعه قرار گرفت. آزمایش هیدروپونیک با افزودن HA (۰، ۵۰۰ و ۱۰۰۰ میلی گرم در لیتر) و کلسیم3.5 و ۷ (برابر با غلظت آن درمحلول غذایی) میلی اکی والان بر لیتر) به محلول غذایی روی پاسخ رشد ژربرا طراحی شد. طرح کاملاً تصادفی در سه تکرار انجام شد. اثرات تیمارها بر رشد ، مقدار پروتئین ، مقدار پرولین ، تعرق ، جذبCO2 ، فتوسنتز ، مقدار SPAD ، تعداد گل برداشت شده و فعالیت آنتی اکسیدانی در ژربرا رقم مالیبو ارزیابی شد. نتایج نشان داد که کاهش سطح کلسیم به ۳.۵ میلی اکی والان بر لیتر باعث کاهش سوپراکسید دیسموتاز (SOD) ، پراکسیداز (POD) و جذب CO2 می شود. با این حال ، باعث افزایش مالون دی آلدئید (MDA) ، محتوای پروتئین ، محتوای پرولین ، کلروفیل ، فتوسنتز می شود. تعرق و تعداد گلهای برداشت شده به طور معنی داری تحت تأثیر غلظت کلسیم قرار نگرفت. بالاترین سطح HA (۱۰۰۰ میلی گرم در لیتر)

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باعث افزایش POD و تعرق (به ترتیب ۳۰ و ۱۱ درصد) شد. با این حال ، مقدار SOD و پروتئین در ۵۰۰ و ۱۰۰۰ میلی گرم بر لیتر هومیک اسید افزایش یافت. هنگامی که HA با کلسیم همراه بود ، مقدار SPAD، تعرق و جذب CO2 به ویژه در سطوح بالای HA (۵۰۰ و ۱۰۰۰ میلی گرم در لیتر) و سطح بالاتر کلسیم (۷ میلی اکی والان بر لیتر) بهبود یافت. نتایج نشان می دهد که HA می تواند با تقویت سیستم دفاع آنتی اکسیدانی آنزیمی گیاه ، تعداد گلهای برداشت شده و سلامت گیاه را افزایش دهد.

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