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Promoted growth and improved quality of *Gerbera jamesonni* L. flowers using exogenous application of amino acids

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Abstract
Commercially available amino acids mixtures have several advantages such as enhancing assimilation of fertilizer, facilitating uptake of water and nutrients and improving photosynthesis of plants. To investigate the response of gerbera (*Gerbera jamesonni* L.) flowers 'Saltino' to foliar application of a mixture of 19 essential amino acids (0.25, 0.50 and 0.75 mg L^{-1}) and ammonium nitrate (200 mg L^{-1}) as nitrogen source, a pot experiment was carried out in the research greenhouse of Eram Botanical Garden of Shiraz University. Number of flowers, flower diameter, stems length, chlorophyll, proline, protein and nitrogen contents, photosynthesis rate, stomatal conductance, and vase life of flowers were significantly improved by amino acids treatments. No significant difference was observed between ammonium nitrate and amino acids mixture for flower fresh and dry weights. Results suggested that application of amino acids mixture can induce acetyl CoA, which plays an improvingrole in physiological processes in plants. Application of amino acids mixture as a promising and sustainable approach can be recommended to promote quality and quantity of gerbera flowers.

Keywords: amino acid, ammonium nitrate, gerbera, morphological, physiological, vase life.

Introduction

Gerbera as one of the most important cut flowers is successfully grown in several areas of the world and meeting the requirements of various markets. Gerbera is also commercially important. After rose, carnation, chrysanthemum, and tulip, gerbera is the fifth most used cut flower in the world. Gerbera flowers are widely used in the cut flower industry due to its long vase life and beautiful flowers. Most of the studies on gerbera have focused to improve its quality and quantity when it faces to environmental problems (Zhang and Ervin, 2004).

Nitrogen is an essential element for plant that usually obtained from the soil. Gaseous form of N is used only by few species such as legumes; therefore, plants generally use other forms of N containing compounds. Some organic N containing compounds could be used as a source of nitrogen for plants.

For a long time, it has been assumed that inorganic nitrogen (in the form of NH^{4+} and NO^{3-}) was the only sources of N for plants (Virtanen and Linkola, 1946; Schobert *et al*., 1988). However, during the last two decades, amino acids are also provided as another source of N. Both laboratory and field experiments confirmed the ability of plants to acquire

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amino acids (Näsholm *et al*., 2009). It has been shown that plants can take up intact amino acids (Weigelt *et al*., 2003). Several factors such as type and concentration of amino acid influence the uptake of amino acids from the soil (Jones *et al*., 2005). Adequate amounts of mino acids is needed to enhance the performance and general quality of the agricultural products.

For instance, Gamal El-Din *et al*. (1997) showed that lemongrass vegetative growth increases as a result of ornithine and phenylalanine application. Moreover, fresh and dry weights of *Datura* during vegetative and flowering stages were significantly increased by phenylalanine application (Youssef *et al*., 2004). Improving effects of phenylalanine on all vegetative growth parameters of crops and ornamental plants such as *Pelargonium graveolens*, *Lupinus termis* and *Matricaria chamomilla* have been previously reported (Mona and Mervat, 2005; Karima *et al*., 2005).

Furthermore, increased plant growth as a result of foliar application of tryptophan on Iberis amara L., *Chatharanthus reseus* and *Philodendron erubescens* has been also reported (Attoa *et al*., 2002; Iman *et al*., 2005; Abou Dahab and Abd El-Aziz, 2006). In a report on *Capsicum annuum* L. it was showed that all used amino acids resulted in remarkable increase in leaf photosynthetic pigments (Rashad *et al*., 2002).

In another investigation on *Catharanthus roseus* L., Iman *et al*. (2005) showed that photosynthetic pigments (chlorophyll a, b and carotenoids) were increased as a result of tryptophan application. Abou Dahab and Abd El-Aziz (2006) showed that foliar application of different amino acid treatments caused a significant increase in the content of total free amino acids in *Philodendron erubescens.*

Furthermore, application of the amino acids as a foliar spray caused an increase in the contents of total soluble sugars that applied on *Ocimum basilicum* L. and *Philodendron erubescens* plants (Talaat and Youssef, 2002; Abou Dahab and Abd El-Aziz, 2006). In studies conducted on gerbera production for different purposes, only nutrient solution was used or effect of some nutrient elements on plant growth was analyzed (Savvas and Gizas, 2002; Issa *et al*., 2001). Therefore, to investigate the improving role of amino acids on plant growth and quality, it is necessary to analyze the response of plants to amino acid feeding. Although, many studies have been done on the effects of amino acids on plant characteristics, very few have been conducted on the effects of an amino acids mixture and its effects on plant physiological and morphological characteristics. Therefore, this study was focused to investigate the effects of a amino acids mixture on physiological and morphological characteristics of gerbera flowers.

Materials and Methods

This experiment was conducted during 2011-2012 in the greenhouse of Eram Botanical Garden of Shiraz University. The averages for day and night temperatures of the greenhouse were 24±1 and 12.5±2.5 °C, respectively. During the experiment, the photoperiod of greenhouse was 12/12h light/dark and relative humidity was about 70%. Three months old yellow gerbera ‘Saltino’ plants were purchased from a greenhouse in Varamin, Tehran. Plants were cultured in pots with 14 cm diameter, 10.5 cm height and drainage holes in the bottom. Pots were filled with cocopeat and perlite (1:1 v/v). Plants were irrigated twice a week with tap water. Concentrations of 0, 0.25, 0.50 and 0.75 mg L^{-1} of an amino acids mixture (Folammina™) were sprayed every two days on the leaves of gerbera plants for duration of two months. Constituents of amino acids mixture are presented in Table 1. Ammonium nitrate fertilizer at a concentration of 200 mg L^{-1} was used as a nitrogen source as a comparison for amino acids mixture. Fresh and dry weights of flowers, chlorophyll (a, b and

total) contents, proline, protein and nitrogen contents, photosynthesis rate, and stomatal conductance of plants and vase life of cut flowers were measured.

Table 1. Constituents of a mixture of amino acids used in this experiment

	Amino Acid	%
1	Hydrosiproline	8.30
2	Threonine	1.00
3	Valine	2.60
4	Tryptophan	0.38
5	Tyrosine	1.30
6	Serine	1.70
7	Proline	13.80
8	Methionine	0.92
9	Lysine	4.40
10	Leucine	3.50
11	Histidine	2.60
12	Isoleucine	1.50
13	Glycine	25.20
14	Phenylalanine	2.30
15	Cysteine	0.30
16	Arginine	6.40
17	Alanin	9.10
18	Glutamic acid	10.50
19	Aspartic acid	5.60

Fresh and dry weights of flowers, flowers' stem length and capitulum diameter

Fresh and dry weights of four flowers were measured using a digital balance (AND, LH100, Japan). Whole flower, together with the stem was cut and was immediately weighted and was recorded as fresh weight. Flowering stem length and diameter of flower capitulum were measured using a digital caliper (Mitutoyo, Japan). To obtain the dry weight, after putting flowers into paper bags, they were placed in an oven for 48 h, with the temperature of 70 °C.

Chlorophyll content

Leaf greenness was quantified using a SPAD 502 chlorophyll meter, which represents an index for relative measurement of leaf chlorophyll content. To convert this data into real chlorophyll content, chemical method of Arnon (1949) was used. Solution absorbance was spectrophotometerically measured (using Spectrophotometer-Spectronic 20D). Acetone 80% was used as a blank sample. Wavelengths of 645 and 663 nm were used for chlorophyll a and b, respectively.

Mg Chl a /g fw= $(12.7(A_{663nm})-2.69(A_{645nm}))\times V/(fw\times 1000)$

Mg Chl b /g fw= $(22.9(A_{645nm})-4.68(A_{663nm}))\times V/(fw\times 1000)$

Mg Chl Total /g fw= mg Chl a /g fw + mg Chl b /g fw

Proline content

Extraction and determination of proline was done according to the method of Bates *et al*. (1973). Leaf samples (1 g) were extracted with 3% sulphosalicylic acid. Extracts (2 ml) were held for 1 h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Proline content was measured by Spectrophotometer at 520 nm and calculated as mmol g^{-1} fresh weight against standard proline.

µmol proline/g fw material= ((µg proline ml^{-1}×ml toluene)/115 µmole))/ (g sample)

Total protein and N contents

In order to determine protein content, Bradford (1976) method was used. Colorimetric protein assay of Bradford is based on an absorbance shift of the dye Coomassie Brilliant BlueG-250. The absorbance at 595 nm was transformed into mg of BSA (Bovin Serum Albumin) by the use of a calibration curve constructed on the same day of the assay. Also nitrogen content of plants was measured by using the following formula:

N content = total Protein content / 6.25.

Photosynthetic rate and stomatal conductance

Photosynthetic rate and stomatal conductance was measured using a portable photosynthesis system (Bioscientific Ltd. ADC). Photosynthetic rate and stomatal

conductance of fully expanded leaves were measured in the middle of a sunny day.

Vase life of flowers

Four flowers of each treatment were randomly selected and were placed in vases filled with distilled water. Flowers were evaluated for their petal wilting and also bent neck disorder. Days after harvest until complete flowers wilting were considered as the end of vase life of flowers. Fresh weights of flowers were daily measured for calculation of relative fresh weight and uptake of vase solution (Nikbakht *et al*., 2008). During the vase life evaluation period, the weights of vases without their cut flowers were daily recorded using a digital balance. Average daily vase solution uptake was calculated by the formula: vase solution uptake rate (g $stem^{-1}$ day^{-1}) = (S_{t-1}-S_t); where, S_t is the weight of vase solution (g) at t = day 1, 2, 3, etc., S_{t-1} is the weight of vase solution (g) on the previous day. Relative fresh weight of stems was calculated using formula: RFW (%) = (W_t/W_{t0}) × 100; where W_t is the weight of stem (g) at t= day 0, 1, 2, etc., and W_{t0} is the weight of the same stem (g) at day 0.

Statistical analysis

This study was conducted using complete randomized design (CRD) with five treatments (0, 0.25, 0.5 and 0.75 mg L^{-1} Folaminna and 200 mg L^{-1} ammonium nitrate) and in four replications (each replication included 2 pots). Finally analysis of obtained data was done using SAS software and mean comparisons performed by LSD test at 5 and 1% probability.

Results

Analysis of variance is indicative of the significant effects of the treatments on most of the studied parameters. According to the ANOVA table, effects of treatments were significant for nitrogen, chlorophyll, protein content, photosynthesis rate, stomatal conductance, stem length, number of flowers and vase life at 1% of probability level, while for proline content and flower diameter it was significant at 5% of probability level.

Number, diameter and Stem length of Flowers

The highest number of flowers was observed in plants treated with 0.25 mg L^{-1} amino acids mixture, which was significantly higher than the number of flowers in control and ammonium nitrate treatments (Table 2). Highest concentration (0.25 mg L^{-1}) of amino acids mixture caused a decrease in the number of flowers when compared to the number of flowers in plants treated with lower concentration of amino acids mixture. Regarding flowers diameter, highest flowers capitulum diameter was observed in plants treated with 0.50 mg L^{-1} amino acids mixture, while the lowest diameter was observed in control flowers (Fig. 1). Stem length in the plants treated with 0.25 mg L^{-1} and 0.50 mg L^{-1} amino acids mixture was higher than the length of stem in the other treatments (Fig. 1). Smallest length of the stem was observed in plants treated with ammonium nitrate.

Table 2. Effects of amino acids mixture (AA) and ammonium nitrate (AN) on measured traits and vase-life of gerbera

Treatments	NF	P_n (µmol m^{-2} s^{-2})	g_s (mmol m^{-2} s^{-2})	Vase life (days after harvest)
Control	3.75^{b*}	6.9^{b}	0.25^{b}	3.3^{b}
AA 0.25 mg L^{-1}	7.25^{a}	8.06^{b}	0.22^{b}	3.3^{b}
AA 0.50 mg L^{-1}	5.75^{ab}	10.12^{b}	0.32^{b}	4.0^{b}
AA 0.75 mg L^{-1}	5.00^{ab}	22.18^{a}	0.76^{a}	9.0^{a}
AN 200 mg L^{-1}	4.75^{b}	9.73^{b}	0.26^{b}	3.3^{b}

*Mean values of number of flowers (NF), photosynthesis rate (P_n), stomatal conductance (g_s) and vase life. Means with similar letters are not significantly different at 5% probability level of LSD test.

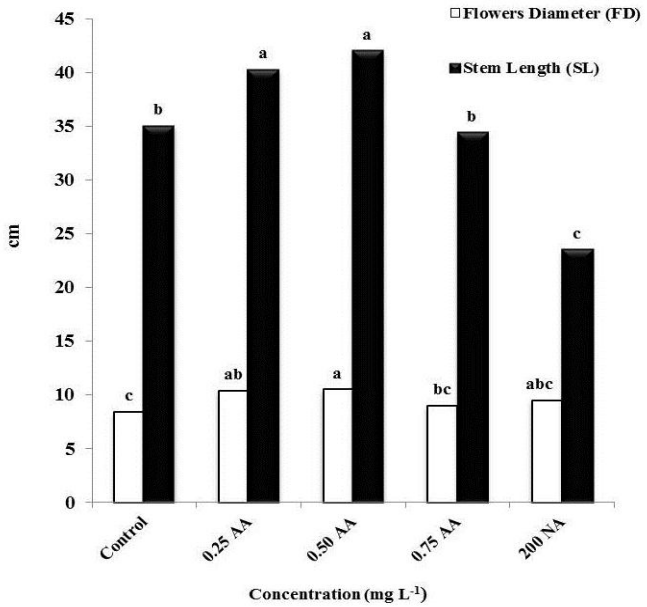

Fig. 1. Effects of amino acids mixture and ammonium nitrate on diameter and stem length of gerbera flowers. Means with similar letters are not significant at 1% probability level for stem length and 5% for flowers diameter. AA and NA represent amino acids and ammonium nitrate, respectively.

Protein and Nitrogen Contents

Highest N content was obtained in the plants treated with 0.50 mg L^{-1} of amino acids mixture, which was not significantly different from N content of plants treated with 0.75 mg L^{-1} of amino acids mixture (Fig. 2). The lowest N content was detected in control plants. No statistical difference was observed among control, ammonium nitrate and 0.25 mg L^{-1} of amino acids mixture treatments. This indicates that mixture of amino acids, as an N source, provides higher levels of nitrogen for gerbera plants than ammonium nitrate. Similar results were obtained for protein content of gerbera flowers (Fig. 2).

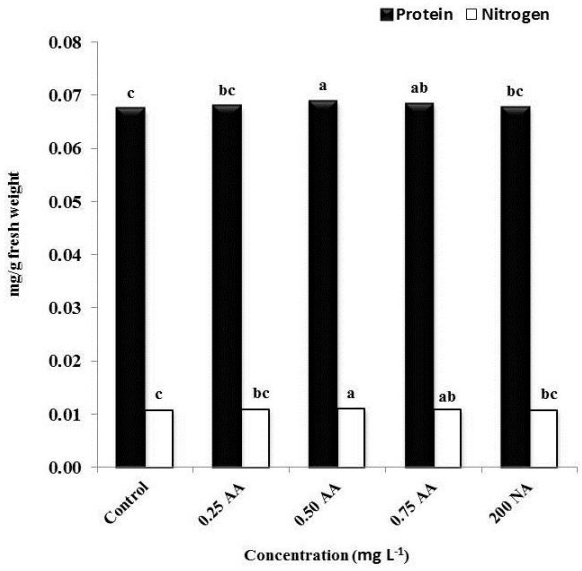

Fig. 2. Effects of amino acids mixture and ammonium nitrate on protein and nitrogen contents of gerbera. Means with similar letters are not significant at 1% probability level of LSD test. AA and NA represent amino acid and ammonium nitrate, respectively.

Chlorophyll and proline contents

Highest amount of chlorophyll was observed in plants treated with ammonium nitrate, while no significant difference was observed between ammonium nitrate and 0.25 and 0.50 mg L^{-1} of amino acids mixture (Fig. 3). The lowest chlorophyll content was detected in 0.75 mg L^{-1} of amino acids mixture. The highest proline content was observed in plants sprayed with 0.50 mg L^{-1} of amino acids mixture which was significantly different from other treatments (Fig. 4). Apart from 0.50 mg L^{-1} of amino acids mixture, no significant difference was detected among other treatments.

Photosynthetic rate and stomatal conductance

Application of amino acids mixture caused an increase in the rate of photosynthesis (Table 2). Highest rate of photosynthesis was obtained in plants treated with 0.75 mg L^{-1} amino acids mixture (22.18 µmol m^{-2} s^{-1}). Results of stomatal conductance Highest stomatal conductance was observed in plants treated with 0.75 mg L^{-1} of amino acids mixture (0.76 mmol m^{-2} s^{-2}) which was significantly different from other treatments. The lowest stomatal conductance was related to foliar application of 0.25 mg L^{-1} amino acids mixture (Table 2).

Postharvest life parameters

Flowers of the plants treated with 0.75 mg L^{-1} of amino acids mixture had significantly longer vase life in comparison with other amino acids mixture treatments. No sign of wilting but a small bending was observed after nine days of cutting from the mother plants that had been sprayed with 0.75 mg L^{-1} of amino acids mixture. Apart from 0.75 mg L^{-1} of amino acids mixture, no significant difference was detected among other treatments (Table 2). Application of 0.75 mg L^{-1} resulted in the highest uptake of vase solution and the highest relative fresh weight as well. However, flowers were completely wilted in day four of vase life assessment; therefore, except for 0.75 mg L^{-1}, no data was recorded following day four of vase life for the other treatments (Figs. 5 and 6).

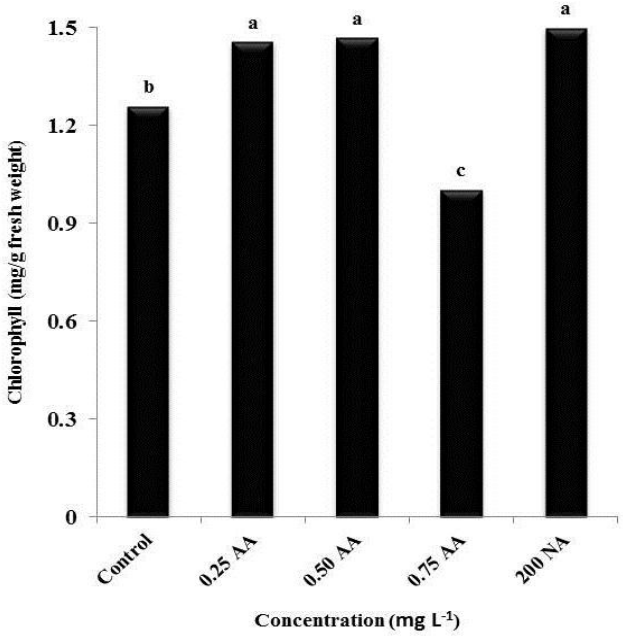

Fig. 3. Effects of amino acids mixture and ammonium nitrate on chlorophyll content of gerbera. Means with similar letters are not significant at 1% probability level of LSD test. AA and NA represent amino acids and ammonium nitrate, respectively.

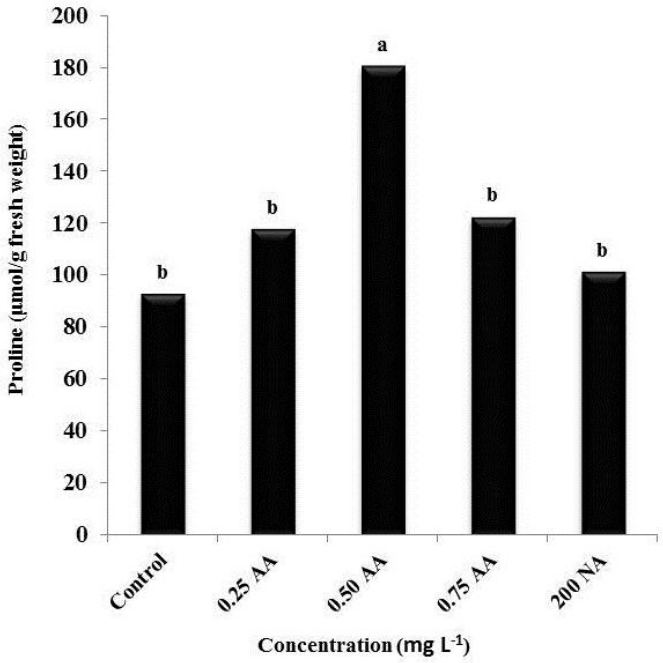

Fig. 4. Effects of amino acids mixture and ammonium nitrate on proline content of gerbera. Means with similar letters are not significant at 5% probability level of LSD test. AA and NA represent amino acids and ammonium nitrate, respectively.

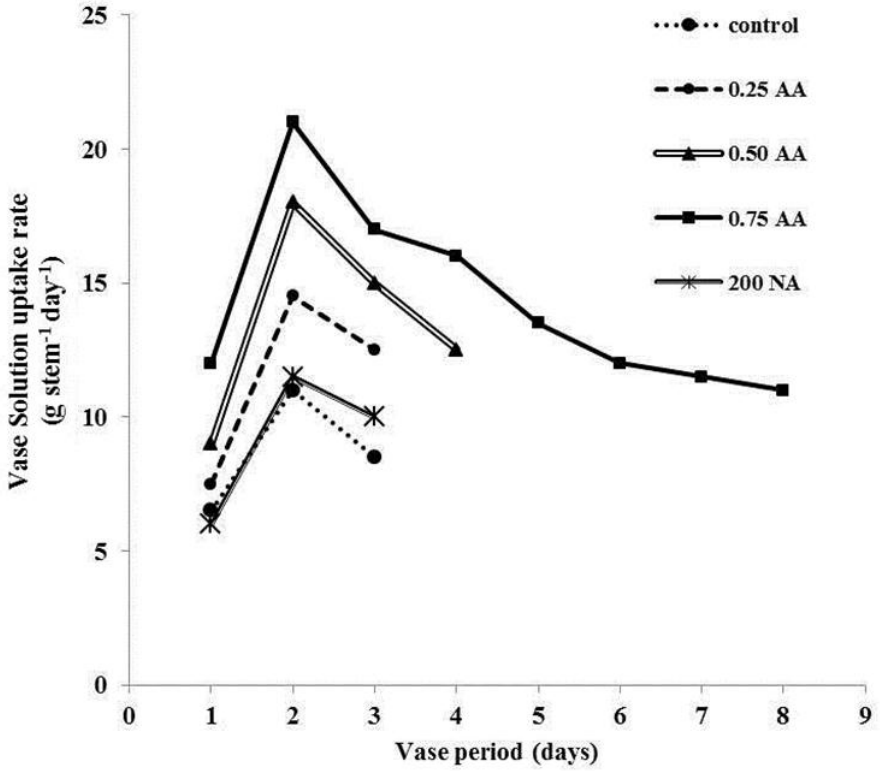

Fig. 5. Effect of amino acids mixture and ammonium nitrate on vase solution uptake of gerbera flowers. AA and NA represent amino acids and ammonium nitrate, respectively.

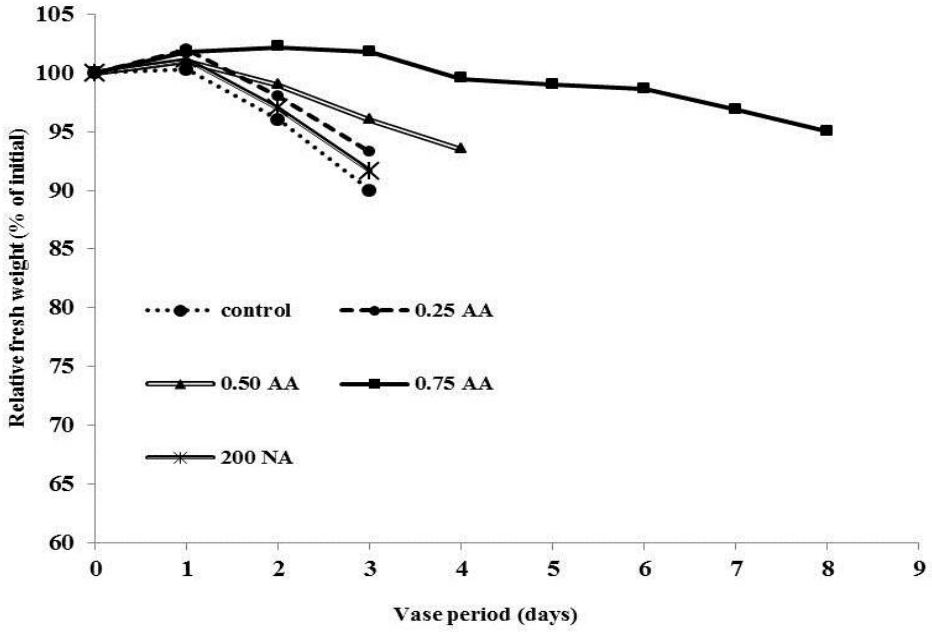

Fig. 6. Effect of amino acids mixture and ammonium nitrate on relative fresh weight of gerbera flowers. AA and NA represent amino acids and ammonium nitrate, respectively.

Discussion

Number, diameter and Stem length of Flowers

Nahed *et al*. (2009b) studied the effects of foliar application of amino acids on *Antirrhinum majus* flowers and showed that plant height increased following amino acids application. The same results have been also reported in *Gladiolus grandiflorum* which are in agreement with the results obtained in our study. Regarding the effect of foliar amino acids application on plant height, the results of this experiment are in agreement with those obtained by El-Bahar *et al*. (1990) on *Datura metel*, Talaat and Youssef (2002) on *Ocimum basilicum*, and Attoa *et al*. (2002) on *Iberis amara* L., who reported improvement of plant growth following foliar application of amino acids. Abou Dahab and Abd El-Aziz (2006) also reported that amino acid application significantly increased the height of *Philodendron erubescens* plants. Nahed *et al*. (2009a) showed that application of amino acids caused increase in the number of flowers in *Gladiolus grandiflorum*. Similar results have been reported by El-Fawakhry and El-Tayeb (2003) in chrysanthemum and Attoa *et al*. (2002) in *Iberis amara*.

Furthermore, our results are in accordance with those reported by Bekheta and Mahgoub (2005) on *Dianthus caryophyllus*, Mona *et al*. (2005) on *Pelargonium graveolens* L. and Nahed *et al*. (2007) on *Salvia farinacea* plants. They indicated that application of amino acids led to induction of flowering parameters and generation of high quality inflorescences. The stimulatory effect of amino acids is related to increase in content and activity of endogenous promoters such as gibberellins and IAA which are known as plant growth promoters (Wilkins, 1989).

Protein and nitrogen

Hua-Jing *et al*. (2007) reported that replacement of nitrate with amino acids caused a decrease in nitrate accumulation in *Brassica chinensis*. They reported increase in plant growth and in concentrations of trace elements by this replacement. Increase in nitrogen and protein contents were reported in plant species such as lupine (Tarraf, 1999), chamomile (Karima *et al*., 2005),

Gladiolus grandiflorum (Nahed *et al*., 2009a), *Thuja orientalis* (Nahed *et al*., 2010) and *Codiaeum variegatum* (Mazher *et al*., 2011). Our results are in agreement with those achieved by Talaat and Youssef (2002), which showed increase in nitrogen content following foliar application of amino acids in rosemary. In some plant species such as carnations, increase in N content results in a change in amino acid and protein contents. This change plays an important role in cell division and growth. Furthermore, production of these proteins, in turn, stimulates the plant vegetative growth. Obtained results from current study are similar to those obtained by Youssef and Talaat (2003) who reported increase in the total nitrogen proportion by foliar application of thiamine in rosemary plants. This increase led to quantitative changes in amino acids and specific proteins which acted positively in cell division and cell elongation (Bekheta and Mahgoub, 2005) on carnation plants.

Proline and Chlorophyll

Although, assessment of tolerance to environmental stresses was not done in the current study, it is likely that accumulation of proline following treatment of plants by amino acids mixture result in improvement of plant tolerance under drought and salinity stresses. Yagi (2006) observed that arginine application facilitate germination and growth of *Eruca sativa* seeds under salinity conditions. After measuring the proline content of gerberas, it was observed that plants treated with amino acids, had higher proline content in comparison with control, which caused higher stress resistance in plants. In agreement, accumulation of proline and restoration of vegetative growth by amino acids application under salinity stress conditions has been reported in tomato plants (Heuer, 2003). Our results are in agreement with those reported in canola (Mona *et al*., 2005), *Eruca sativa* (Yogi, 2006), *Philodendron erubescens* (Abou Dahab and Abd El-Aziz, 2006), *Antirrhinum majus* (Nahed *et al*., 2009b) and *Gladiolus grandflorum* (Nahed *et al*., 2009a), which amino acids application resulted in higher chlorophyll content in plants. Farshid *et al*. (2013) reported an increase in chlorophyll content of rose flowers by application of gluthamine and suggested that this increase could be due to increased discharge of alpha-ketogelutaric acid from Krebs cycle. The effects of glutamine can be due to its role in glutamate conversion to delta-aminolevulinic acid as the chlorophyll precursor (Beale, 1990). Rashad *et al*. (2002) indicated that all used amino acids led to marked increase in leaf photosynthetic pigments in *Capsicum annuum* plants. Iman *et al*. (2005) showed that photosynthetic pigments (chlorophyll a, b and carotenoids) of *Catharanthus roseus* leaves were increased as a result of application of tryptophan.

Photosynthesis and stomatal conductance

Results obtained in this study were in agreement with the findings of Abou Dahab and Abd El-Aziz (2006) on *Philodendron erobescens*. They reported that foliar application of tryptophan, led to an increase in the content of photosynthetic pigments. Abd El-Aziz *et al*. (2009) have suggested that this stimulating effect of tryptophan may be due to involvement of succinyl *CoA* and glycine in initiating of chlorophyll formation and ultimately elevation of photosynthetic rate. Amino acids are particularly important for stimulation cell growth, they act as buffers and maintain favorable pH within the plant cell, moreover, they contain both acid and basic groups; they take out the ammonia from the cell (Nahed *et al*., 2010). This function is associated with amid formation, so they protect plants from ammonia toxicity.

Vase life of flowers

Stem break, a sudden bending of the stem, occurs in many gerbera cultivars and is a practical problem affecting the sale of the flowers (van Meeteren, 1978).

As it was clearly shown in Table 2, the longest vase life of gerbera flowers were observed in plants treated with 0.75 mg L^{-1}. It seems that concentrations higher than 0.50 mg L^{-1} of amino acids can enhance vase life of flowers. It can be suggested to test concentrations between 0.50 and 0.75 mg L^{-1} in order to determine the optimal concentration of amino acid mixture for vase life assessment. Apparently by increasing the concentration of amino acids to a certain extent (higher than 0.50 mg L^{-1}) not only the nitrogen supply of the plants for the optimal growth is provided but also nitrogen in form of amino acids can contribute in other biological responses to prevent early wilting of flowers. It has been proved that nine amino acids (directly or indirectly) are converted to acetyl CoA (Stryer, 1995). Therefore, it seems physiological changes induced by application of acetyl CoA resulted from increased terpenoids content especially GA. In addition, acetyl CoA application, as a component of Krebs cycle, could change the status of ATP and reduced coenzymes in cells. GA stimulates activity of antioxidant enzymes, as a result, accumulation of active oxygen species and decrease in lipid peroxidation would occur (Qing Zhu *et al*., 2011). It seems that the application of acetyl CoA could lead to promoted longevity of gerbera cut flowers via induction of especial physiological changes, consequently senescence is postponed. As far as we know, there is no direct evidence for improvement of vase solution uptake and subsequently increase in relative fresh weight of flowers. However, it is accepted that improved nutritional status (*i.e.* nitrogen) during growth of horticultural crops can directly affect the postharvest life of their harvested products (Chang *et al*., 2010; Ali, 2012).

Conclusion

Application of amino acids mixture as a nitrogen source resulted in improved morphological and physiological responses in gerbera 'Saltino' plants and the vase life of their cut flowers. This investigation showed that significant improvements in the number of flowers, flower diameter, stem length, proline, chlorophyll, protein and nitrogen contents, and gas exchange parameters of plants and vase life of cut flowers could be attributed to the application of amino acids mixture. To achieve high quality gerbera flowers, difference individual amino acid concentration needs to be adjusted in the final mixture for commercial production of gerbera.. It is obvious that foliar application of amino acids mixture can enhance physiological and postharvest quality of gerbera flowers, however in order to optimize this horticultural practice it can be suggested that combination of this mixture with other minerals to reduce soil application of fertilizers. This approach can be an environmental friendly practice towards sustainable agriculture.

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