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Changes in Physiological Properties and Vase Life of Gerbera (*Gerbera jamesonii*) Under Co-Application of Foliar Applied L-Glutamic Acid, Nitrogen and Potassium

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Abstract

Vase life of cut flowers of many gerbera (*Gerbera jamesonii*) cultivars is typically short because of stem bending; therefore, stem bending that occurs during the early vase life period is a major problem in gerbera. Foliar application of amino acids and inorganic fertilizers is an effective practice to improve plant yield in agriculture and horticulture. This experiment was carried out to improve vase life of gerbera cut flowers through changes in relative fresh weight (RFW), anthocyanin content, electrolyte leakage (EL) and stem bending. The plants were treated by foliar application of 4 and 8 g L^{-1} L-glutamic acid (GA), 5 and 10 g L^{-1} potassium (K) and nitrogen (N). The results showed the greatest RFW was observed in flowers sprayed with 4 g L^{-1} GA, 5 g L^{-1} N and 5 g L^{-1} K to be 86.7 %. The 20 % reduction of EL was observed in flowers treated with co-application of 8 g L^{-1} GA, 10 g L^{-1} N and 5 g L^{-1} K as compared to control. The main enhancement of anthocyanin concentration was corresponded to GA application. Stem bending ranged from 63.1° in gerbera flowers supplied with non-GA, 5 g L^{-1} N and 5 g L^{-1} K to 73.9° in control plants. To sum up, 8 g L^{-1} GA in combination with 5 g L^{-1} N and 5 g L^{-1} K was chosen as an optimum treatment to improve vase life and decreased stem bending of gerbera cut flowers.

Keywords: Anthocyanin, Electrolyte leakage, Stem bending, Vase life.

INTRODUCTION

Due to various flowers color and shape, gerbera is a popular genus of Asteraceae family (Naing *et al*., 2017). *Gerbera jamesonii* L., is a herbaceous perennial plant species, which its flowering stalks in between the leaves, ranging from 12 to 37 cm (Rashmi *et al.,* 2018). Single daisy like flowers of gerbera are mostly applied for garden and home decoration in early stages (Deng and Bhattarai, 2018). Recently, gerbera as cut flowers has been widely used due to its ability to propagate through seed, rapid growth and the demand for cheap labor supplies (Deng and Bhattarai, 2018; Leman *et al.* 2020); however, its vase life is often short due to stem bending, which precedes wilting of the ray petals (Naing *et al*., 2017). In general, stem bending is related to the lack of mechanical support, particularly in the xylem and the lack of sclerenchyma cells in the stem below the floral head, which contains high levels of lignin (Naing *et al*., 2017).

To overcome the problems that cause stem bending of cut gerbera flowers, various practices of combined fertilizers have been tested (Naing *et al*., 2017). The foliar application of inorganic and organic fertilizers extended the vase life of cut flowers by strongly improvising nutritional value and antioxidant capacity. The increases in antioxidant potential and plant nutrient might improve vase life of cut flowers plants (Shabanian *et al*., 2018). Amino acids can directly and indirectly affect plant growth and development. Glutamic acid (GA) is important in nitrogen transport and storage, and mainly in pollination and fruit formation (Chen *et al.,* 2020). GA is involved in auxin synthesis and also is considered as a common precursor for chlorophyll and proline (Chen *et al*., 2020). In addition, pre-harvest treatment with macronutrients has significant effects on plant growth and flowering. Nitrogen (N) as a main essential mineral leads to create many organic compounds such as amino acids, proteins, enzymes and nucleic acids (Hristov *et al.*, 2019). N participates in chlorophyll molecule structure directly and it is a positive and significant relation between leave’s N and chlorophyll content (Samaresh *et al*., 2020). A second important nutrient for plants is potassium (K), which is a critical macro nutrient in controlling plant productivity (Adhikari *et al*., 2020). K can stimulate many enzymatic reactions in plants. It requirements are high and affect floral characteristics in terms of flower development and flower coloration (Alvarado-Camarillo *et al.,* 2018). The interaction effect of N and K on plant metabolism are several times stronger than their individual action (Guo *et al*., 2019).

The integrated nutrients have been used to improve the post-harvest life of cut flowers (Dalawai and Naik, 2018). Giri and Beura *et al.* (2021) showed the better vase life of gerbera cut flowers under organic and inorganic sucrose of nutrients. However, there is little information combination of GA and inorganic fertilizers on postharvest life of cut flowers. Therefore, the present study was carried out to different concentrations of GA, N and K on plant growth, physiological and biochemical properties, nutritional value and longevity of gerbera plants under a soilless system.

MATERIALS AND METHODS

Plant material and growth conditions

The 4-leaf seedlings of gerbera plants were purchased from a tissue culture company in Varamin, Iran. The seedlings were transferred to the 4-liter pots in a greenhouse in Imam Khomeini Higher Education Center, Karaj, Iran. Each pot contained one plant of gerbera with a median containing perlite/coco peat (1:1 ratio). The average temperature greenhouse during the experiment were 25/18 °C (day/night) with photoperiod of 16/8 (lightness/ darkness) and relative humidity of 65%-80%. All plants were uniformly nourished by 500 ml of Hoagland's nutrient solution divided in three times in day. After emerging the flowers, they were cut and placed in the distilled water with sucrose.

Experimental design and treatments

The experiments were carried out as factorial based on completely randomized design (CRD) with three replications. The gerbera plants were treated with foliar application of GA (L-glutamic acid from sigma) in three levels (0, 4 and 8 g L^{-1}), N in three concentration of urea (0, 5 and 10 g L^{-1}) and K in three concentrations of potassium sulfate (0, 5 and 10 g L^{-1}). The foliar application was started 15 days after transplanting for three times in 10-day intervals. The first bud appeared 50 days after transplanting. The 10 days after last foliar application, the samples were collected during three days to measure further analysis.

Vase life

The vase life of each flower was determined when flower stems showed bending (He *et al*., 2006).

Relative fresh weight (RFW)

From the control stems and those treated with GA, N and K optimal concentrations, the fresh weight was measured every 3 days and RFW was calculated using the formula: RFW (%) = (FWt/FW0) × 100, where FWt is the fresh weight of a stem (g) at days 3, 6, or 9 and FW0 is the initial fresh weight of the stem (g) at day 1 (He *et al*., 2006).

Electrolyte leakage measurement

The freshly petal discs (0.5 cm^2) were rinsed 3 times (2–3 min) with demineralised water and subsequently floated on 10 mL of demineralised water. The electrolyte leakage in the solution was measured after 22 h of floating at room temperature using a conducti meter (Crison 522, Crison Instruments, S.A., Spain). Total conductivity was obtained after keeping the flasks in an oven (90 ℃) for 2 h. Results were expressed as percentage of total conductivity (Agarie *et al*., 1995).

Determination of anthocyanins

Petal discs (1 cm in diameter) were selected to assay anthocyanins concentration in acidified methanol (HCl: Methanol, 1:99, v/v) as determined by Havaux and Kloppstech (2001). Absorption spectra of the extracts were determined at 530 nm after centrifugation (4000 rpm for 10 min) using a UV-1700 spectrophotometer (Shimadzu, Tokyo, Japan).

Stem bending

To determine the degree of stem bending during the experiment, a protractor was used to measure the bending through the angle difference between the flowering stem and the apex of the flower and was expressed in degrees (Ferrante *et al*., 2007).

Data analysis

All data was statistically analyzed SAS software in three replicates. Duncan's multiple range tests represented the comparison of mean values. The data were statistically investigated at 5% probability level.

RESULTS AND DISCUSSION

Relative fresh weight (RFW)

RFW was significantly ($P<0.05$) changed under GA, N and K (Table 1).

Table 1. Analysis of variance for postharvest traits under glutamic acid (GA), nitrogen (N) and potassium (K).

SoV	df	MS				
		Relative fresh weight	**Electrolyte leakage**	**Anthocyanin**	**Stem bending**	**Vase life**
Time	2	21711**	5311**	0.231**	11214**	-
GA	2	261.3**	662.2**	0.216**	16.95**	12.33**
N	2	46.07**	142.1**	0.016**	16.01**	5.48**
K	2	37.42**	25.55**	0.014**	0.89^{ns}	0.33^{ns}
GA×N	4	36.88**	47.75**	0.001**	12.51**	0.09^{ns}
GA×K	4	55.01**	16.99**	0.001**	5.78**	0.22^{ns}
N×K	4	16.65**	59.59**	0.003**	16.79^{ns}	0.09^{ns}
GA×N×K	8	7.88**	9.23**	0.002**	6.27**	0.2^{ns}
Error	52	3.1	1.43	0.0002	0.78	0.19
CV (%)	-	2.14	2.53	5.24	2.83	5.75

** and ^{ns}: significant at $P < 0.01$ and insignificant, respectively.

RFW was significantly increased under foliar applied of these treatments. The greatest RFW was observed in flowers sprayed with 4 g L^{-1} GA, 5 g L^{-1} N and 5 g L^{-1} K to be 86.7%. However, the minimum RFW was recorded as 78.7% in control plants. Totally, 4 g L^{-1} GA followed by 5 g L^{-1} K and N were significantly better than other treatments (Table, 2).

Table 2. Relative fresh weight, electrolyte leakage, anthocyanin content and stem bending under foliar application of glutamic acid (GA), nitrogen (N) and potassium (K).

GA	N	K	Electrolyte leakage (%)	Relative fresh weight (%)	Anthocyanin (mg g^{-1})	Stem bending (degree)
0	0	0	53.3 ± 0.52^{a}	$78.8\pm0.34^{h-j}$	0.21 ± 0.007^{q}	$73.9\pm1.39^{a-c}$
		5 g L^{-1}	53.3 ± 0.52^{a}	$79.5\pm0.27^{g-f}$	$0.24\pm0.003^{j-m}$	$71.2\pm1.83^{c-e}$
		10 g L^{-1}	51.7 ± 0.11^{b}	$79.3\pm0.34^{g-f}$	$0.22\pm0.002^{n-q}$	75.1 ± 0.69^{a}
	5 g L^{-1}	0	52 ± 0.50^{b}	$78.5\pm0.37^{h-j}$	$0.23\pm0.007^{l-p}$	$72.7\pm1.2^{a-d}$
		5 g L^{-1}	$47.6\pm0.88^{f-h}$	$80.8\pm0.44^{c-f}$	0.26 ± 0.005^{l}	63.1 ± 0.69^{i}
		10 g L^{-1}	49.2 ± 0.19^{cd}	$79.5\pm0.35^{f-j}$	$0.24\pm0.003^{j-m}$	65.5 ± 1.2^{hi}
	10 g L^{-1}	0	47.9 ± 0.69^{fg}	78.3 ± 0.27^{ij}	$0.24\pm0.003^{j-m}$	$67.9\pm1.83^{f-h}$
		5 g L^{-1}	$48.5\pm0.88^{d-f}$	78 ± 0.54^{j}	$0.23\pm0.005^{k-o}$	$72.7\pm1.2^{a-d}$
		10 g L^{-1}	49.9 ± 0.50^{c}	78.3 ± 0.33^{ij}	$0.23\pm0.004^{j-m}$	$71.5\pm1.83^{b-e}$
4 g L^{-1}	0	0	$47.5\pm0.33^{f-h}$	$78.9\pm0.35^{g-j}$	0.22 ± 0.003^{pq}	66.7 ± 0.69^{gh}
		5 g L^{-1}	$47.6\pm0.66^{f-h}$	$81.5\pm0.43^{c-e}$	$0.22\pm0.003^{m-q}$	$72.7\pm1.2^{a-d}$
		10 g L^{-1}	46.6 ± 0.6^{hi}	$80.8\pm0.45^{c-f}$	$0.24\pm0.004^{j-l}$	65.5 ± 1.2^{hi}
	5 g L^{-1}	0	46.9 ± 0.19^{gh}	$81.5\pm0.46^{c-e}$	0.24 ± 0.003^{j}	65.5 ± 1.2^{hi}
		5 g L^{-1}	45.3 ± 0.5^{j}	86.8 ± 0.22^{a}	0.28 ± 0.007^{gh}	65.5 ± 1.2^{hi}
		10 g L^{-1}	44.9 ± 0.19^{j}	82 ± 0.34^{cd}	0.27 ± 0.01^{hi}	65.5 ± 1.2^{hi}
	10 g L^{-1}	0	45.6 ± 0.33^{ij}	81.8 ± 0.47^{cd}	0.24 ± 0.005^{jk}	$69.1\pm1.2^{e-g}$
		5 g L^{-1}	$47.9\pm0.50^{g-h}$	84 ± 0.32^{b}	0.28 ± 0.005^{fg}	$70.3\pm1.83^{d-f}$
		10 g L^{-1}	$48.9\pm0.50^{c-e}$	$80\pm0.47^{e-h}$	$0.22\pm0.002^{o-q}$	$69.1\pm1.2^{e-g}$
8 g L^{-1}	0	0	45.3 ± 0.19^{j}	81.8 ± 0.54^{cd}	0.29 ± 0.002^{e}	65.5 ± 1.2^{hi}
		5 g L^{-1}	44.9 ± 0.19^{j}	82 ± 0.37^{cd}	0.31 ± 0.003^{d}	$71.8\pm1.2^{b-e}$
		10 g L^{-1}	$47.6\pm0.33^{f-h}$	82.3 ± 0.32^{c}	0.31 ± 0.006^{d}	$71.8\pm1.2^{b-e}$
	5 g L^{-1}	0	46.6 ± 0.33^{hi}	82 ± 0.45^{cd}	0.29 ± 0.005^{ef}	74.5 ± 1.2^{ab}
		5 g L^{-1}	42.7 ± 0.33^{m}	$80.5\pm0.65f^{d-g}$	0.34 ± 0.005^{ab}	$69.1\pm1.2^{e-g}$
		10 g L^{-1}	44.7 ± 0.34^{jk}	81.8 ± 0.36^{cd}	0.34 ± 0.003^{a}	$69.1\pm1.2^{e-g}$
	10 g L^{-1}	0	43.7 ± 0.32^{kl}	81.9 ± 0.76^{cd}	0.31 ± 0.007^{d}	$67.9\pm0.69^{f-h}$
		5 g L^{-1}	43 ± 0.50^{lm}	$79.8\pm0.47^{f-i}$	0.33 ± 0.007^{cd}	$71.5\pm0.69^{b-e}$
		10 g L^{-1}	44.7 ± 0.33^{jk}	$80.1\pm0.32^{e-h}$	0.32 ± 0.007^{c}	$69.1\pm1.2^{e-g}$

Means with different letters on the same column are significantly different ($P<0.05$) based on Duncan's test.

K affects stomatal function in photosynthesis and transpiration like maintenance of plant turgor, enzyme promotion and photo-assimilates transportation (Shahzad *et al*., 2017). RFW in nutritional treatments was higher than control plants, which is due to increased leaf chlorophyll content and consequently enhanced photosynthetic activity. GA and N foliar application likely contributed to improved plant weight as a result of increased leaf area and better absorption/utilization of photosynthetically active radiation (Mahlangu *et al*., 2016). Increased plan weight with N fertilization are in agreement with several previous works (Nemadodzi *et al*., 2017; Zikalala *et al*., 2017; Mahlangu *et al*., 2016). GA strengthens the plants by improving the synthesis of chlorophyll and carotenoid contents, which subsequently results in increased plant growth. The RFW decreased over postharvest period. It decreased to 88, 71 and 64% in days 3, 6 and 9, respectively (Fig. 1a). The change in RFW is the main indicator of water relations in cut flowers. The gradual decrease in relative fresh weight during postharvest is predictable, but the lower the reduction, the better the effect of the treatments applied. Weight loss of cut flowers is one of the beginning stages of aging in flowers and in fact, as the flowers get closer to the aging stage, the ability to absorb water and transpiration, cell turbulence disappears Rivers and flowers wither (Reid and Jiang, 2012). A decreased fresh weight of anthurium over time but its increase by N and K application have been reported by Sumathi *et al*. (2017).

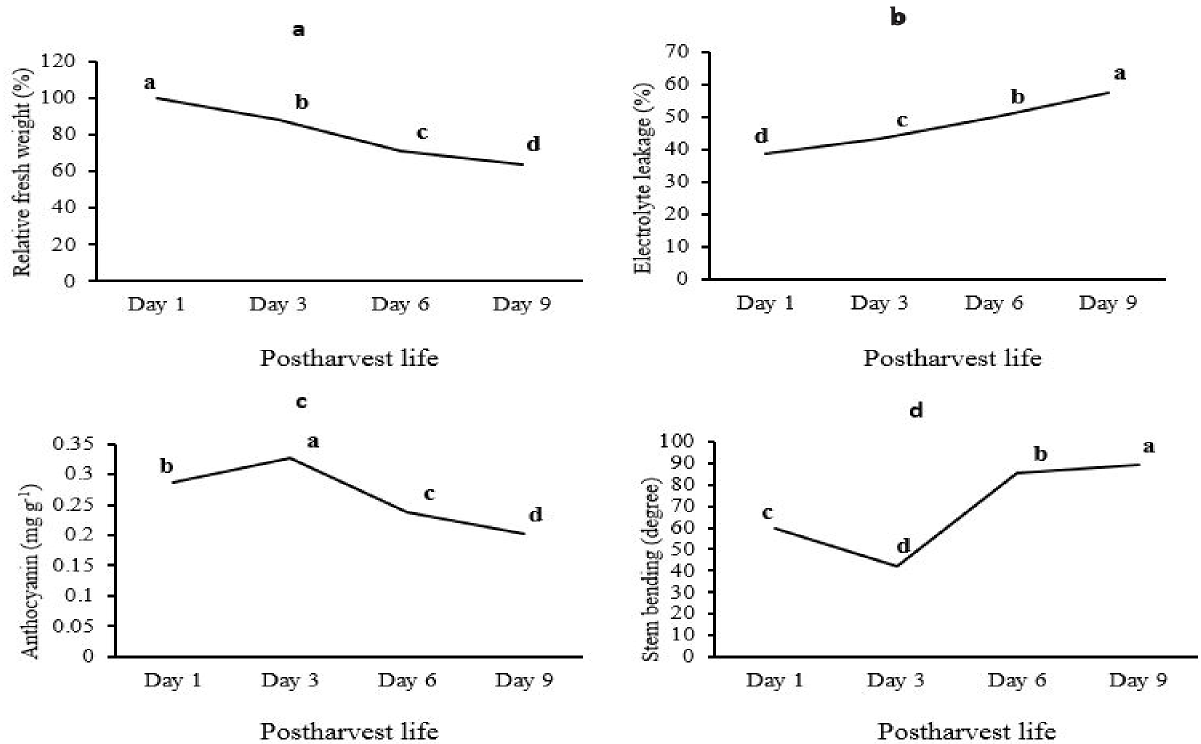

Fig. 1. Relative fresh weight (a), electrolyte leakage (b), anthocyanin content (c) and stem bending (d) at postharvest life. The values are represented with mean ±SD, n=3. Small letters show mean comparison by Duncan's multiple range test at a 5% probability level.

Electrolyte leakage (EL)

Foliar application of GA, N and K significantly ($P<0.05$) affected EL of gerbera flowers (Table 1). It decreased under co-application of GA, N and K compared to non-sprayed flowers (control). The 20% reduction of EL was observed in flowers treated with co-application of 8 g L^{-1} GA, 10 g L^{-1} N and 5 g L^{-1} K as compared to control. Under non-N and K application, 4 and 8 g L^{-1} GA decreased EL by 10 and 15% when compared with non-GA application (Table 2). The effect of K and N in the absence of GA was not extremely significant. The main reduction EL without GA application was obtained in flowers supplied with 5 g L^{-1} N and 5 K and g L^{-1}

as 11% decline relative to control (Table 2). The EL increased over time of postharvest period. Compared to day 1, EL increased up to 12, 21 and 48% at days 3, 6 and 9 (Fig. 1b). The EL indicates the initial signs of aging (Wei *et al*., 2019). An increase in EL a decrease in the ability of cell membranes to maintain permeability and is one of the most important signs of aging in tissue (Hniličková *et al*., 2019). Cell membrane failure promotes physiological changes that lead to the aging of petals and flowers. Similar to our results, increased EL over time have been reported in different plants during postharvest storage (Dokhanieh and Aghdam, 2016; Wei *et al*., 2019).

Anthocyanin content

The interaction of GA, N and K significantly ($P<0.05$) changed anthocyanin content of gerbera flowers (Table 1). The main enhancement of anthocyanin concentration was corresponded to GA application. Under non-K and N application, 4 and 8 g L^{-1} GA increased anthocyanin content. Enhanced vase life of rose flowers with polyamines spray treatments can be attributed to increased water uptake in cut roses, higher retention of fresh weight and petal sugar status and lower EL from the petal tissue. During storage life, chlorophyll breakdown leads to the decrease in photosynthetic capacity, resulting in an increased susceptibility to light-induced oxidative damage by the cells (Li *et al*., 2019). Anthocyanins have protective role as 'sunscreen' and as scavengers for active oxygen species (ROS) (Liang and He, 2018). The Anthocyanin content increased at day 3 and then decreased to day 6 (Fig. 1c). They protect the photosynthetic system from photo inhibition by absorbing green light and reducing excess excitation energy (Li *et al*., 2019). The increased anthocyanin content at strawberries have been reported by Li *et al*. (2019), which is line with our results. Haghighi (2012) showed the increased antioxidant activity of lettuce plants under N and GA application. The ameliorative role of anthocyanins in different plant parts (*e.g.* leaf and fruit skin) under stress conditions has been reported (Fang *et al*., 2020; Aliniaeifard *et al*., 2020).

Stem bending

Stem bending was significantly affected by the interaction of GA, N and K (Table 1). It ranged from 63.1° in gerbera flowers supplied with non-GA, 5 g L^{-1} N and 5 g L^{-1} K to 73.9° in control plants (Table 2). The vase life of cut gerbera flowers is ended when cut stems exhibit bending or breaking. Although the mechanism that causes stem bending remains unclear, previous studies have demonstrated that several factors, such as turgor loss, ethylene production, xylem blockage, PAL activity, lignin content and genetic background, are associated with the mechanism that causes stem bending (Naing *et al*., 2017). Recently, the combination of organic and inorganic fertilizers has been widely used in improving the vase life of cut flowers, such as rose, gladiolus and gerbera (Niyokuri *et al.*, 2017; Sable, 2018). The stem bending showed different responses to time of postharvest. It decreased to 43° at day 3 and then increased to 85.6° and 89.1° at days 6 and 9, respectively (Fig. 1d).

Vase life

Vase life of gerbera flowers was influenced by the GA and N ($P<0.05$, Table 1). The 8 g L^{-1} GA showed higher vase life (8.37 days) compared with 4 g L^{-1} GA and control. However, there was no significant change of vase life between control and 4 g L^{-1} GA (Fig 2a). In addition, 10 g L^{-1} N significantly had higher vase life as compared to that reported at 5 g L^{-1} N and control (Fig. 2b). GA and N had a significant role in the stimulation of cell division and in the delay of senescence (Zhang *et al.,* 2017) and is known for its anti-senescence effects during ageing sequence of plant tissue (Chen *et al*., 2020). Mirzaei Mashhoud *et al*. (2017) showed increased vase life of rose cut flowers under AG application. Similar to our results, the improved vase life of gladiolus due to the use of N have been reported (Dhakal *et al*., 2017).

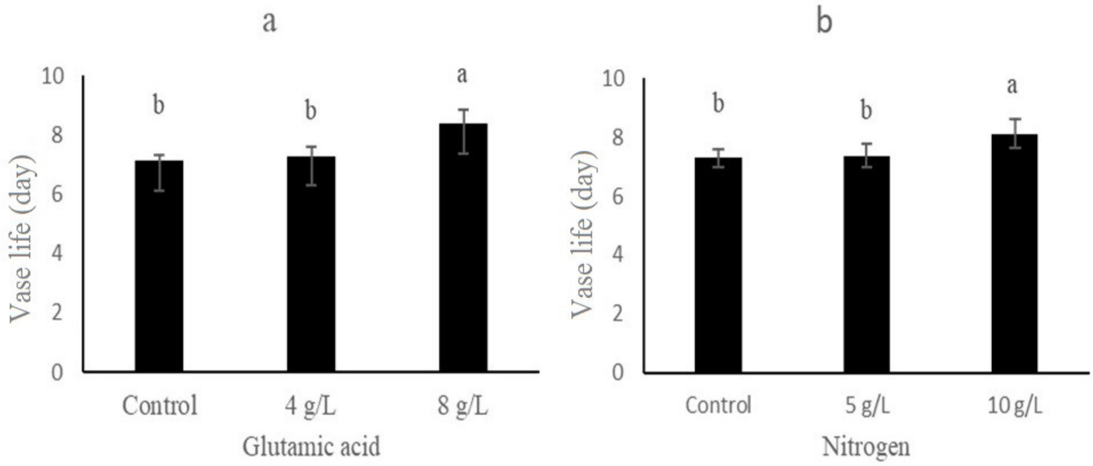

Fig. 2. Vase life of gerbera cut flowers under foliar application of glutamic acid (GA), nitrogen (N) and potassium (K). The values are represented with mean ±SD, n=3. Small letters show mean comparison by Duncan's multiple range test at a 5% probability level.

CONCLUSIONS

Many attempts have been used to overcome stem bending of gerbera cut flowers. In present study we used different concentrations of GA, K and N to improve vase life and reduced stem bending of gerbera cut flowers. The 8 g L^{-1} GA in combination with 5 g L^{-1} N and 5 g L^{-1} K was selected as optimum treatment to improve vase life and decreased stem bending.

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