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Pre-Harvest Application of γ-Aminobutyric Acid (GABA) and $CaCl_2$ Improves the Vase Life of Rose Cut cv. 'Jumilia'

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Abstract

Rose is an important cut flower crop throughout the world in the floriculture industry. One of the most important problems of cut roses is their short post-harvest vase life, which imposes huge losses for cut plant production and trade in the market. This study aimed to investigate the effects of γ-aminobutyric acid (GABA) and calcium chloride ($CaCl_2$) on vase life and the ACC synthase activity of cut rose cv. 'Jumilia' flowers. A factorial experiment based on a completely randomized design (CRD) with 12 treatments and 3 replications was carried out in a soilless culture greenhouse. The treatments included GABA in four concentrations (0, 20, 40, and 60 mM) and $CaCl_2$ in three concentrations (0, 0.75, and 1.5% as foliar application). The combined application of GABA and $CaCl_2$ significantly prolonged the vase life of the cut rose flowers compared to the control. The results showed that the highest and lowest vase life were obtained from the flowers treated with 20, 40, or 60 mM GABA and 0.75 % $CaCl_2$ and the untreated flowers, by 19.1, 18.86, 18.74, and 13.85 days, respectively. The pre-harvest application of GABA and $CaCl_2$ inhibited the ACC synthase activity. The highest and lowest ACC synthase activity were observed in plants treated with 40 mM GABA and 1.5 and 0.75% $CaCl_2$ and the untreated flowers by 3.57, 3.96, and 8.18 nmol g^{-1} FW, respectively. The combined application of GABA and $CaCl_2$ increased the Ca content by 100.95% compared to the control plants. The highest and lowest total chlorophyll contents in the leaves were related to the plants treated with 60 mM GABA and 0.75 and 1.5% $CaCl_2$ and the untreated flowers by 8.4, 8.24, and 6.22 mg g^{-1} FW, respectively. The foliar application of GABA and $CaCl_2$ significantly ($P < 0.01$) increased the polyamines (PAs) contents, such as putrescine (Put), spermidine (Spd), and spermine (Spm), in the petals of the treated rose flowers. So, the use of foliar application GABA 60 mM and $CaCl_2$ 1.5% is recommended due to the 38% increase in the vase life of cut rose flower.

Keywords: ACC synthase activity, Calcium, Chlorophyll content, Polyamines.

INTRODUCTION

Roses (*Rosa hybrida* L.) which belong to the Rosaceae family are the most diverse and extensively cultured commercial flower in the ornamental industry around the world. This plant species is important for its growing habit, flower color, and flower shape. Regarding production and economic value, roses have the first ranked in the ornamental plant industry (Chamani and Wagstaff, 2018; Gaurav *et al.*, 2021). They are cultivated worldwide for many goals in the ornamental industry, e.g., to produce beautiful flowers, cut flowers, pot plants, and bedding plants, so this flower has numerous usage in various industries including beverage, food, pharmaceutical, health, and cosmetic industries (Datta, 2022). For the rose, key quantitative and qualitative characteristics such as stem length and diameter, flower bud height and diameter, flower color, and vase life persistence are important factors that can satisfy consumers and increase its value and economy in marketing and business (Hosseini Farahi *et al.,* 2019).

One of the most important problems of cut roses is their short post-harvest shelf life, which imposes huge losses for cut plant production and trade in the market (Mirzaei Mashhoud *et al.,* 2016). Different variables affect the post-harvest vase life and longevity of roses, including cultivar, hereditary background, cultivation conditions, harvesting operation, and post-harvest handling management (Fazli and Ahmadi, 2024).

The vase life is one of the most important quality characteristics of cut roses and is directly related to customer satisfaction and increasing economic value. Some factors such as environmental factors, water quality, flower maturity stage, transportation, fungal infections, and vascular occlusion can influence and limit the vase life of cut rose flowers (Fazli and Ahmadi, 2024; Pun and Ichimura, 2003; Rasouli *et al.,* 2015).

An effective and environmentally friendly compound that extends the shelf life/vase life of horticultural products is γ-aminobutyric acid (GABA). GABA is a non-proteinaceous amino acid with four carbon atoms in its shape widely found in most prokaryotes and eukaryotes organisms (Hayat *et al.,* 2023; Mohammadi *et al.,* 2020; Nazoori *et al.*, 2020). Some researchers have reported that the pre-harvest and post-harvest application of GABA extends the vase life of some ornamentals including rose (Mirzaei Mashhoud *et al.*, 2016), gerbera (Mohammadi *et al.,* 2020; Mohammadi *et al.,* 2021), carnations (Molaei *et al*., 2021); protea (Vardien *et al*., 2018), anthurium (Mahjoory *et al*., 2019; Soleimani Aghdam *et al*., 2015), narcissus (Heidari Krush and Rastegar, 2022) and *Polianthes tuberose* (Babarabie *et al*., 2019).

The application of GABA significantly extended the vase life of two carnation cultivars ('Delphi' and 'Dob Pedro') by increasing catalase, ascorbate peroxidase, superoxide dismutase, and guaiacol peroxidase activities, as well as petal tissue, and reducing lipid peroxidation (Molaei *et al.,* 2021). Inhibition of MDA formation during lipid peroxidation by GABA is another mechanism proposed by Deng *et al*. (2010). Similarly, it reportedly improved the quality and vase life of narcissus by inhibiting the activities of peroxidase and polyphenol oxidase, delaying petal browning during storage, and enhancing the relative water content (Heidari Krush and Rastegar, 2022). It was reported that the pre-harvest and post-harvest treatment by GABA prolonged the vase life of cut *Anthurium* flowers, which was due to the effect of GABA on decreasing the activity of phospholipase D, reducing the accumulation of hydrogen peroxidase (H_2O_2) and reactive oxygen species (ROS), increasing the ratio of unsaturated to saturated fatty acids, and alleviating the damage of chilling by protecting the membrane integrity (Soleimani Aghdam *et al.,* 2015).

Another compound that is used to extend the post-harvest quality and maintenance of

horticultural crops is calcium. Calcium (Ca^{2+}) is an essential and widely used plant micronutrient and plays an important role in maintaining cell wall structure and membrane integrity, as well as cell signaling responses. So, it is a secondary messenger that plays fundamental roles in regulating physiological functions, such as plant growth and development, and delaying the senescence process in horticultural crops. It can inhibit the activities of ACC synthase and ACC oxidase, which leads to less endogenous ethylene production in the plant. Calcium chloride ($CaCl_2$) has been widely used to maintain the integrity of the fruit cell wall and its marketability. Other forms of calcium ions, such as calcium phosphate, calcium citrate, calcium oxide, and calcium lactate, can be used for pre- and post-harvest treatments (Aghdam *et al.,* 2012; Han *et al.,* 2021; Li *et al.,* 2020; Saeedi *et al.,* 2022). Increases have been reported in post-harvest shelf life and vase life by calcium application in many horticultural crops such as loquat (Li *et al.,* 2020), rose (Abdolmaleki *et al.,* 2015; Hosseini Farahi and Aboutalebi Jahromi, 2018; Schmitzer *et al.,* 2012), *Gerbera* (García-González *et al*., 2022) and *Alstroemeria* (Samadzadeh and Kamiab, 2017).

Therefore, this study was conducted to evaluate the effects of GABA and $CaCl_2$ treatments alone or in combination on improving the qualitative and quantitative traits and vase life of cut rose cv. 'Jumilia' flowers.

MATERIALS AND METHODS

Plant materials and experimental setup

The experiment was conducted in the hydroponic greenhouse of Sida Rose Company located in Yasuj, Iran in 2022. The *Rosa hybrid* cv. 'Jumilia' were grafted on Natal Briar as a rootstock cutting purchased from a local commercial producer and cultured in plastic pots (100 cm× 40 cm) filled with a cocopeat/perlite mixture (50:50) in a hydroponic system. The plants were fed based on the nutritional solution described in Table 1. Nutrient solutions were applied to the plants by a pump and an open drip irrigation system five times a day at an interval of 2 hours. Operations such as pruning, pest and disease control, and branch bending were carried out according to the standard methods during the growth period. The average day/night temperature of the greenhouse was 24±4 / 15±2 ºC and the relative humidity was 40–60%. The properties of the water and nutrition solution are shown in Tables 1 and 2, respectively.

Table 1. The characteristics of the tap water used for fertigation and treatment preparation.

Minerals (meq L^{-1})						SSP	SAR	TH	TA	EC	pH
Ca^{+2}	Mg^{+2}	Cl^-	SO_4^{-2}	CO_3^{-2}	HCO_3^{-2}				(mg L^{-1})	(µS/cm)	
3.40	1.10	0.40	1.01	0.00	3.40	2.58	0.08	225	170	401	7.23
Na^+	K^+	SO_4^{2-}	B	Fe	NH_4^+	$PO4_3^-$	NO_3^-	Zn	Cu	Al	Mn
meq L^{-1}										(Mg/L)	
0.12	1.17	0.218	0.08	0.019	0.13	0.56	15.01	0.17	0.01	0.03	0.011

SSP: Soluble Sodium Percent; SAR: Sodium Adsorption Ratio; TH: Total Hardness; TA: Total Alkalinity.

Experimental design and treatments

The study was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications and five plants per replication. The first factor was assigned to γ-amino butyric acid (GABA) at four rates of 0, 20, 40, and 60 mM, and the second factor to calcium chloride ($CaCl_2$) at three levels of 0, 0.75, and 1.5% (Table 3). The plants were sprayed by using a hand-sprayer until the flowers were wet to runoff with GABA and $CaCl_2$

solution. Additional flowers were also sprayed with distilled water as the control. The sprays were applied three times at 7-day intervals before harvest. The flowers were cut at commercial stage and put into water and kept in a room with average day and night temperatures of 24±4 / 15±2 ºC and the relative humidity was 40 – 60 %.

Table 2. The chemical fertilizer used in the nutrient solution of the plants based on greenhouse conditions.

Fertilizers	Tank A: To prepare 2500 litters of nutrient solution (g)	Fertilizers	Tank B: To prepare 2500 litters of nutrient solution (g)
Ammonium nitrate	7000	Mono-potassium phosphate	10500
Potassium nitrate	13000	Potassium nitrate	13000
Calcium nitrate	40000	Magnesium nitrate	11000
Iron chelate	2800	Ammonium nitrate	7000
pH	5-5.5	Zinc	170
EC	1600-1800	Sodium molybdate	7
		Boric acid	100
		Copper sulfate	75
		Nitric acid	3 lit

Table 3. The treatments used in the experiment.

No	Treatment	No	Treatment
1	Control	7	GABA 40 mM
2	GABA 0 + $CaCl_2$ 0.75 %	8	GABA 40 mM + $CaCl_2$ 0.75 %
3	GABA 0+ $CaCl_2$ 1.5 %	9	GABA 40 mM + $CaCl_2$ 1.5 %
4	GABA 20 mM	10	GABA 60 mM
5	GABA 20mM + $CaCl_2$ 0.75 %	11	GABA 60 mM + $CaCl_2$ 0.75 %
6	GABA 20mM + $CaCl_2$ 1.5 %	12	GABA 60 mM + $CaCl_2$ 1.5 %

Measurements

Vase life

Harvesting cut flower is done in the evening with sharp secateur at the tight bud stage when the colour is fully developed and the petals have not yet started unfolding. To evaluate the vase life, 15 flower stems were harvested from each treatment. They were recut to 50 cm in length and were individually placed in water and stored at room temperature (25 °C and 85–90 % RH). The vase life was determined by assessing the appearance of such symptoms as the bent neck, petal wilting, and shedding according to Jowkar *et al.* (2017).

ACC synthase activity

ACC synthase activity was measured according to the used of Jiang *et al*. (1994). At first, 2 g of tissue were homogenized in 10 ml of cold buffer containing 50 mmol of Tris/HCl, 1 mmol of dithiothritol, 1 mmol of phenylmethylsulfonyl fluoride and centrifuged in 20 ml for 20 minutes at 1°C and then the solution A column was passed through Sephadex G-25 column to desalt. The extracted solution was placed in a test tube and 50 mmol of S-adonosylmethionine, 4 mmol of pyridoxal phosphate and 50 mmol of Tris/HCl, 1 mmol of dithiothritol were added to it and incubated for 2 hours at 37 °C. The reaction was stopped by adding 20 µmol of mercury

chloride and the amount of ACC produced was evaluated by converting it to ethylene. The ACC was converted to ethylene by injecting 200 ll of an ice-cold mixture of 5 mol/l NaOCl and 15 mol/l NaOH (2:1, v/v) into each tube, vortex-mixing for 10 s and incubating the samples for 3 min. The ethylene in the headspace was then measured using a Shimadzu GC 9A gas chromatograph. A unit of ACC synthase activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of ACC per hour under the stated assay conditions.

Total chlorophyll

Total chlorophyll was measured by the method of Loranty *et al.* (2010) with some modifications. Briefly, 0.1 g of frozen leaf tissue was mixed with 0.1 g of magnesium oxide and then homogenized in 10 ml of 80% acetone diluted with water. Then, the resulting solution was centrifuged for 10 minutes at 3000 rpm, and the absorbance of the supernatant extract was read at 470, 646, and 663 nm using a spectrophotometer (Unico, UV-2100, USA). The total chlorophyll was calculated in terms of mg/g FW using the following equations:

$$\text{Chlorophyll a (mg g}^{-1}) = (12.25\times A_{663}) - (2.79\times A_{646})$$

$$\text{Chlorophyll b (mg g}^{-1}) = (21.21\times A_{646}) - (5\times A_{663})$$

$$\text{Total Chlorophyll (mg g}^{-1}) = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Polyamines (PAs)

Polyamines including putrescine (Put), spermidine (Spd), and spermine (Spm) were measured using an HPLC device based on the method described by Tassoni *et al.* (2006). For this purpose, 0.15 g of petal powder was mixed with 10 units of perchloric acid (PCA) 4% for 1 h at room temperature and was centrifuged at 27,000 g for 30 min at 48°C. The sediments were washed 3 times and mixed in fresh PCA solution. Then, the supernatant solution was kept in 6 normal hypochlorous acid (HClO) in fire-resistant vials at 110 °C for 20 h in order to separate the polyamines from the conjugate state. Then, the precipitates were mixed with dansyl-chloride (3 mg mL^{-1} of acetone), extracted with toluene, and measured with Unicam crystal 200 model (HPLC device with an C18 column) and ultra-detector (Spherisorb ODS2, 5 µM violet particle (4.66250 mm, diameter).

Calcium content in petals

Calcium concentrations in the petal samples of each treatment were determined after drying (48 h at 70 °C), grinding, and digesting with nitric acid and perchlorate or a sulfuric acid/ salicylic acid mixture. The Ca concentration in the extract was determined with a Perkin-Elmer 460 atomic absorption spectrophotometer (Hosseini Farahi and Aboutalebi Jahromi, 2018).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and the values were reported as mean. A two-way ANOVA was employed to test the interaction GABA × $CaCl_2$. Duncan's multiple range test (DMRT) was used to determine significant differences at $P < 0.05$. ANOVA and the comparison of means were performed using Statistical Analysis System (SAS) package version 9.4 (SAS Institute, 2017).

RESULTS

Vase life

The results of ANOVA indicated that the vase life of the cut rose flowers was significantly ($P < 0.05$) affected by the foliar application of GABA and $CaCl_2$. Fig. 1 displays the effects of GABA and $CaCl_2$ on the vase life of the cut rose flowers. The vase life was extended by the application of GABA and $CaCl_2$ as compared to the untreated flowers. It was found that increasing GABA and $CaCl_2$ increased the vase life. The longest and shortest vase life were obtained from the plants treated with GABA at 20, 40, and 60 mM and 0.75% $CaCl_2$ and the untreated flowers (19.1, 18.86, 18.74, and 13.85 days, respectively). In this study, the use of GABA alone increased the vase life, but the increase in the vase life was more and more effective when combined with calcium. Also, lower concentrations of GABA (20 mM) were more effective than higher concentrations (40 and 60 mM) in increasing the vase life. In fact, the application of the combination of GABA + $CaCl_2$ increased the vase life by 38% compared to the untreated plants.

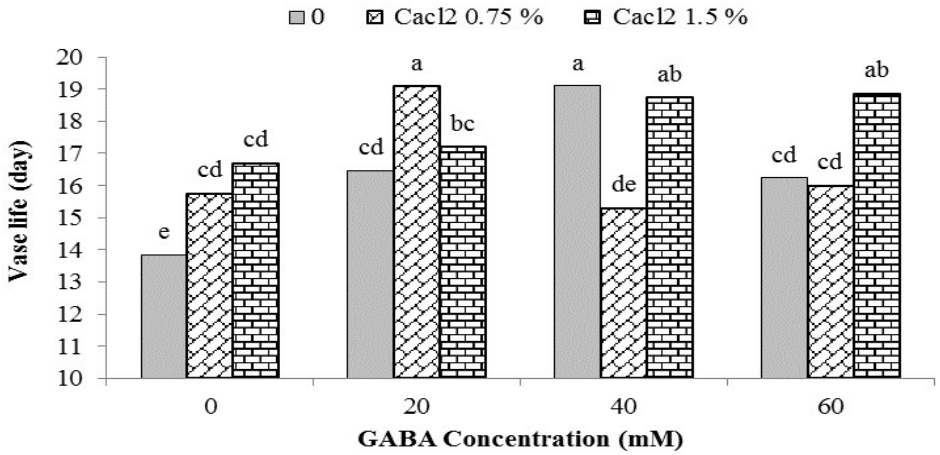

Fig. 1. The interaction effects of pre-harvest application of GABA + $CaCl_2$ on the vase life of cut rose flowers. *Means with the same letters are not significantly different at 5% level of probability using DMRT test.

ACC synthase activity

The results indicated that enzyme ACC synthase activity in the petals of the cut rose flowers was significantly ($P < 0.01$) affected by the foliar application of GABA and $CaCl_2$. Fig. 2 indicates the effects of GABA and $CaCl_2$ on ACC synthase activity. The results revealed that the pre-harvest application of GABA and $CaCl_2$ inhibited the ACC synthase activity. The highest and lowest level of ACC synthase activity was obtained from the plants treated with GABA at 40 mM and Ca at 1.5 and 0.75% rates and the untreated flowers (3.57, 3.96, and 8.18 nmol g^{-1} FW, respectively). In fact, the combined application of GABA and $CaCl_2$ decreased ACC synthase activity by 151% compared to the untreated plants (Fig. 2).

Calcium content

The amount of Ca in rose petals was significantly ($P < 0.01$) affected by the pre-harvest application of GABA and $CaCl_2$. Petal analysis indicated that the pre-harvest application of GABA and $CaCl_2$ significantly increased Ca absorption and accumulation. Among the applied treatments, GABA at the rate of 60 mM and $CaCl_2$ at the rate of 1.5% significantly increased Ca content as compared to the untreated flowers. The highest and lowest Ca contents

(10.57 and 5.26 mg/100 g FW, respectively) were related to the plants treated with GABA at the rate of 60 mM and $CaCl_2$ at the rate of 1.5% whereas the combined application of GABA and $CaCl_2$ increased the Ca content by 100.95% compared to the untreated plants (Fig. 3).

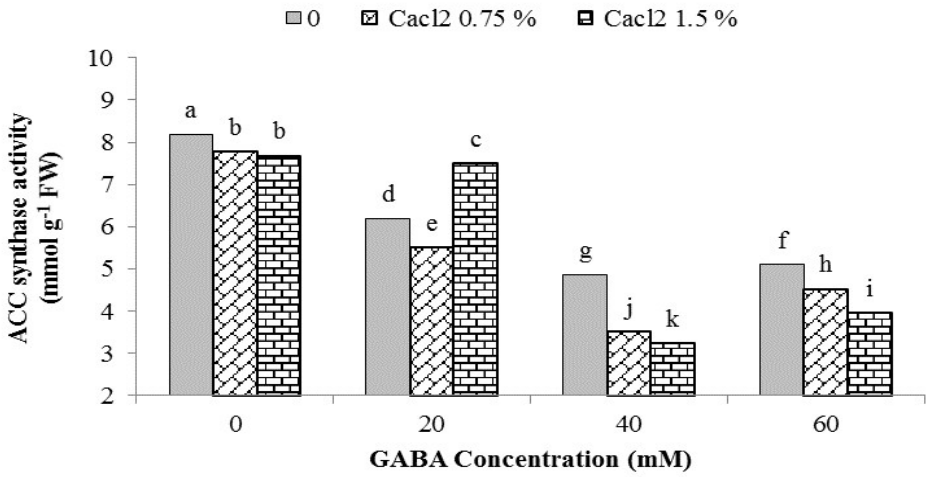

Fig. 2. The interaction effects of the pre-harvest application of GABA + $CaCl_2$ on ACC synthase activity of cut rose flowers. *Means with the same letters are not significantly different at 5% level of probability using DMRT test.

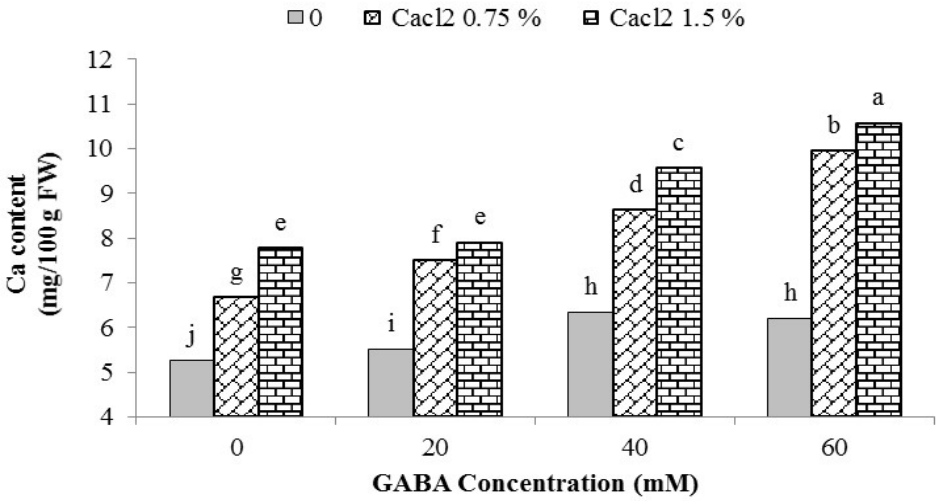

Fig. 3. The interaction effects of the pre-harvest application of GABA + $CaCl_2$ on the Ca content of cut rose flowers. *Means with the same letters are not significantly different at 5% level of probability using DMRT test.

Total chlorophyll

In the present study, the total chlorophyll content was significantly ($P < 0.01$) affected by the pre-harvest application of GABA and $CaCl_2$. As shown in Fig. 4, the flowers treated with GABA and $CaCl_2$ exhibited significantly higher total chlorophyll content. The highest and lowest total chlorophyll contents in the leaves were obtained from the plants treated with GABA at the rate of 60 mM and $CaCl_2$ at the rates of 0.75 and 1.5 % and the untreated flowers by 8.4, 8.24, and 6.22 mg g^{-1} FW, respectively. The total chlorophyll content increased by 35% with the application of GABA and $CaCl_2$ compared to the untreated plants.

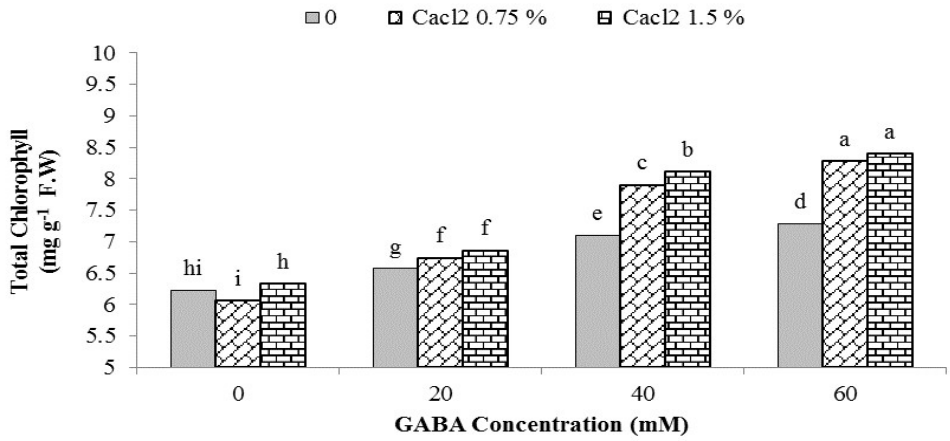

Fig. 4. The interaction effects of the pre-harvest application of GABA + $CaCl_2$ on total chlorophyll of cut rose flowers. *Means with the same letters are not significantly different at 5% level of probability using DMRT test.

Polyamines content in petals

The application of GABA and $CaCl_2$ significantly ($P < 0.01$) increased the content of polyamines such as putrescine (Put), spermidine (Spd), and spermine (Spm) in the petals of the treated rose flowers. Petal analysis indicated that the pre-harvest application of GABA and $CaCl_2$ significantly increased the endogenous Put content. By increasing the concentration of GABA and $CaCl_2$, the Put content in the petals increased so that the highest and lowest amount of Put was observed in the flowers treated with 60 mM GABA + 1.5% $CaCl_2$ and the untreated flowers (16.1 and 9.14 nmol g^{-1} FW), respectively. The amount of endogenous Spd in the flowers treated with GABA and $CaCl_2$ showed a significant increase compared to the untreated flowers. The results in Table 4 showed that the amount of endogenous Spd increased from 21.34 nmol g^{-1} FW in the untreated flowers to 33.15 nmol g^{-1} FW in the flowers treated with 60 mM GABA + 1.5% $CaCl_2$, respectively. Similar results were obtained for the endogenous Spm content. As is seen in table 4, the application of GABA + $CaCl_2$ increased the Spm content in the petals of the treated flowers. As shown in Table 4, the amount of endogenous Spd increased from 17.26 nmol g^{-1} FW in the untreated flowers to 29.44 nmol g^{-1} FW in the flowers treated with 60 mM GABA + 1.5% $CaCl_2$, respectively (Table 4).

Table 4. The interaction effects of the exogenous application of GABA × $CaCl_2$ on the main polyamines content of rose's petal.

Treatment	Putrescine (nmol g^{-1} FW)	Spermidine (nmol g^{-1} FW)	Spermine (nmol g^{-1} FW)
Control	9.14^{i}	21.34^{k}	17.26^{k}
CaCl2 0.75 %	9.08^{i}	20.94^{l}	17.52^{j}
CaCl2 1.5 %	9.17^{i}	21.88^{j}	17.96^{i}
GABA 20mM	10.19^{h}	23.55^{i}	19.6^{h}
GABA 20mM+$CaCl_2$ 0.75 %	10.66^{g}	24.26^{h}	20.24^{g}
GABA 20mM+$CaCl_2$ 1. 5 %	11.37^{f}	25.05^{g}	21.88^{f}
GABA 40mM	11.21^{f}	25.82^{f}	23.17^{e}
GABA 40mM+$CaCl_2$ 0.75 %	14.11^{d}	28.82^{d}	25.2^{d}
GABA 40mM+$CaCl_2$ 1. 5 %	14.73^{c}	30.13^{c}	26.04^{c}
GABA 60mM	12.3^{e}	27.21^{e}	25.05^{d}
GABA 60mM+$CaCl_2$ 0.75 %	15.37^{b}	31.44^{b}	28.17^{b}
GABA 60mM+$CaCl_2$ 1. 5 %	16.1^{a}	33.15^{a}	29.44^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the DMRT test.

DISCUSSION

This study explored the effects of GABA and $CaCl_2$ on the vase life and related parameters such as ACC synthase activity, Ca content, and endogenous polyamines. The results showed that the pre-harvest application of GABA in combination with $CaCl_2$ increased vase life, total chlorophyll, endogenous polyamines (Put, Spd, and Spm), and Ca content and decreased ACC synthase activity. The increase in vase life was due to the positive effects of GABA and $CaCl_2$ on increasing Ca uptake in the petals (Fig. 3), increasing the total chlorophyll (Fig. 4) and the synthesis of polyamines (Table 4), and also decreasing enzyme ACC synthase activity in rose petals (Fig. 2).

One of the most crucial problems in the production and trade of cut flowers is their short post-harvest vase life (Mirzaei Mashhoud *et al.,* 2016). An important factor limiting the post-harvest vase life is petal browning and shrinkage due to senescence (Heidari Krush and Rastegar, 2022). The results of the present study showed that GABA alone or in combination with $CaCl_2$ could significantly prolong the post-harvest vase life of the roses. Similar results have been reported for two cultivars ('Delphi' and 'Dob Pedro') of carnations (Molaei *et al.,* 2021), *Narcissus* cv. 'Shahla-e-Shiraz' (Heidari Krush and Rastegar, 2022), *Anthurium* cv. 'Sirion' (Mahjoory *et al.,* 2019; Soleimani Aghdam *et al.,* 2015), tea plants cv. 'Zhongcha 108' (Ren *et al.,* 2021), and *Gerbera* cv. 'Stanza' (Mohammadi *et al.,* 2020).

Exogenous GABA application affects ethylene biosynthesis thru adjustments in transcript abundance of ACC synthase and ACC oxidase, two key enzymes within the biosynthetic pathway (Kaspal *et al*., 2021). Calcium is a vital plant macronutrient and performs a critical role in maintaining cellular-wall structure and membrane integrity as well as cellular signaling responses. It is able to inhibit the activities of both ACC synthase and ACC oxidase leading to decrease production of endogenous ethylene in the fruit (Saeedi *et al*., 2022). GABA causes the expression of genes encoding antioxidant enzymes. Also, this compound increases the activity of glutathione S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) and reduces ROS. So, it may protect the cellular membrane integrity against deleterious effects of ROS, such as superoxide radicals, H_2O_2, hydroxyl radicals, and singlet oxygen (Heidari Krush and Rastegar, 2022; Mahjoory *et al.,* 2019; Mittler 2002; Yang *et al.,* 2011). In another research, the longest vase life of cut *Anthurium* flowers was observed in 1 mM GABA at 10 °C, and the shortest was obtained from the untreated flowers (Mahjoory *et al.,* 2019). GABA may be critical in maintaining flower quality through the maintenance of cell integrity and reduction of polyphenol oxidase interest in petals (Aghdam *et al.,* 2019; Soleimani Aghdam *et al.,* 2015). A previous study has shown that the accumulation of endogenous GABA via the GABA shunt is another mechanism to enhance resistance to biotic and abiotic stresses in crops (Zhu *et al.,* 2022).

In the present research, the application of GABA and $CaCl_2$ inhibited ACC synthase activity in rose petals during vase longevity. Decreases were reported in ethylene production in apple 'Cripps Pink' fruits by applying GABA (Han *et al.,* 2018). So, pre-harvest and post-harvest dip foliar application of Ca can delay ripening and senescence of many horticultural crops (Al Shoffe *et al.,* 2021). Interestingly, an increase in the activity or associated gene expression of GAD in fruit and the accumulation of GABA with the application of calcium has been reported by some researchers. So, post-harvest Ca treatment additionally improved the activity of the GABA pathway (Han *et al.,* 2021). A study reported enhanced disease resistance in the post-harvest quality of apple fruits due to the role of GABA through the regulation of GABA shunt,

ROS, and polyamine metabolism (Zhu *et al.,* 2022).

The present study demonstrated that GABA + $CaCl_2$ enhanced the Ca absorption in rose petals and consequently extended the vase life. Calcium can reduce ethylene production in fruits and cut flowers by inhibiting and reducing the activities of both ACC synthase and ACC oxidase (Saeedi *et al.,* 2022). Calcium can increase the post-harvest shelf life or vase life of horticultural crops due to the decreased respiration rate and microbial activity. Also, increases have been reported in maintained fruit firmness, phenolic content, ascorbic acid, and antioxidant capacity with the post-harvest Ca application (Choi *et al.,* 2019). Calcium (Ca^{2+}) is an essential and widely used element and a secondary messenger that actively participates in the cell wall structure, cell effector responses, and membrane function and acts as a counteraction inside storage organelles. It also plays a vital role in plant growth, development, response to environmental signals, regulation of physiological functions, regulation of the activities and metabolisms of numerous crucial enzymes in cells, and postharvest longevity of fruits, vegetables, and cut flowers (Aghdam *et al.,* 2012; Khalaj *et al.,* 2023; Li *et al.,* 2020). There are some reports about the effect of pre-harvest Ca foliar application on prolonging the vase life of cut flowers (Aghdam *et al.,* 2019; Hosseini Farahi and Aboutalebi Jahromi, 2018; Khalaj *et al.,* 2023; Li *et al.,* 2020). It has been reported to increase the vase life of roses 'Dulce Vita' with the use of polyamines + Ca (Hosseini Farahi and Aboutalebi Jahromi, 2018). The combined application of calcium oxide (CaO) + GABA maintained the post-harvest quality of fresh in-hull pistachio (Saeedi *et al.,* 2022).

In the present study, the application of GABA and $CaCl_2$ enhanced the endogenous PAs in rose petals. Increasing the vase life has a direct and significant relationship with the amount of endogenous PAs such as Put, Spd, and Spm. It is noteworthy that in this research, the combination of GABA and $CaCl_2$ increased the amount of endogenous PAs compared to the untreated plants. The roles of GABA are in fact closely related with polyamines and proline in plants in reaction to abiotic stress. The catabolism of polyamines is a crucial cause of GABA production, and proline shares the common synthetic precursor glutamic acid with GABA (Kaur *et al.*, 2020). Hu *et al*. (2015) confirmed that exogenous GABA use positively eased polyamines biosynthesis and enhanced endogenous GABA content. PAs play a positive defensive role in the cellular membrane by enhancing ROS scavenging capability in plants (Zhu *et al.,* 2022). GABA application also inhibited membrane lipid peroxidation by altering the activity of the lipoxygenase enzyme, leading to higher cell wall stability as reported by (Yu *et al.,* 2014).

CONCLUSION

In this study, we focused on the effect of GABA and $CaCl_2$ on various characteristics of cut rose 'Jumilia' flowers. Interestingly, the combination of GABA and $CaCl_2$ had a synergistic effect on all the measured traits. Different concentrations of GABA, in combination with $CaCl_2$, prolonged vase life, increased total chlorophyll, polyamines (Put, Spd, Spm), and Ca contents. In fact, the application of GABA and $CaCl_2$ increased the vase life by 38%, total chlorophyll by 35%, and Ca content in the petals by 100.95% and decreased enzyme ACC synthase activity by 151% as compared to the untreated flowers. So, the use of foliar application GABA 60 mM and $CaCl_2$ 1.5% is recommended due to the 37% increase in the vase life of cut rose flower.

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