Purpose: Carnation (Dianthus caryophyllus L.) is one of the most popular and economically important cut flowers worldwide ranking the third after rose and chrysanthemum. The vase life of many carnation cultivars is short due to sensitivity to ethylene or other factors. This research was performed to study effect of gamma aminobutyric acid (GABA 0.5, 0.75 and 1mM) on postharvest quality of two famous carnation cultivars viz. Delphi (white) and Dob Pedro (Red). Research method: The experiment was conducted as completely randomized design (CRD) with five replications per treatment. Findings: The results indicated that all concentrations of GABA significantly prolonged the vase life in both cultivars. GABA decreased lipid peroxidation of petal tissue while increased the activity of some antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and guaiacol peroxidase (GPX). Polyphenoloxidase (PPO) activity was also decreased in GABA-treated cut flower. Interestingly, both cultivars responded similarly to GABA treatments. The findings also revealed that GABA had antioxidant properties capable of increasing defense ability of the carnation cut flower. Meanwhile, the applied concentration is very critical and should be taken into account. Based on the present findings, the best treatment is GABA at 0.75 mM. Limitations: No limitations were encountered. Originality/Value: The results of this research indicated that GABA had antioxidant properties capable of increasing defense ability of the carnation cut flower. It appears that the applied GABA operates through membrane conservation, increasing the activity of antioxidants enzymes and decreasing PPO activity.
INTRODUCTION

_Dianthus_ L. (Caryophyllaceae) consists of more than 300 species worldwide distributed in Eurasia and Africa, but the Mediterranean is the main diversity center for this genus (Farsi et al., 2015; Vaezi et al., 2014). _D. caryophyllus_ commonly known as carnation and its numerous cultivars are of significant importance particularly for floriculture market. Carnation is one of the most popular and economically important cut flowers worldwide ranking the third after rose and chrysanthemum (Teixeira da Silva, 2006; Roodbaraki et al., 2012). The potential vase life of cut flowers is one of the most important quality affecting consumer satisfaction and market dynamism (Onozaki et al., 2001). Senescence of cut flower is associated with a series of highly regulated physiological and biochemical processes such as increment of reactive oxygen species (ROS), degradation of proteins and nucleic acid, peroxidation of membrane lipids and membrane leakage, regulation of oxidative enzymes activities, imbalance of plant hormones, floral abscission, color change, chlorosis or weight loss (Buchanan-Wollaston et al., 2003). The commercial value of cut flowers is extremely important which is determined by a multidisciplinary network of genetic factors, preharvest and nutritional conditions and postharvest management (Ren et al., 2017). Long postharvest life and high quality are two crucial factors for successful marketing of cut flowers (Ahmad et al., 2013). Some cut (fresh) flowers are highly perishable, hence improving their vase life by retarding senescence process through chemical or genetic means is of particular importance (Ezhilmathi et al., 2007).

Commercial preservatives are widely used to prolong the vase life of cut flowers (Ahmad et al., 2013). There exists a rich literature review about the treatments applied to improve carnation postharvest quality and extending its vase life. Some compounds such as 1-MCP has been used by researchers to improve postharvest quality and vase life of cut carnation flowers (Hassanpour Asil et al., 2013; Karimi et al., 2012). So, vase life of carnation flower can be prolonged by various postharvest chemical treatments (Onozaki et al., 2001). Carnation is a climacteric cut flower and most of its cultivars are highly sensitive to ethylene (Kazemi & Ameri, 2012). Early senescence is the main limiting factor during the marketing of cut flowers (Rahmani et al., 2015). The onset of flower senescence may be significantly delayed by using compounds such as aminooxyacetic acid, aminoethoxyvinyl glycine that prevent ethylene biosynthesis or inhibit ethylene action such as silver thiosulfate (Onozaki et al., 2001).

Gamma aminobutyric acid (GABA) is a quaternary carbon non-protein amino acid with regulatory and signaling mechanism affecting various processes in plants (Sheng et al., 2018; Nonaka et al., 2017; Shelp et al., 2017; Soleymani Aghdam et al., 2015; Bouche et al., 2003). Exogenous application of GABA is very effective in ameliorating plant stress condition (Vajayakumari, 2016; Kinnersley & Turano, 2000). This compound is a potential compound produced in different plant tissues and plays vital role in coping with various abiotic and biotic stressors in plants. Numerous studies have confirmed the protective role of GABA against abiotic stressors such as chilling (Soleymani Aghdam et al., 2016), cold (Yang et al., 2011), salt stress (Shi et al., 2010; Zhang et al., 2011), heat and drought (Li et al., 2016). In plants GABA may acts as a metabolite or signaling molecule. Dramatic increase in endogenous GABA after its exogenous application in plants, obviously indicates its signaling role in plant metabolism particularly stress conditions (Shelp et al., 2017; Vajayakumari et al., 2016; Michaeli & Fromm, 2015).

Delphi and Dob Pedro are two carnation cultivars with high popularity and demand among Iranian consumers for many occasions. Many carnation cultivars have short lifespan;
therefore, the objective of this study was to investigate the effect of GABA on postharvest quality of flowers of the mentioned carnation cultivars.

MATERIALS AND METHODS

In this experiment, fresh cut flowers of two cultivars of carnation (Delphi and Dob Pedro with white and red color respectively were obtained from a commercial greenhouse. Similar flowers were selected and recut to stem with length of 35 cm. All experiments were carried out in the growth chamber with the light/dark photoperiod of 16/8 h day/night, and at 25°C. The relative humidity was 50% and photon flux density was 200 μmol m⁻² s⁻¹. Flowers were placed in solution containing 0.5, 0.75 and 1mM GABA. Distilled water was used as control. Ten cut flowers were placed in a 250 ml flask with 200 ml of solution. After three days of vase life, when all of flowers were still fresh, the petals of five flowers for each treatment were selected and used to measure physiological indicators (five samples from each treatment were used for biochemical analysis while another five flowers were left to evaluate vase life). After the loss of ornamental value, the vase life of cut flowers under each treatment was determined.

Vase Life
Vase life was determined as the number of days needs to wilt the flowers. Appearance of symptoms such as shrinkage and brown edges was used for vase life determination. The petals were judged to have senesced when they wilted or became necrotic at the edges.

Relative fresh weight
The relative fresh weight (RFW) of flowers was calculated as follow (1):

\[
RFW(\%) = \frac{FW_t}{FW_0} \times 100
\]

where FWt is the fresh weight of a flower (g) at t = days 0, 1, 2, etc., and FWt 0 is the fresh weight of a flower (g) at t = day 0

Lipid peroxidation of membrane
The content of malondialdehyde (MDA) was measured as lipid peroxidation index according to Heath and Packer (1968). Petal samples (0.1 g) were homogenized in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 4000 ×g for 10 min. The supernatant (0.5 ml) was mixed with 1.5 ml of 20% (w/v) TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA). Mixtures were heated at 95 °C for 30 min then quickly cooled in an ice bath. Mixtures were centrifuged at 10000 ×g for five min, and their supernatant absorbance was measured at 532 nm. The value of non-specific absorption at 600 nm was subtracted from the 532 nm reading. The MDA content was calculated using the Lambert-Beer law, with an extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as µmol MDA per g fresh weight.

Enzyme extraction and activity determination
Three hundred mg of petal tissue (from same flowers of five samples) were homogenized in an ice-cold mortar using 50 mM potassium phosphate buffer (pH 7.0) containing 1mM EDTA and 1% (w/v) soluble PVP. After centrifugation (10000 ×g, 20 min, 4 °C), the supernatant was used for determination of enzymes activity.
**Superoxide dismutase activity (SOD)**
Superoxide dismutase (EC 1.15.1.1) activity was assayed using Rao and Sresty method (Rao & Sresty, 2000). The reaction mixture was 3 ml, which contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM Na-EDTA and 0.1 ml of enzyme extract. The reaction was started by adding 2 μM riboflavin and placing the reaction tubes under 15 W fluorescent lamps for 15 min. A complete reaction mixture which no enzyme extracts added was used as the control. The reaction was stopped by switching off the lamp. The photoreduction of NBT was measured at 560 nm. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of NBT oxidation under the experimental conditions. The specific activity of enzyme is expressed as U mg\(^{-1}\) protein.

**Catalase activity (CAT)**
CAT (EC 1.11.1.6) activity was measured according to the method of Dhindsa et al. (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H\(_2\)O\(_2\), and 100 μL of the enzyme extract. The decline in absorbance of mixture was measured at 240 nm (Ɛ=40 mM\(^{-1}\)cm\(^{-1}\)). The enzyme activity was recorded in U per milligram protein (1 μM of H\(_2\)O\(_2\) reduction min\(^{-1}\) mg\(^{-1}\) protein).

**Guaiacol peroxidase (GPX)**
GPX (EC1.11.1.7) activity was measured by a method described by Plewa et al. (1991). Reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.3% (v/v) H\(_2\)O\(_2\), 1% (v/v) guaiacol and the enzyme extract. The amount of enzyme that produced 1 μmol of tetraguaiacol per minute was considered as one unit (U) of enzyme activity. The concentration of tetraguaiacol measured by the absorbance at 470nm and using an extinction coefficient of 25.5 mM\(^{-1}\) cm\(^{-1}\). The enzyme activity was recorded as U per milligram protein.

**Ascorbate peroxidase (APX)**
APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H\(_2\)O\(_2\) were mixed in test tube and then 150 μL of enzyme extract was added to it. The absorbance was recorded at 290 nm (Ɛ=2.8 mM\(^{-1}\)cm\(^{-1}\)). The enzyme activity was expressed in U per milligram protein.

**Polyphenol oxidase (PPO) activity assay**
Polyphenol oxidase (EC 1.14.18.1) activity was determined according to the method of Nicoli et al. (1991). The reaction solution contained 50 mM potassium phosphate buffer (pH 7.0), 20 mM pyrogallol and 100 μl enzyme extract. Solution absorbance was recorded at 420 nm after three min and the activity was determined by using an extinction coefficient of 6.2 mM\(^{-1}\) cm\(^{-1}\).

**Total soluble proteins**
Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

**Statistical analysis**
The experiments were performed in a randomized performed based on complete randomized design (CRD). Data were comprised five replications per treatment. Means were compared by one-way analysis of variance and Duncan’s multiple range tests. Differences at P<0.05 were considered to be significant.
RESULTS AND DISCUSSION

In this research, GABA treatments increased the vase life of both cultivar of carnation (Fig. 1). These results agree with those reported by Soleymani Aghdam et al. (2015) in anthurium flowers under cold stress. In this experiment the highest vase life was obtained with 0.75 mM GABA (Fig. 1). In this research, weight loss was also significantly affected by treatments, and all concentrations of GABA decreased weight loss in comparison with control (Fig. 1). Plant hormones play critical roles in adaptation of plants to adverse environmental conditions through a sophisticated crosstalk among them. Several studies have provided evidence for the relationships between GABA, polyamines and hormones such as abscisic acid, cytokinins, auxins, gibberellins and ethylene (Podlešáková et al., 2019). Exogenous application of GABA has conferred higher stress tolerance by modulating the expression of genes involved in plant signaling, transcriptional regulation, hormone biosynthesis, reactive oxygen species production and polyamine metabolism (Bouche & Fromm, 2004). Postharvest performance of many ornamental plants considerably varies among cultivars (Maenish et al., 2010). This variation can often be related to differences in the ability of floral tissues to synthetize and/or perceive ethylene (Maenish et al., 2010). Variations among carnation cultivars in terms of vase life, have been reported by some workers (Onozaki et al., 2001; Hassanpour Asil et al., 2013; Roodbaraki et al., 2013). Although most cultivars of carnation are sensitive to ethylene but some cultivars have a long vase life which is associated with low ethylene production and low ethylene sensitivity (Tanase et al., 2015). Genotypic variation among rose cultivars also has reported considering postharvest performance and ethylene sensitivity (Maenish et al., 2010). So, the difference between the vase life of these two cultivars in this experiment, could be attributed to genetic background resulting in physiological characteristics. These findings also confirm the similar results reported on roses recently (In & Lim, 2018).

In this study, increasing vase life was associated with a decrease in malondialdehyde in GABA treated flowers, where 0.75 mM treatment was the most effective (Fig. 2).

Decrease of malondialdehyde content is one of the parameters that indicate greater integrity of cell membranes. Protection of cell membrane and maintaining its integrity against deteriorating effects of oxidative stress is generally accepted as the mode of action of plant hormones and signaling molecules (Ezhilmathi et al., 2007). The senescence of petal is linked with a series of highly regulated physiological and biochemical processes. This process accompanying with oxidative stress, and macromolecule degradation (lipids, proteins and nucleic acids) (Buchanan-Wollaston, 2003; Ezhilmathi et al., 2007). In previous research, Soleymani Aghdam et al. (2015) reported that the amount of malondialdehyde in the cut flower of anthurium stored at 4 °C, was much lower in GABA-treated flowers. Petal senescence is hastened by some other factors such as ROS. ROS cause membrane lipid peroxidation, disturb membrane integrity and increase electrolyte leakage. The measurement of MDA in carnation petals in this experiment indicated that GABA preserved membrane stability and we here assume that prolonging the vase life of these carnation cultivars could be pertained to GABA role in protection of cell membrane against oxidative stress. The antioxidant status reflecting by antioxidant enzymes, confirm this assumption.
Fig 1. Effect of different concentrations of Gamma Aminobutyric Acid on vase life and weight loss of Dob Pedro (red) and Delphi (white) (two carnation cultivars) cut flowers. Data are means of five replicates. The mean comparisons among treatments were determined by DMRT taking $P < 0.05$ as significant. Different letters indicate significant differences among treatments.

Data of this experiment showed that flowers which were in GABA solution had higher antioxidant enzymes activity than control. All concentrations of GABA increased the activity of SOD, CAT, APX and GPX. The highest activity of these enzymes was found in 0.5 mM and 0.75 mM of GABA solutions. The findings also indicated that the activity of SOD, GPX and CAT were higher in Delphi compared to Dob Pedro cultivar while the higher activity of APX was in Dob Pedro than Delphi cultivar (Fig. 3). Antioxidant enzymes have an important role in postharvest quality improvement of cut flowers and preservative compounds are used to alter their status particularly during senescence (Gerailoo & Ghasemnezhad, 2011). Ameliorative effects of GABA treatments have been reported for cut anthodium (Soleimani Aghdam et al., 2015, Mahjoori et al., 2019), tuberose (Babarabie et al., 2018) and gerbera (Mohammadi et al., 2021). This positive effect of GABA on prolonging vase life and improving postharvest quality of cut flowers relates to strengthening antioxidant systems including the higher activity of antioxidant enzymes.
Fig. 2. Effect of different concentrations of Gamma Aminobutyric Acid on lipid peroxidation of Dob Pedro (red) and Delphi (white) cut flowers. Data are means of five replicates. The mean comparisons among treatments were determined by DMRT taking $P < 0.05$ as significant. Different letters indicate significant differences among treatments.

Fig. 3. Effect of different concentrations of Gamma Aminobutyric Acid solutions on antioxidant enzymes (SOD, CAT, APX and GPX) activity in carnation cultivars Dob Pedro (red) and Delphi (white) cut flowers. The significant of different between treatments was determined by DMRT method taking $P < 0.05$ as significant. Different letters indicate significant differences among treatments.

The results of the present investigation indicated that all applied GABA solutions reduced PPO activity in petal and the flowers which were treated with 0.75 mM GABA had the lowest activity of PPO (Fig. 4).
The browning phenomenon observed in cut flowers, is an indication of PPO activity capable of degrading phenolic compounds and has negative effect on postharvest quality of flowers. Decreased PPO activity in the treatments used in this study could also be one of the reasons for prolonged postharvest life of cut flowers.

**CONCLUSION**

The results of this research indicated that GABA had antioxidant properties capable of increasing defense ability of the carnation cut flower. Meanwhile, the applied concentration is very critical and should be taken into account. Based on the present findings, the best treatment was GABA at 0.75 mM especially in Delphi cultivar. It appears that the applied GABA operates through protecting cell membranes, increasing antioxidant enzymes activity and decreasing PPO activity increasing the activity of antioxidants enzymes and decreasing PPO activity.

**Declarations of interest**

The authors declare that they have no conflict of interest.

**REFERENCES**


