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Improvement of morpho-physiological and biochemical characteristics of stock flower (*Matthiola incana* L.) by Gamma-aminobutyric acid and spermine

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Abstract
Common stock (*Matthiola incana* L.) is an important cut flower, bedding and aromatic plant. Increasing its flower number and size is a desired objective among florists. Plant growth regulators including γ-aminobutyric acid (GABA) and spermine have proved beneficial for improving flowers' characteristics. This study was designed on a completely randomized design with 4 replicates and 16 treatments. GABA (0, 2.5, 5 and 10 mM) and spermine (0, 1, 2 and 3 mM) were sprayed on six leaves seedlings. Increasing concentration of GABA and spermine significantly decreased the flowering time and conversely increased plant height, number of flowers, stem diameter, flower diameter, shoot and root dry weight, leaf relative water content, chlorophyll, and antioxidant enzymes activity. The highest number of flower shoot and root dry weight, chlorophyll, and antioxidant enzymes in addition to the lowest electrolyte leakage were obtained by 10 mM GABA in combination with 3 mM Spermine. Also, maximum plant height and earliest flowering time were gained using 3 mM spermine, while the greatest leaf relative water content was obtained by 10 mM GABA. Application of GABA and spermine could be suggested as a promising way to improve stock flower's aesthetic characteristics and possibly enhance their stress tolerance.
Keywords: Flower characteristics, GABA, γ-aminobutyric acid, Ornamental plant, Plant growth regulator, Spermine, Stock flower, *Matthiola incana* L.

Introduction
The economic value of ornamental plants has a significant worldwide increase and will be continued at an annual rate of 8-10%. Among them, productions of cut flowers and potted plants have faced a substantial increase. Stock flower (*Matthiola incana* (L.) W.T. Aiton) has been known as a main crop in the floriculture trade and it has become increasingly important in recent years. It is widely cultivated as a cut flower and bedding plant due to its strong aroma, fascinating form and petal number, and also in a great variety of colors. Stock flowers are native to Europe, south western and central Asia and northern Africa. This ornamental plant belongs to the *Brassicaceae*, and it is divided in to multiple groups based on the type of use including cut flowers, potted plants and bedding plants. Commonly it is found in two forms of single-flowered and double-flowered plants, which the latter is more popular in the market (Ahmadi Hesar *et al.*, 2011).
A well-known practice to increase the yield and quality of ornamental plants is the exogenous application of plant growth regulators (PGRs). They are considered as useful agrochemical tools that

help plants efficiently consume nutrients and successfully demonstrate their genetic and physiological potentials (Syed Sarfraz *et al.*, 2011).

Polyamines such as spermine are characterized as a group of natural compounds with a low molecular weight that plays an important role in a wide range of physiological processes of plants, animals and microorganisms. Plant cell division and growth greatly relies on polyamine resource of an organism. It has been indicated that aging of many plant organs is associated with decreased levels of polyamines (Valero *et al.*, 2002). In fact, spermine is effective in preventing cell death processes and it is referred to as a rejuvenating agent (Thomas & Thomas, 2001). It acts as a compatible osmolyte conferring stress tolerance and show antimicrobial activity against plant pathogens (Syed Sarfraz *et al.*, 2011). Spermine has been used in a number of ornamental plants in order to enhance their growth and development including rose (Tatte *et al.*, 2016) *Zoysia* turfgrass (Sun *et al.*, 2019) and gerbera (Palagani & Singh, 2017).

γ-amino butyric acid (GABA) is found in bacteria, plants and animals (Fait *et al.*, 2007). It is a plant growth regulator that can control growth and yield of various crops. GABA increases the level of endogenous hormones of plants, which in turn affects their growth and physiological traits. In plants, several signaling roles have been also attributed to GABA, including involvement in pH regulation, nitrogen storage and plant growth (Shelp *et al.*, 2012). Exogenous application of GABA may stimulate antioxidant responses of plants and further provide multiple stress tolerances (Krishnan *et al.*, 2013). Few reports exist on GABA application in the ornamental plants including anthurium (Soleimani Aghdam *et al.*, 2016), perennial ryegrass (Krishnan *et al.*, 2013), chamomile (Sadeghiani *et al.*, 2019), periwinkle (Bayanloo *et al.*, 2020), *Agrostis* turfgrass (Li *et al.*, 2019) and narcissus (Heidari Krush & Rastegar, 2021) where it has improved their morpho-physiological responses.

The objective of this research was to investigate the effects of exogenous application of GABA and spermine on some morpho-physiological and biochemical characteristics of stock flower. The plant's aesthetic features and antioxidant enzymes activity is studied in this research for later use in the cultivation practices and stressful environments of *M. incana*.

Materials and Methods

Plant materials and treatments

This study was carried in the greenhouse of College of Agriculture at Shiraz University. It was implemented based on a completely randomized design with 4 replicates. Stock flower seedlings were transferred to pots containing equal proportions of fine sand and loam soil (1:1) in 6 leaves development stage. Leaves were later sprayed every month with GABA (0, 2.5, 5 and 10 mM) and spermine (0, 1, 2 and 3 mM). Foliar application continued until 4 months. After the plants grew to the flowering stage, morpho-physiological and biochemical traits were measured. Average day and night temperature during the cultivation was set at 24 (± 2) ˚C and 16 (± 2) ˚C, respectively. The plants grew under natural photoperiod during winter and spring and relative humidity was controlled at 60 %.

Morphological measurements

Flowering time, number of flowers, plant height, stem diameter and flower diameter were measured by day count, flower count, ruler and digital caliper, respectively. Plant roots and shoots were removed instantaneously after harvesting of the plants. In order to determine the dry weight, roots and shoots were placed in an oven at 60 ˚C for 48 h and later weighed using a scale.

Physiological and biochemical measurements

At the end of spring the physiological and biochemical measurements were carried out. Leaf relative water content (RWC) and electrolyte leakage (EL) were measured according to the following equations:

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$$RWC=\frac{FW-DW}{TW-DW}\times 100 \qquad EL=\frac{EC1}{EC2}\times 100$$

FW = fresh weight, TW = turgid weight, DW= dry weight.
EC1=primary ion leakage, EC2=second ion leakage

Total chlorophyll content was measured using the method described by adding 10 ml of methylsolfoxide to 100 mg of fresh leaves. It was kept in dark place for 24 h. the absorbance was read at 470, 663 and 645 nm using a spectrophotometer device and total chlorophyll content was calculated according to the following equation:

$$\text{Total chlorophyll (mg/ g F.W.)} = \frac{20/2(A645)+8/02(A663)\times Volume\ made}{Wt.of\ the\ sample}$$

Total protein was calculated and enzyme unit activity was measured accordingly. Superoxide dismutase (SOD) enzyme activity was determined using the method of Beyer JR & Fridovich (1987). It was evaluated by measuring the decrease in light absorption of nitroblue tetrazolium superoxide complex under effect of enzyme extract. Three ml of the reaction mixture contained 50 mM potassium phosphate buffer, 0.01 mM EDTA, 13 mM L-methionin, 75 µl nitroblue tetrazolium, 40 µl riboflavin and 20 µl of enzyme extract.

Catalase (CAT) enzyme activity was measured after decomposition of H_2O_2, detected by a spectrophotometer at 240 nm as described by Chandlee & Scandalios (1984). The reaction mixture consisted of 3ml enzyme extract, 50 mM potassium phosphate buffer, 10 mM H_2O_2 and 50 µl enzyme extract.

Peroxidase (POD) enzyme activity was measured following the decomposition of H_2O_2 at 240 nm by a spectrophotometer as described by Upadhyaya *et al.* (1985). The reaction mixture consisted of 0.05 ml of 20 mM guaiacol, 2.5 ml potassium phosphate buffer and 50 µl enzyme extract.

Ascorbate peroxidase (APX) enzyme activity was measured by the procedure of Nakano & Asada (1981). The activity of this enzyme was determined by a spectrophotometer device at 290 nm.

Statistical analysis

Data were subjected to analysis of variances. Means were compared using LSD test at the significant level of $p \leq 0.05$ by SAS 9.0 software.

Results

Flowering time

The earliest flowering time was obtained in the treatment of 3 mM spermine with an average of 119 days and the most delayed flowering time was seen in the control plants with an average of 143 days (Figure 1). As the concentrations of GABA and spermine were increased, flowering time conversely decreased. Spermine treatment decreased the flowering time for 24 days, compared to the control. It showed a greater reduction in flowering time than GABA. (Figure 1).

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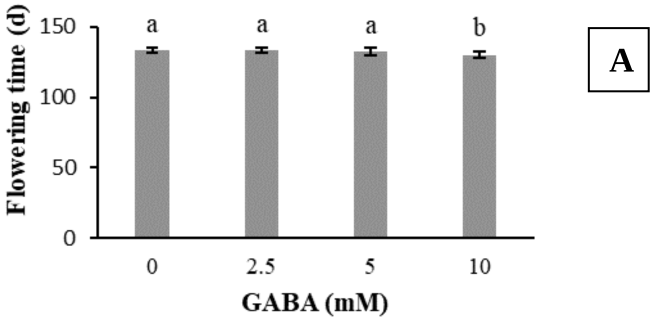

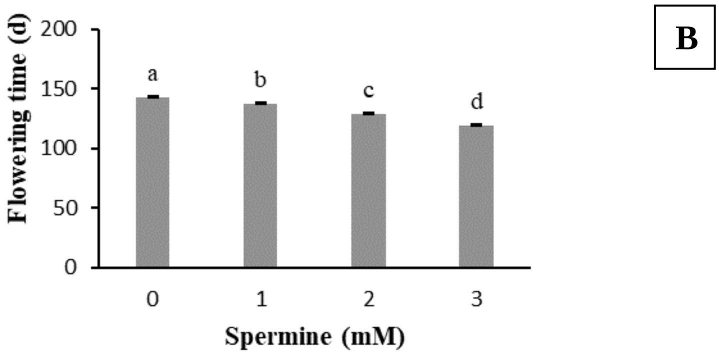

Figure 1- Effect of GABA (A) and spermine (B) on flowering time of stock flower. Each value represents the means ± SE. Different letters indicate significant differences at *P* < 0.05 using the LSD test.

Flower number

Combined treatment of GABA and spermine at their highest concentration doubled the number of flowers per inflorescence, compared to the control. The highest number of flowers (37 flowers) was obtained in the combined treatment of 10 mM GABA and 3 mM spermine, while the lowest number of flowers (16 flowers) was observed in the control. Although both PGRs increased the number of flowers, spermine had a greater effect on number of flowers than GABA (Figure 2).

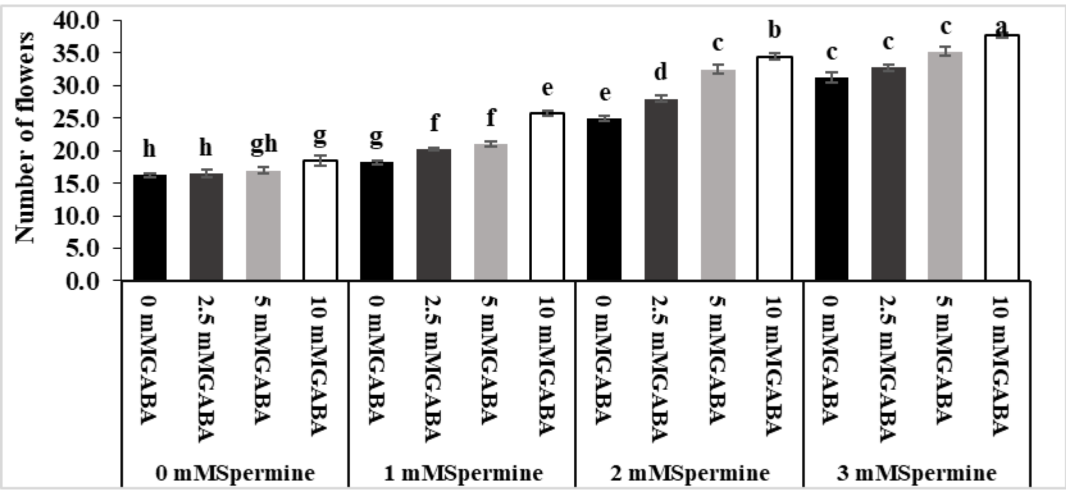

Figure 2- Effect of GABA and spermine on flower number of *M. incana*. Each value represents the means ± SE. Different letters indicate significant differences at *P* < 0.05 using the LSD test.

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Plant height

The maximum plant height (42 cm) was obtained by 3 mM spermine treatment, while the lowest plant height (32 cm) was detected in the control. Both GABA and spermine enlarged the plant height, but spermine showed a larger increase of plant height than GABA (Figure 3).

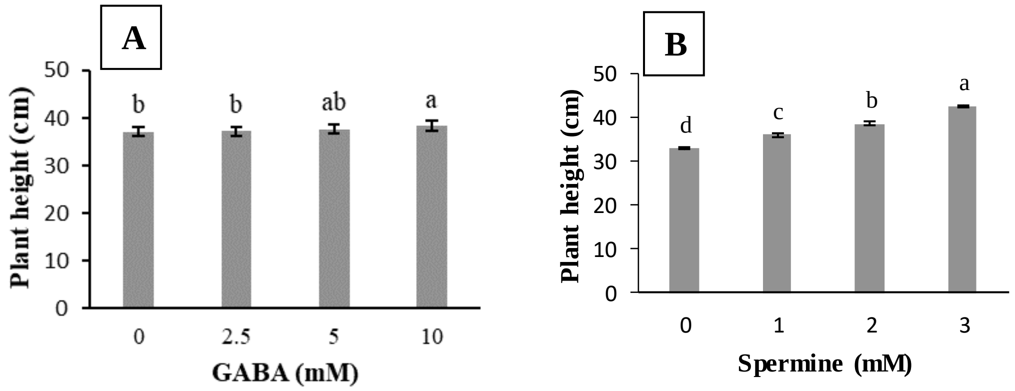

Figure 3- Effect of GABA (A) and spermine (B) on stock flower height. Each value represents the means ± SE. Different letters indicate significant differences at *p* < 0.05 using the LSD test.

Stem diameter

The greatest stem diameter (7 mm) was gained in the combined treatment of 10 mM GABA and 3 mM spermine, while minimum stem diameter was seen in the control. Although both GABA and spermine increased the stem diameter, but spermine had a greater effect on stem diameter than GABA (Figure 4).

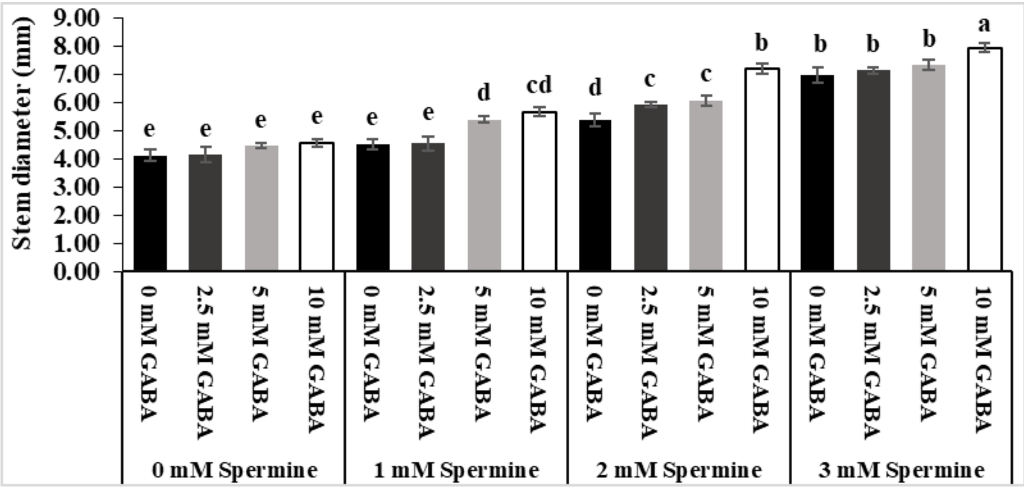

Figure 4- Effect of GABA and spermine on stem diameter of *M. incana*. Each value represents the means ± SE. Different letters indicate significant differences at *P* < 0.05 using the LSD test.

Flower diameter

The lowest flower diameter (2 cm) was observed in the control, while combined treatment of 10 mM GABA and 3 mM spermine doubled the flower diameter (5 cm). Both GABA and spermine improved the flower diameter, though spermine displayed a stronger effect on flower diameter than GABA (Figure 5).

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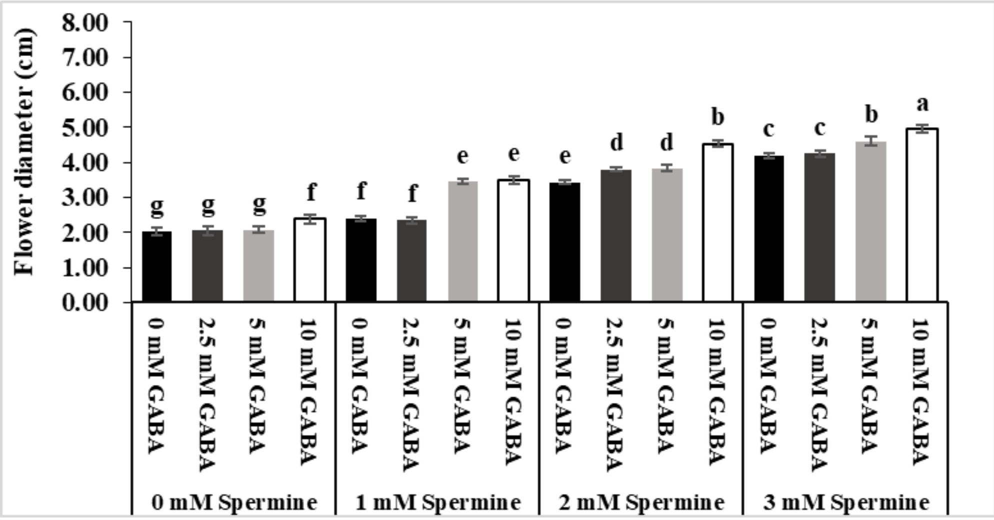
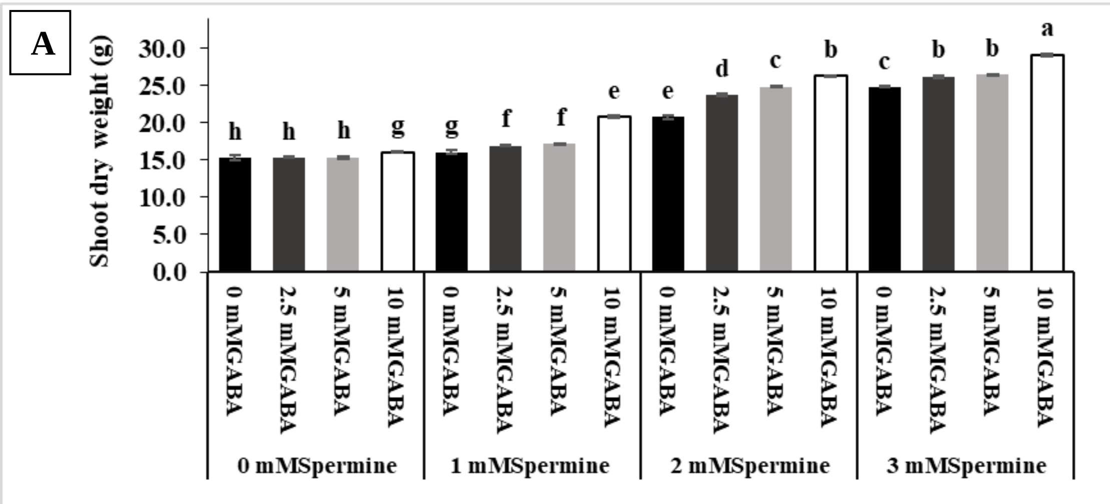


Figure 5- Effect of GABA and spermine on flower diameter of stock flower. Each value represents the means ± SE. Different letters indicate significant differences at *P* < 0.05 using the LSD test.

Shoot dry weight (SDW) and root dry weight (RDW)

The maximum SDW and RDW (29 g and 10 g, respectively) were found in the combined treatment of 10 mM GABA and 3 mM spermine. The minimum SDW and RDW (15 g and 4g, respectively) were gained in the control. GABA and spermine increased shoot dry weight and root dry weight, but spermine accumulated a greater dry mass (Figure 6).



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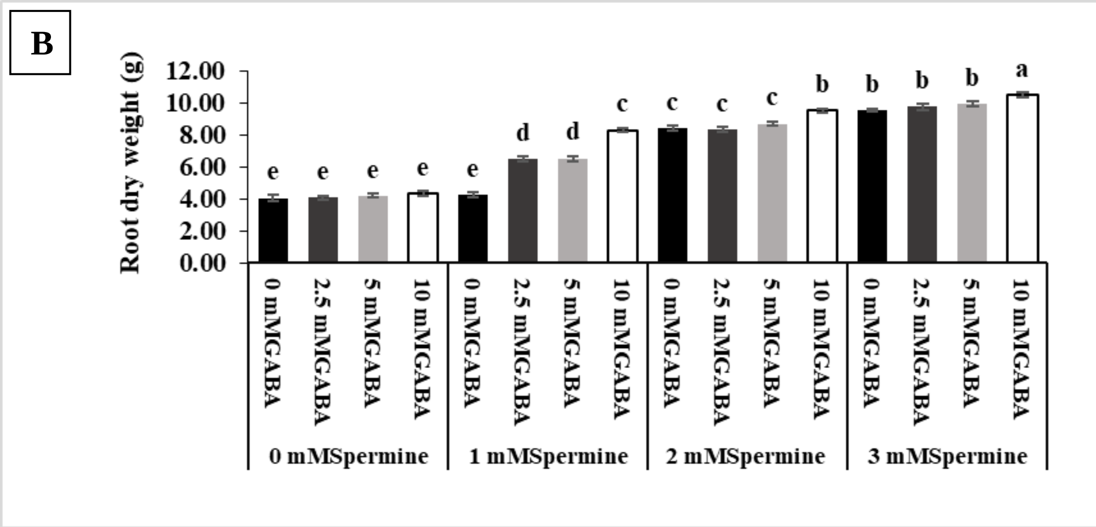

Figure 6- Effect of GABA and spermine on shoot dry weight (A) and root dry weight (B) of *M. incana*. Each value represents the means ± SE. Different letters indicate significant differences at *P*< 0.05 using the LSD test.

Relative water content (RWC)

The highest RWC (80 %) was gained by 10 mM GABA, which showed a significant difference with other treatments. The lowest RWC (68 %) was detected in the control. Both GABA and spermine increased the RWC in a similar pattern (Figure 7).

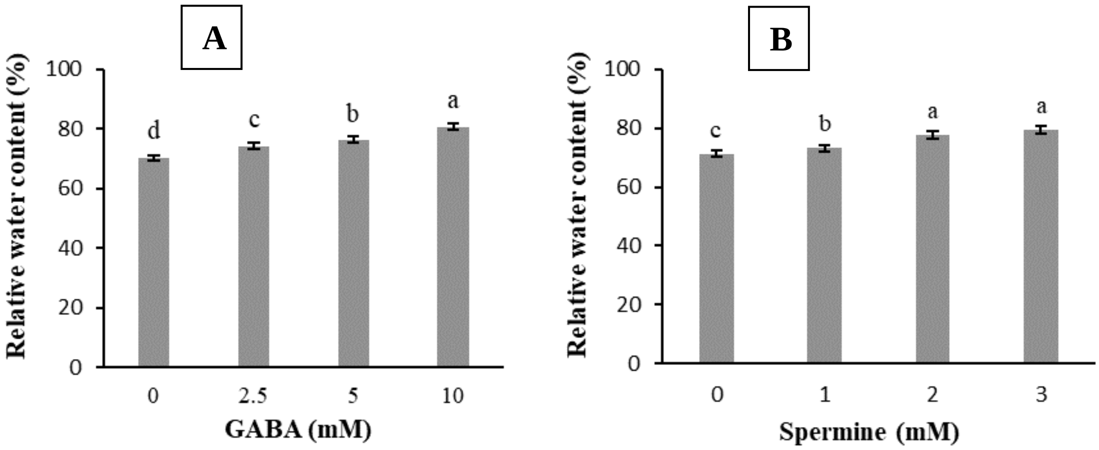

Figure 7- Effect of GABA (A) and spermine (B) on relative water content of stock flower. Each value represents the means ± SE. Different letters indicate significant differences at *P*< 0.05 using the LSD test.

Electrolyte leakage (EL)

The lowest electrolyte leakage (20 %) was seen in the combined treatment of 10 mM GABA and 3 mM spermine. On the other hand, the highest electrolyte leakage (34 %) was observed in the control. Both GABA and spermine decreased the electrolyte, but spermine had a greater effect on electrolyte leakage than GABA (Figure 8).

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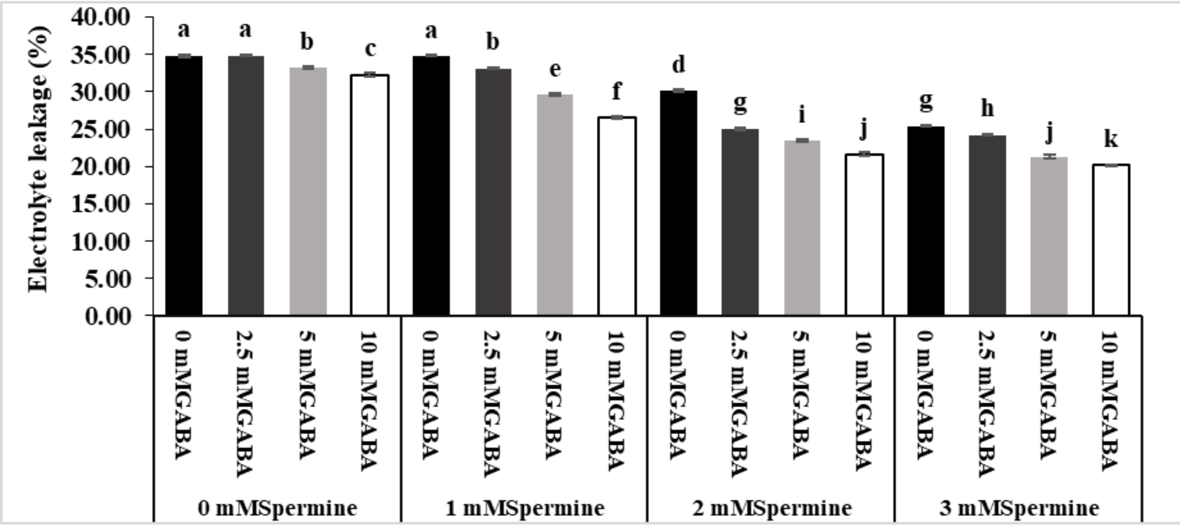

Figure 8- Effect of GABA and spermine on electrolyte leakage of *M. incana*. Each value represents the means ± SE. Different letters indicate significant differences at *P*< 0.05 using the LSD test.

Total chlorophyll content

The combined treatment of 10 mM GABA and 3 mM spermine increased the leaves chlorophyll to the maximum content. However, the lowest chlorophyll amount was seen in the control. Both GABA and spermine increased the total chlorophyll content, but GABA had a greater effect than spermine (Figure 9).

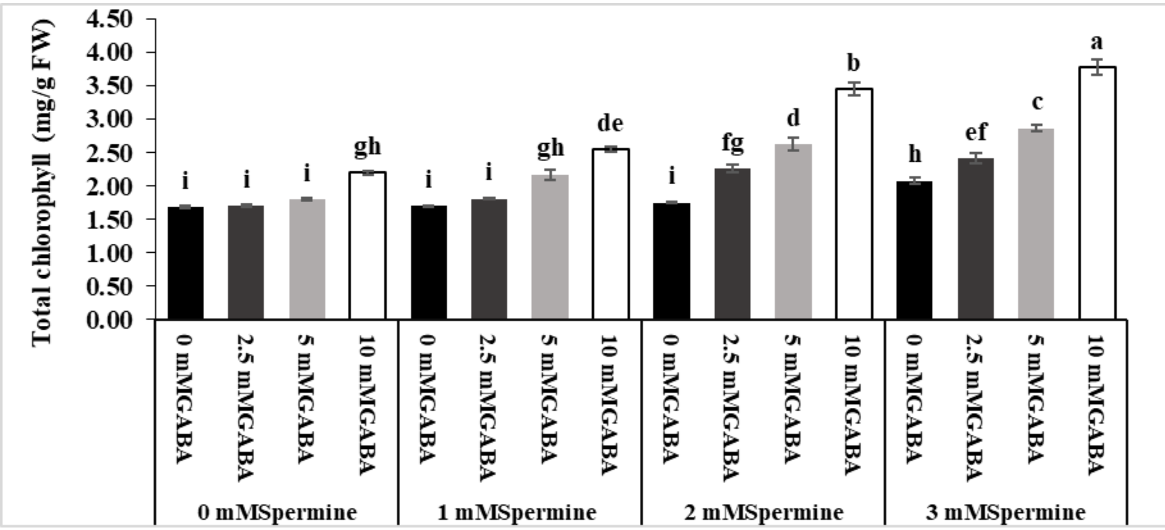

Figure 9- Effect of GABA and spermine on total chlorophyll content of stock flower. Each value represents the means ± SE. Different letters indicate significant differences at *P*< 0.05 using the LSD test.

Antioxidant enzymes activity

The highest activity of CAT, POD, SOD and APX enzymes was seen in the combined treatment of 10 mM GABA and 3 mM spermine, which compared to the control demonstrated a 3-fold, 5-fold, 5-fold and 2-fold increase, respectively (Figure 10).

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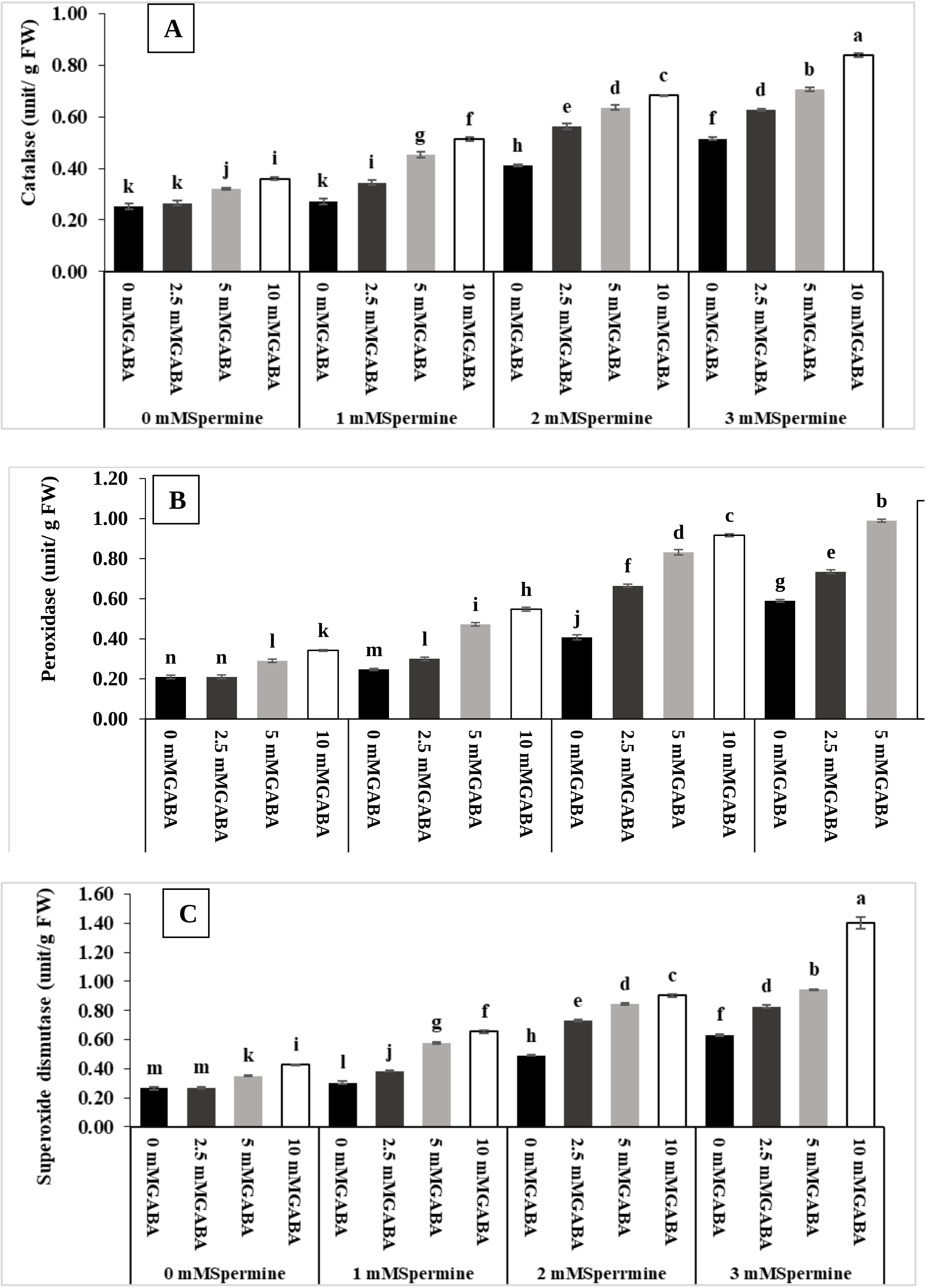

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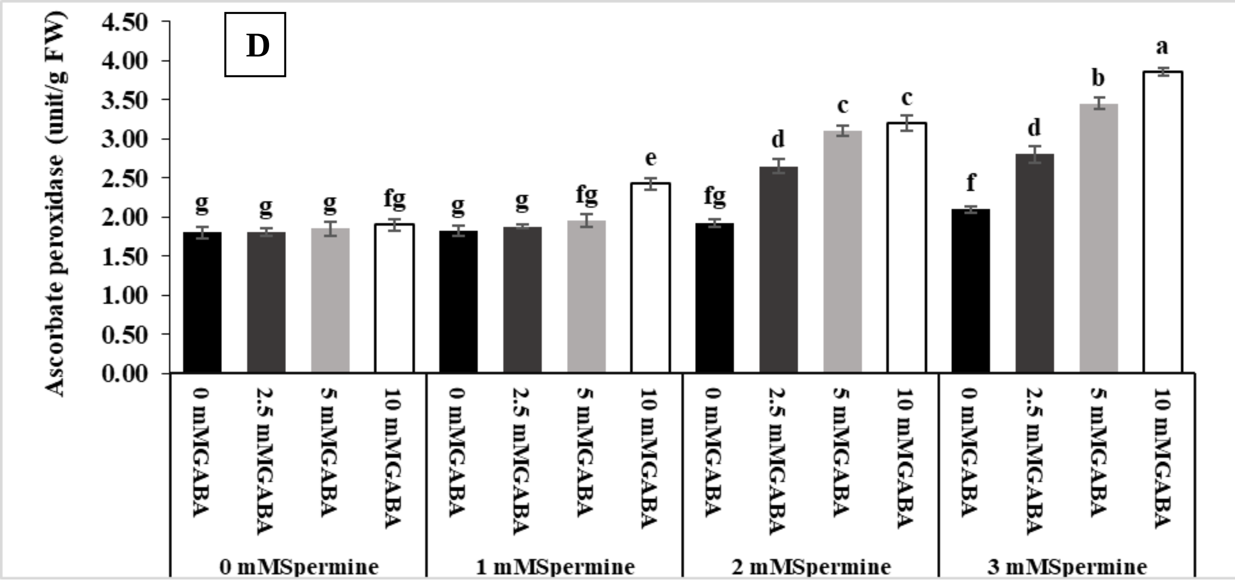

Figure 10- Effect of GABA and spermine on antioxidant enzymes activity of *M. incana*. Each value represents the means ± SE. Different letters indicate significant differences at *P*< 0.05 using the LSD test.

Discussion

Plant growth regulators are used exogenously to increase the yield and quality of ornamental plants. They can affect plants' enzyme activities resulting in enhanced production of protein, chlorophyll, carbohydrates and etc. The role of spermine and GABA in improving the morpho-physiological traits of plants has been proven before. Polyamines enhance the morphological traits by increased cell division and subsequent accelerated plant growth and development (Walden *et al.*, 1997; Valero *et al.*, 2003; Takahashi *et al.*, 2010). Their nitrogen structure as well as antioxidant effect can lead to increased plant growth (Khosrowshahi *et al.*, 2008; Tang *et al.*, 2015). It is believed that exogenous application of polyamines can also result in endogenous increase of polyamines. They are considered essential for the normal development of the reproductive structures and improving the plant yield (Lie *et al.*, 2009). In a previous report on roses, Tatte *et al.* (2016) indicated that 10 ppm spermine has beneficial effects on vegetative and reproductive characteristics. In another study, Ekinci *et al.* (2019), demonstrated that spermine improved the plant height, leaf number, stem diameter and fresh and dry weight of pepper plant. Similar outcomes were also obtained in the stock flower of the current study.
In *Zoysia* turfgrass (Sun *et al.*, 2019) and rose flower (Tatte *et al.*, 2016), spermine increased the total chlorophyll content. GABA also increased the total chlorophyll content of *Agrostis* turfgrass (Li *et al.*, 2019) and garlic (Yousef *et al.*, 2019). In the present study on stock flower, chlorophyll content was improved which then resulted in enhanced plant growth and dry weight. Moreover, application of GABA has increased the vegetative growth of garlic plant (Yousef *et al.*, 2019). It also increased the fresh and dry weight of chamomile flowers (Sadeghiani *et al.*, 2019). Bayanloo *et al.* (2020) showed shat GABA use has increased periwinkle's stem height and diameter. Comparable results were obtained in the current research on stock flower. Moreover, Hugo (2000) reported that GABA has increased endogenous levels of hormones in the plant, which in turn has affected the growth and physiological traits. In general, the increase in growth parameters due to GABA use can be explained by the fact that GABA may play a role in carbon and nitrogen metabolism and also as integral part of the TCA cycle (Fait *et al.*, 2007). Another report suggests that GABA can be used as a source of nitrogen for nitrogen metabolism, storage and transport (Shelp *et al.*, 2012). Therefore, considering

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the role of GABA on morphological traits, storage and nitrogen metabolism, the improvement of morphological traits of stock flower in the present study can be justified.

In previous studies, spermine application in rose (Tatte *et al.*, 2016), gerbera (Palagani & Singh, 2017) and *Zoysia* turfgrass (Sun *et al.*, 2019) has increased the RWC. Furthermore, GABA treatment in perennial ryegrass (Krishnan *et al.*, 2013), tomato (Zarei *et al.*, 2020), anthurium (Soleimani Aghdam *et al.*, 2016) and *Agrostis* turfgrass (Li *et al.*, 2019) has increased the RWC as well. In the current investigation on stock flower, similar findings were observed. It can be inferred that polyamines reduce moisture loss by reducing the rate of respiration and production of ethylene, and simultaneously maintaining cell turgidity and membranes integrity (Khosrow Shahi *et al.*, 2008). It is suggested that polyamines act as compatible osmolytes by stimulating water uptake and reducing the osmotic stress (Hosseini Farahi, 2013).

Furthermore, spermine use in *Zoysia* turfgrass (Sun *et al.*, 2019) and wheat (Hassan *et al.*, 2020) has increased membrane stability and reduced electrolyte leakage. Also, GABA application in anthurium (Soleimani Aghdam *et al.*, 2016) and *Agrostis* turfgrass (Li *et al.*, 2019) has increased membrane firmness and further decreased electrolyte leakage. Similar results have been observed in the current study on stock flower. Polyamines maintain membranes stability and reduce the electrolyte leakage by increasing membrane fluidity and binding to negatively charged molecules (i.e., phospholipid and pectin polysaccharides) (Pazoki *et al.*, 2017). In fact, polyamines exhibit antioxidant properties and poly-cationic activity to stabilize cell membranes and protect them from destruction. GABA may reduce lipid peroxidation through increased antioxidant enzymes activity and therefore eliminates free radicals (Zarei *et al.*, 2020).

GABA has been reported to protect photosynthetic apparatus and chlorophyll pigments under stress by reducing the accumulation of reactive oxygen species (Vijayakumari & Puthur, 2016). In the present study on *M. incana*, GABA treatments increased the activity of antioxidant enzymes which led to increased chlorophyll production. Due to the anti-ethylene role of polyamines, they inhibit the degradation of chlorophyll by preventing the production of enzymes essential for ethylene biosynthesis (Valero *et al.*, 2002).

Exogenous application of spermine has led to synthesis of antioxidant metabolites, which is an increasing force for interaction as well as neutralizing the harmful effects of reactive oxygen species (Shu *et al.*, 2013; Sajjad *et al.*, 2015). Furthermore, spermine use in *Zoysia* (Sun *et al.*, 2019) and gerbera (Palagani & Singh, 2017) has increased the activity of antioxidant enzymes. Also, GABA application in *Agrostis* turfgrass (Li *et al.*, 2019) and *Lolium* turfgrass (Krishnan *et al.*, 2013) has increased the antioxidant enzymes activity. In the same way, spermine and GABA showed increased antioxidant enzymes activity in stock flower as well. Researchers have revealed that GABA acts as the first defense barrier against reactive oxygen species and reduces phospholipase and lipoxygenase activity by increasing the ratio of saturated fatty acids to unsaturated fatty acids (Soleimani Aghdam *et al.*, 2016).

Conclusion

Exogenous application of GABA and spermine improved the morpho-physiological and biochemical characteristics of stock flower. The aesthetic features such as plant height, stem diameter, flowering time, flower number and flower diameter were enhanced. Thus, GABA and spermine can be suggested as potent PGRs to enhance the flowers' growth and attractiveness. Its use to enhance drought stress tolerance merits further investigations.

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