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REGULATION OF MORPHO-PHYSIOLOGICAL AND VASE QUALITY ATTRIBUTES OF CARNATION (*DIANTHUS CARYOPHYLLUS*) CV. TABASCO MEDIATED BY GA_3

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Abstract

Gibberellic acid (GA_3) is an excellent plant growth regulator and a promoter of vase life that accelerates the antioxidant activities in plants. In the current study, GA_3 was tested for its hypothesized influences on growth and vase life attributes of the carnation plant. Plants were treated with triple-frequency six levels of GA_3, i.e., controls (no GA_3 treatment), 25, 50, 100, 200, and 400 mg/L initiated one-month post-transplantation. The results helped to infer several floral traits following GA_3 treatments. Early flower initiation, flower diameter, stalk length, stalk diameter, flower yield per plant, and maximum fresh and dry weight of cut flowers were grossly improved with the highest concentration of GA_3. The variance in the improved traits of plant parameters was explained by the increasing concentrations of GA_3 foliar spray. The parameters enormously influenced by GA_3 treatments were the percentage of flower opening and the vase life of cut flowers, which were likely attributable to the reduced ethylene synthesis and improved membrane integrity of petals. The activities of antioxidant enzymes varied between 25 mg/L and 400 mg/L GA_3 treatments versus control. The study provided a sound insight into the favorable effects of GA_3 applications on overall growth and antioxidant activities of carnation cut flowers, which prolonged their vase life.

Key words: Gibberellic acid; Carnation (*Dianthus caryophyllus*); Plant growth parameters; Floral traits; Vase quality attributes; Antioxidant enzymes activity.

Introduction

Plant growth-promoting hormones play a vital role in regulating the morpho-physiological and biochemical parameters of plants. Gibberellic acid-3 (GA_3) is a tetracyclic di-terpenoid plant hormone involved in the regulation of plant growth. GA_3 is chiefly known for its growth, development, and early anthesis in many plant species (Mahmoody and Noori, 2014). It also cross-talks to other hormones and activates their production, which may affect growth (Arney and Mancinelli, 1995; Weiss & Ori, 2007). Moreover, it could stimulate photosynthesis via CO_2 assimilation, and the synthesis of chlorophyll and rubisco enzyme (Taiz & Zeigar, 2006; Aftab *et al*., 2010). Plants tend to cope with environmental stresses via synthesizing certain antioxidants with the help of endogenous GA_3 interaction with environmental clues. GA_3, which is synthesized in stamens, translocated to the floral organs and pedicel, helps to determine the male and female fertility and thereby influences the overall flower development (Gupta & Chakrabarty, 2013). It is, therefore, necessary for light dependant plants that prefer lower temperatures and long days to transit from vegetative to onset flowering such as carnations (Cerny-Koenig, 2004). The vase life is affected by various GA_3-mediated pathways. Over the last few years, various studies have evidenced that GA_3 promoted membrane integrity by inhibiting the electrolyte ions-leakage through interfering with the peroxidation of polyunsaturated lipids and by reducing the synthesis of malondialdehyde (MDA) contents. Additionally, the increased calcium ions absorption, affecting the stabilization of membrane structures by GA_3 is also reported (Gururani *et al*., 2015).

Carnation (*Dianthus caryophyllus)* is one of the 10 most magnificent and economically important cut flowers in the world that ranks second to rose flower. The carnation, rose, and chrysanthemum contributes 50% in world floral trade (Yashaswini *et al*., 2011; Chandra *et al*., 2016). Commercially, carnation is attributed to garden, pot, bed, and edge plant that adds colors to rock gardens and indoors. Several varieties of the carnation are used in food items just because of their peppery aroma and taste; likewise, other varieties are used in pharmaceutical industry to formulate the ailing medicines for various diseases. Carnation is demanded widely in Netherland and France perfume formulations. It is a member of Caryophyllaceae family whose species are called carnations or pinks (Jurgens *et al*., 2003; Hamidimoghadam *et al*., 2014).

Moreover, it is a long day ornamental plant, which is half-hardy herbaceous specie with diploid chromosome number 2n=30 (Blake, 1955; Gharge *et al*., 2011). Post-harvest longevity and quality appearance of cut flowers are the fundamental features that appeal to customers and are of utmost importance for marketing (Mansouri, 2012). Application of GA_3, therefore, is used to promote active seed germination and also to influence the phase transition, including the development of meristem to shoot, juvenile to adult, and shift from vegetative to the reproductive growth cycle. The current study was designed to optimize the best dose of GA_3 treatments to improve the plant's physiological character. Furthermore, the cut flower quality attributes viz., vase life, and antioxidants system of carnation cut flowers were also determined.

Materials and Methods

Study design: We designed a polyethylene tunnel to study plant growth and quality of cut flower (Sim carnation cv. Tabasco) by using cuttings (5-10 cm along with 3-5 nodes). We used six concentrations of gibberellic acid, 0 (controls), 25, 50, 100, 200, and 400 mg/L as a foliar spray for plants prepared from cutting

source. The 1st foliar spray was applied after one month of transplantation following 2nd spray after 15 days of 1st spray and 3rd spray just a week before flower bud initiation. Plants in all experiment blocks were fertigated thoroughly with a basal dose of N-P-K (10:10:10) from "Grow More" artificial fertilizer fortnightly till flowering. Soil medium had loamy texture, with pH, 7.1; EC, 0.79 dS m^{-1}; organic matter, 0.75%; available P, 3.1 mg kg^{-1} and available K, 100 mg kg^{-1}. The experiment was performed in a completely randomized block (CRD) design with three replications. Post-harvest analysis of carnation cut flowers was performed over all flowering stages, i.e., open brush bud, fully opened flower, and at the onset of senescence. For vase life analysis, the flower stalks of uniform size were shifted to the laboratory and re-cut underwater and placed in glass bottles containing distilled water at 25±5°C and 14h illumination period.

Plant growth and floral parameters: Data on plant height, number of leaf pairs, number of side shoots and leaf chlorophyll contents were measured in fully matured plants. The number of days taken to the first flower opening, flower diameter, flower stalk length/diameter and flower yield were documented in the field. The fresh and dry weight of cut flowers were also measured at post-harvest stages in the laboratory.

Study of vase quality attributes and ethylene synthesis: Percentage of flowers opening and vase life of carnation cut flowers were studied during the vase holding period with a method reported by Satoh *et al*., (2005). The ethylene synthesis in flowers was measured from fully opened flowers by using an ethylene analyzer (ICA56 Ethylene Analyser, Diaz *et al*., 2017). Briefly, three flowers of carnation were put in 450 ml jars in such a way that each flower was placed in a separate pot. The flowers were kept in jars for 1h while keeping the lids sealed adequately with sealing tape. Ethylene concentration was measured after 1h by inserting the hypodermic syringe inside the vials through rubber septum on the lid.

Measurement of the membrane integrity (%): Membrane integrity of carnation cut flowers was measured by using the method prescribed by Singh *et al*., (2008). In short, five petals (about 1 cm size each) of carnation flower per replication were taken and washed in distilled water for about 1 minute. Petals were dried on filter paper, then inserted into the test tube containing 10 ml of distilled water and incubated at 25°C for 180 minutes. After incubation, initial electrolyte leakage was measured by using a conductivity meter. The solution was autoclaved at 121°C for 15 minutes to isolate all the electrolytes from petals before the final conductivity (total electrolyte leakage) could be found. The percentage of membrane integrity was calculated by using the following equation:

Membrane Integrity (%) = [1 - (Electrolyte leakage after 180 min of incubation /Total electrolyte leakage)]×100

Analysis of antioxidants activities: All antioxidants were measured at three cut flower stages, i.e., open brush bud, fully opened flower, and at the onset of senescence in fresh samples.

Preparation of cell-free enzyme extract: Two grams of each frozen carnation flower sample (preserved at -80ºC), was ground by using a pre-chilled mortar and pestle. Each sample was then suspended in 5 ml of 0.1 M KPO_4 (pH 7.8) containing 0.2 g Polyvinyl pyrrolidone (PVP) and 0.5% Triton. The mixture was centrifuged (HERMLE Z 200 centrifuge machine) at 14000 rpm for 30 minutes at 4°C (Abbasi *et al*., 1998), and the supernatant was separated for further analysis.

Superoxide dismutase (SOD): SOD's enzymatic activity was evaluated by measuring inhibition of photochemical reduction of nitro blue tetrazolium (NBT) by using the method reported by Abbasi *et al*., (1998). Two sets of five cuvettes were used, each containing 0, 50, 100, 200 and 300 µl enzyme extract together with 13 mM Methionine, 75 µM NBT, 0.1 mM EDTA and 2 µM riboflavin (substrate) were added to each cuvette and transposed to allow maximum contact between enzyme and substrate. One set of the cuvette was placed in complete darkness to mimic control. Another set was placed under fluorescent lamps for 10 minutes. Light absorbance was measured at 560 nm with a spectrophotometer (Optima®3000 plus).

Peroxidase (POD): POD was also measured at three flower stages, i.e., open brush bud, fully opened flower, and at the onset of senescence by using the method reported previously (Hassan *et al*., 2007). The assay mixture comprised of 15 mM Na_3PO_4 buffer (pH 6.0) and 100 µl substrate, which contained 0.1 mM guaiacol (O-methoxyphenol) and 1 mM H_2O_2. At a wavelength of 470 nm, the absorbance of the reaction mixture was measured by using a spectrophotometer. POD activity was calculated as the change in optical density (OD) over three minutes and expressed as units per gram fresh weight (U g^{-1} F.W).

Catalase (CAT): The CAT activity was determined by using the method reported by Abassi *et al*., (1998). To this purpose, two buffer solutions were used, i.e., the first solution (buffer A) consisted of 50 mM KPO_4 buffer (pH 7.0), while the second solution (buffer B) consisted of 12.5 mM H_2O_2 in 50 mM KPO_4 buffer (pH 7.0). A 100 µl enzyme extract was added into each of two cuvettes, one containing 1 ml buffer-A and other containing 1 ml buffer-B. Both cuvettes were placed in the dark. The optical density (OD) of spectrophotometer at 240 nm was set to observe readings at 45 and 60s, starting from the time the extract was added to the cuvettes. The difference in optical density between (OD) 45 and 60 sec was used to calculate CAT activity. One unit CAT activity was expressed as a unit per gram fresh weight (U g^{-1} F.W).

Statistical analysis

All the relevant data on growth, physiological, and post-harvest parameters were subjected to a comparative analysis. The regression scatter plots were constructed using MS-Excel. In contrast, SPSS, was used for the analysis of variance (ANOVA), and variations among treatment means were compared through LSD at 5% level of significance.

Results

Plant growth parameters and floral traits: As the concentration of applied GA_3 increased, significant improvements were observed in the morphological traits of carnation plants compared with controls; while the results were highest in plant height (ranging between 58.7 and 60 cm), number of leaf pairs (average leaves 58±0.4), leaf chlorophyll contents (53.1±4.6 SPAD) and the number of side shoots (i.e., 14±0.7) in plants sprayed with triple frequency foliar spray of GA_3 at 400 mg/L (Table 1). The results of preharvest floral attributes, i.e., days took to first flower opening (58 days versus controls, i.e., 77 days), and flower diameter (improved 7.5 cm versus that of controls, i.e., 3.4 cm) were enhanced with highest GA_3 concentration. Additionally, the flower stalk length (58.4 cm versus 39.7 cm in controls), flower stalk diameter (4.80 mm versus 2.77 mm in controls), flower yield per plant (average 7 flowers versus 2 flowers per plant in controls), fresh and dry weight of carnation cut flowers were also significantly improved with GA_3 400 mg/L (Table 2).

The results showed that the percentage of flower opening, vase life, ethylene production, and membrane integrity were significantly improved after the application of 400 mg/L of GA_3 versus controls. A higher percentage of flowers opening and increased vase life (Table 3) by least ethylene synthesis and maximum membrane integrity (%) were observed after 400 mg/L GA_3 treatment (Fig. 1) at open brush bud stage (Table 3). The applied GA_3 concentrations caused a linear rise in flower opening. The membrane integrity also improved from the point of open brush bud stage to fully opened flower stage and steadily declined at the onset of senescence. The ethylene synthesis was least at the open brush bud stage; however, a slight increase in ethylene was observed at a fully opened flower stage. At the onset of senescence, ethylene synthesis was abruptly increased (Table 3). Both parameters had a linear relationship with applied GA_3 concentrations.

Comparative analysis of antioxidants: All antioxidants, including SOD, POD, and CAT, were enhanced at 400 mg/L of GA_3. The highest GA_3 improved the studied traits with a concentration of 400 mg/L at all stages viz., open brush bud, fully opened flower, and at the onset of senescence (Fig. 1) compared with control. In this study, the antioxidants increased at open brush bud and fully opened flower stage, and declined at the onset of senescence. Additionally, the highest enzyme activity was observed in fully opened flowers at 400 mg/L of GA_3 (Table 3).

The antioxidants gradually decreased across observed stages, being highest in concentration at open brush bud stage (20.3 U g^{-1} F. W, 12.6 U g^{-1} F. W, 2.4 U g^{-1} F. W of SOD, POD, and CAT respectively) and least during the onset of senescence (average 6.6 U g^{-1} F. W, 4.7 U g^{-1} F. W and 1.4 U g^{-1} F. W for SOD, POD, and CAT respectively), $p<0.01$ (except that of CAT which was non-significant between first two stages, $p=0.15$).

Table 1. Influence of various GA_3 treatments on the carnation's growth parameters.

GA_3 concentrations (mg/L)		Plant height (cm)	No. of leaf pairs	Leaf chlorophyll contents (SPAD)	No. of side shoots	No. of days taken to first flower opening	Flower diameter (cm)	Ethylene (ppm)
Control	Mean ± S.D	51±2.64	44.5±1.8	23.0±2	5.33±0.58	77±0.1	3.45±0.72	9.33±3.51
		DE	E	D	E	A	E	A
	Min – Max	49-54	43.2-46.6	20.9-24.8	5-6.1	76.3-77.4	2.62-3.96	7.2-12
25	Mean ± S.D	50.3±0.57	47.4±1.1	21.2±2	5.66±0.57	61±1.7	3.82± 0.37	7.4±0.6
		E	D	D	DE	B	DE	AB
	Min – Max	50.2-54	46.6-48.6	19.3-23.4	5.2-6	60-63	3.42-4.16	7-8.10
50	Mean ± S.D	53.5±0.57	49±0.57	27.1±2.97	6.6±0.9	61.3±1	4.12±0.28	6.56±0.5
		CD	CD	D	D	B	D	BC
	Min – Max	48.4-54.4	48.4-49.4	24.7-30.4	5.6-7.4	60-62.3	3.94-4.46	6-7.2
100	Mean ± S.D	54.6±1.31	49.9±0.3	35.9±4.61	8.1±0.64	60.6±1.5	5.04±0.11	5.6±0.6
		BC	C	C	C	BC	C	BCD
	Min – Max	53.2-55.8	49.6-50.2	30.7-39.3	7.4-8.6	59-62	4.94-5.16	4.9-6
200	Mean ± S.D	56.4±1.27	54.1±0.8	44.1±5.2	11.2±0.4	58±2	6.6±0.1	4.93±0.56
		AB	B	B	B	CD	B	CD
	Min – Max	54.6-57.4	53.2-54.8	38.8-49.4	10.8-12	56-60	6.58-6.78	4.3-5.4
400	Mean ± S.D	58.7±6.2	58±0.41	53.1±4.6	14±0.76	58±0.1	7.5±0.18	4.06±0.15
		A	A	A	A	D	A	D
	Min – Max	58-60	57.8-58.8	48.4-57.6	13.2-14.6	57.5-58.5	7.34-7.68	3.9-4.2
LSD values		**1.21**	**0.79**	**3.11**	**0.54**	**1.1**	**0.29**	**0.92**

* Mean values in the respective columns with different letters were significantly different at $p(<0.05)$ when compared among various GA_3 treatments; No. = Number (count); Min-Max = Minimum – Maximum values

Table 2. Influence of various GA_3 treatments on the carnation's growth parameters.

GA_3 treatments (mg/L)		Flower stalk length (cm)	Flower stalk diameter (mm)	Flower yield per plant	Fresh weight of the flowers (g)	The dry weight of the flowers (g)	Vase Life (Days)	Percentage of flowers opening (100%)	Membrane integrity (%)
Control	Mean ± S.D	39.7±3.8 E	2.77±1 C	2.8±0.2 D	15.2±1.8 D	2.93±0.64 D	6.66±0.4 E	37.7±0.1 D	58.1±7.86 D
	Min – Max	35.8-43.4	2.16-3.96	2.78-2.82	14-17.6	2.2-3.4	6.62-6.68	37.6-38.2	50.6-66.3
25	Mean ± S.D	44.2± 1.2 D	3.11±0.61 BC	3.06±0.5 D	15.7±0.6 CD	3.53±0.5 D	9.4±0.5 D	45.3±3 C	75.8±4.07 C
	Min – Max	43.4-45.6	2.5-3.73	2.6-3.6	14-15.9	3.1-4	9.1-10	42-48.7	72.3-80.3
50	Mean ± S.D	48.1±1.9 CD	3.15±0.3 BC	4.06±0.11 C	16.8±0.6 CD	4.6±0.41 C	10.7±0.6 C	47.8±1.9 C	77.4±2.52 C
	Min – Max	46.8-50.4	2.8-3.6	4-4.2	16.3-17.6	4.28-5.08	10-11.3	46.3-50	75.6-80.3
100	Mean ± S.D	50.3±2.5 BC	3.51±0.11 BC	4.06±0.5 C	18.9±1 BC	5.11±0.22 C	11.7±0.9 BC	53.9±5 B	82.4±1.70 BC
	Min – Max	48.2-53.2	3.39-3.6	3.6-4.6	17.8-19.8	4.92-5.36	10.6-12.3	48.2-57.7	81-84.3
200	Mean ± S.D	53.8±1.9 B	3.89±0.31 AB	5.46±0.23 B	21.9±2 B	5.97±0.04 B	12.3±0.8 B	58.1±4.6 B	88.5±2 AB
	Min – Max	52.2-56	3.64-4.24	5.2-5.6	19.8-23.8	5.9-6.02	11.3-13	53.6-63	86.3-90.3
400	Mean ± S.D	58±0.11 A	4.8±0.09 A	7.33±0.3 A	25.2±3.1 A	6.82±0.19 A	13.6±0.7 A	75±0.3 A	92.5±3.82 A
	Min – Max	56.4-58.8	4.74-4.9	7-7.6	22.4-28.6	6.6-6.94	13-14.3	74.7-75.5	87-95.6
LSD values		**1.83**	**0.42**	**0.27**	**1.45**	**0.32**	**0.56**	**2.6**	**3.57**

* Mean values in the respective columns with different letters were significantly different at $p(<0.05)$ when compared among various GA_3 treatments; M= Mean; S.D= Standard deviation; Min-Max = Minimum – Maximum values

Table 3. Summary of post-harvest quality attributes.

Summary of stages at different concentration of treatments (mg/L)			SOD	POD	CAT
Control	Open brush bud	Mean ± S.D	8 ± 1	3.6 ± 0.7	1.73 ± 0.47
		Min – Max	7.3-9	2.9-4.3	1.19-2
	Fully opened flower	Mean ± S.D	16.7 ± 0.4	6.56 ± 0.3	2.23 ± 0.01
		Min – Max	16-17	6.3-6.9	1.19-2.3
	Onset of senescence	Mean ± S.D	3.5 ± 0.5	0.86 ± 0.2	0.8 ± 0.1
		Min – Max	3-4.1	0.6-1.1	0.7-0.9
25	Open brush bud	Mean ± S.D	10.1 ± 0.7	7.3 ± 0.2	2 ± 0.1
		Min – Max	9.5-11	7.1-7.5	1.99-2.2
	Fully opened flower	Mean ± S.D	17.1 ± 0.76	11.6 ± 0.5	2.29 ± 0.1
		Min – Max	16.5-18	11-12	2.24-2.3
	Onset of senescence	Mean ± S.D	5.3 ± 0.4	4.16 ± 0.8	1.19 ± 0.17
		Min – Max	5-5.8	3.3-4.9	1.09-1.4
50	Open brush bud	Mean ± S.D	12 ± 2	8.3 ± 0.3	2.25 ± 0.1
		Min – Max	10.4-14	8-8.6	2.2-2.29
	Fully opened flower	Mean ± S.D	19.7 ± 0.6	12.7 ± 0.6	2.35 ± 0.01
		Min – Max	19.2-20	12-13	2.324-2.37
	Onset of senescence	Mean ± S.D	5.6 ± 0.95	5 ± 0.7	1.42 ± 0.03
		Min – Max	4.6-6.5	4.2-5.5	1.38-1.45
100	Open brush bud	Mean ± S.D	13.6 ± 0.5	9.6 ± 0.3	2.3 ± 0.02
		Min – Max	13-14.3	9.3-9.9	2.28-2.4
	Fully opened flower	Mean ± S.D	22 ± 1.1	14 ± 0.3	2.38 ± 0.02
		Min – Max	21.3-23	13.8-14.5	2.37-2.41
	Onset of senescence	Mean ± S.D	7.16 ± 0.3	5.8 ± 0.1	1.53 ± 0.03
		Min – Max	6.9-7.6	5.7-5.94	1.5-1.56
200	Open brush bud	Mean ± S.D	15 ± 1	9.7 ± 0.4	2.34 ± 0.01
		Min – Max	14-16	9.2-10	2.2-2.5
	Fully opened flower	Mean ± S.D	22.1 ± 1.25	14.5 ± 0.5	2.44 ± 0.01
		Min – Max	21-23.5	14.2-15	2.43-2.45
	Onset of senescence	Mean ± S.D	8.7 ± 0.3	6.23 ± 0.4	1.57 ± 0.1
		Min – Max	8.4-9	5.9-6.8	1.5-1.62
400	Open brush bud	Mean ± S.D	16.6 ± 0.8	9.96 ± 0.05	2.38 ± 0.01
		Min – Max	15-18	9.5-10	2.36-2.39
	Fully opened flower	Mean ± S.D	23.9 ± 0.8	15.8 ± 0.76	2.47 ± 0.01
		Min – Max	23-24.5	15-16.5	2.44-2.49
	Onset of senescence	Mean ± S.D	9.36 ± 0.4	6.4 ± 1.2	1.62 ± 0.02
		Min – Max	8.9-9.7	5-7.2	1.6-1.65

M= Mean; S.D= Standard deviation; Min-Max = Minimum – Maximum values

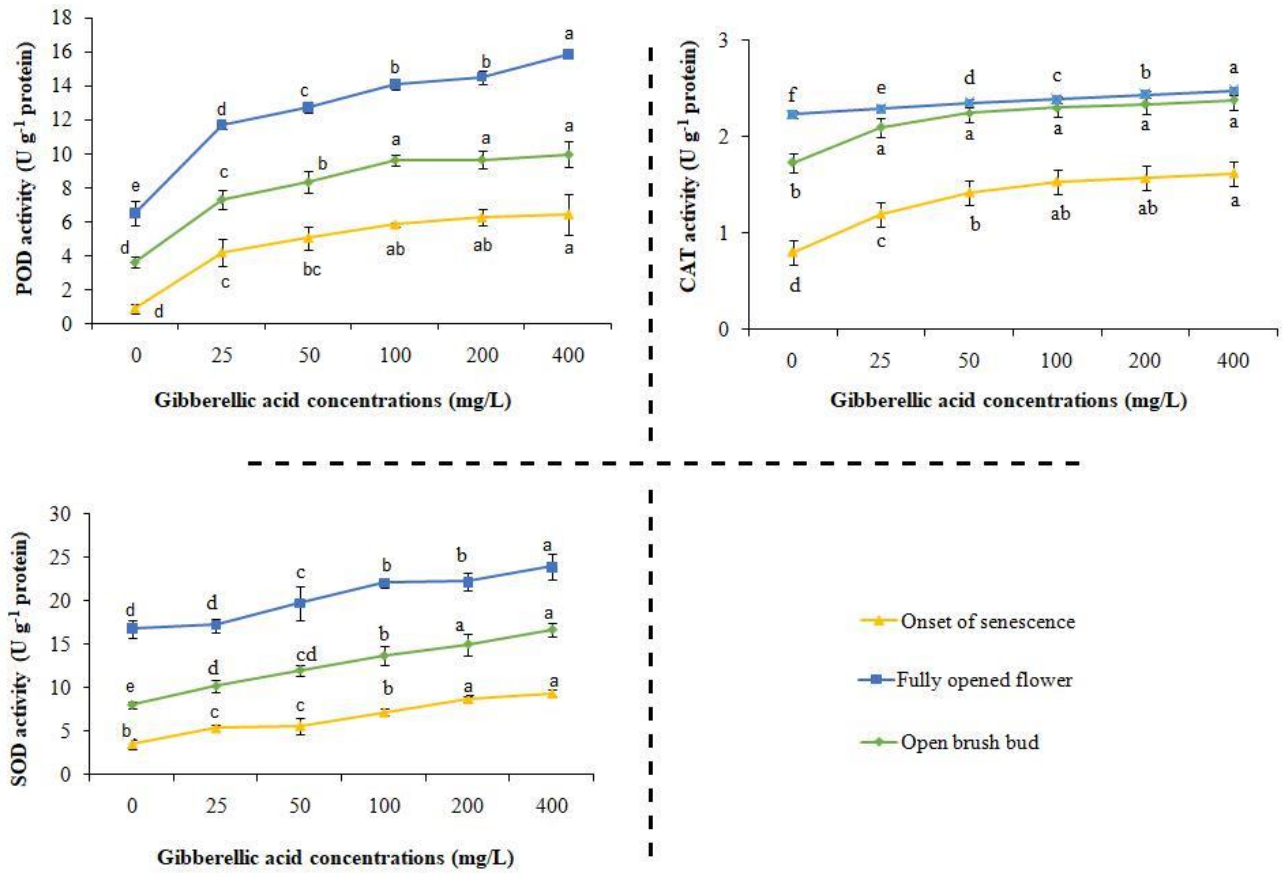

Fig. 1. Effect of GA_3 treatments on antioxidant enzyme activities (POD, CAT, and SOD) as observed in the carnation cut flowers.

Regression analysis between GA_3-treatments and observed parameters: We found a strong association between the applied doses of GA_3 and the studied parameters of carnation plants (Fig. 2). A strong association was found between increasing GA_3 concentrations and the number of side shoots of plants (R^2=0.94), followed by the percentage of flower opening (R^2=0.91), the number of leaf pairs (R^2=0.91) and leaf chlorophyll contents (R^2=0.84). A higher percentage of the variance of the number of days taken to first flower opening was also explained by the treatments.

Discussion

Plant growth parameters, floral traits, and post-harvest quality attributes following GA_3 applications: The current study seeks to explore the beneficial impact of foliar application of exogenous GA_3 on the carnation plant's post-harvest quality. The results were suggestive of several improvements in certain morphological parameters. The GA_3 treatments significantly improved the morphological traits (such as the plant height, number of leaf pairs, number of side shoots, and leaf chlorophyll contents of carnation plants) compared to the control plants (Table 1). The study also gained insight into underlying endogenous GA_3 synthesis, which likely mediates the synthesis of auxin (IAA) in tissues using the tryptophan; thus, it remained widely beneficial to improve the certain traits in the carnation plants. Since IAA is a promoter of cell division/elongation process via activation of cell wall loosening enzymes (Keyes *et al*., 1990; Arney & Mancinelli, 1995; Alhajhoj, 2017; Ali *et al*., 2019; Cornea-Cipcigan *et al*., 2020). Hence, the current results were also suggestive of dose-dependent improvements in the plants, included the percentage of flower opening (100%), prolonged vase life, reduced ethylene production, and enhanced membrane integrity. GA_3 is also a mediator of cell-wall expansion, which lowers the osmotic potential of cell solution. It increases the flow of water towards cells, promotes cell turgidity (Barai *et al*., 2014). These mechanisms improve meristem growth, stem elongation, and initiation of leaf primordia, thus influenced numerous traits in the carnation plants. As carnation frequently responds to gibberellins for flower induction (Cardoso *et al*., 2012; Goldberg-Moeller *et al*., 2013) thus in current findings, the apparent shortening in the number of days to flowering (at 400 mg/L GA_3), the maximized flower diameter, stalk length/diameter, fresh and dry flower weights were grossly improved. These factors represented again in the plant biomass, yield, fresh and dry weight, and flower diameter related to GA_3 spray, known to drive the light-harvesting and food assimilation (Hassanpouraghdam *et al*., 2011; Alhajhoj, 2017; Ali *et al*., 2019) Fig. 1.

The applied GA_3 caused a noteworthy advancement in cell division and elongation while it broadened the spectrum of gene expression imparting flower opening. We evidenced the GA_3-treatments antagonizing ethylene release from the floral tissues. Since, GA_3 is known to

initiate early flowering by inhibiting the abscisic acid functioning (Saeed *et al*., 2013; Sajid *et al*., 2016), enhancing nutrients uptake, carbohydrates filling in sinks, balancing C/N ratio in leaves and by activating the hydrolyzing enzymes (Cardoso *et al*., 2012; Ali *et al*., 2019). The reduced ethylene synthesis remained a noteworthy aspect, promoting flower opening, delaying the aging process (Doorn & Kamdee, 2014), and causing a higher percentage of flower's bloom (Table 3, Fig. 1) with 400 mg/L GA_3 concentration.

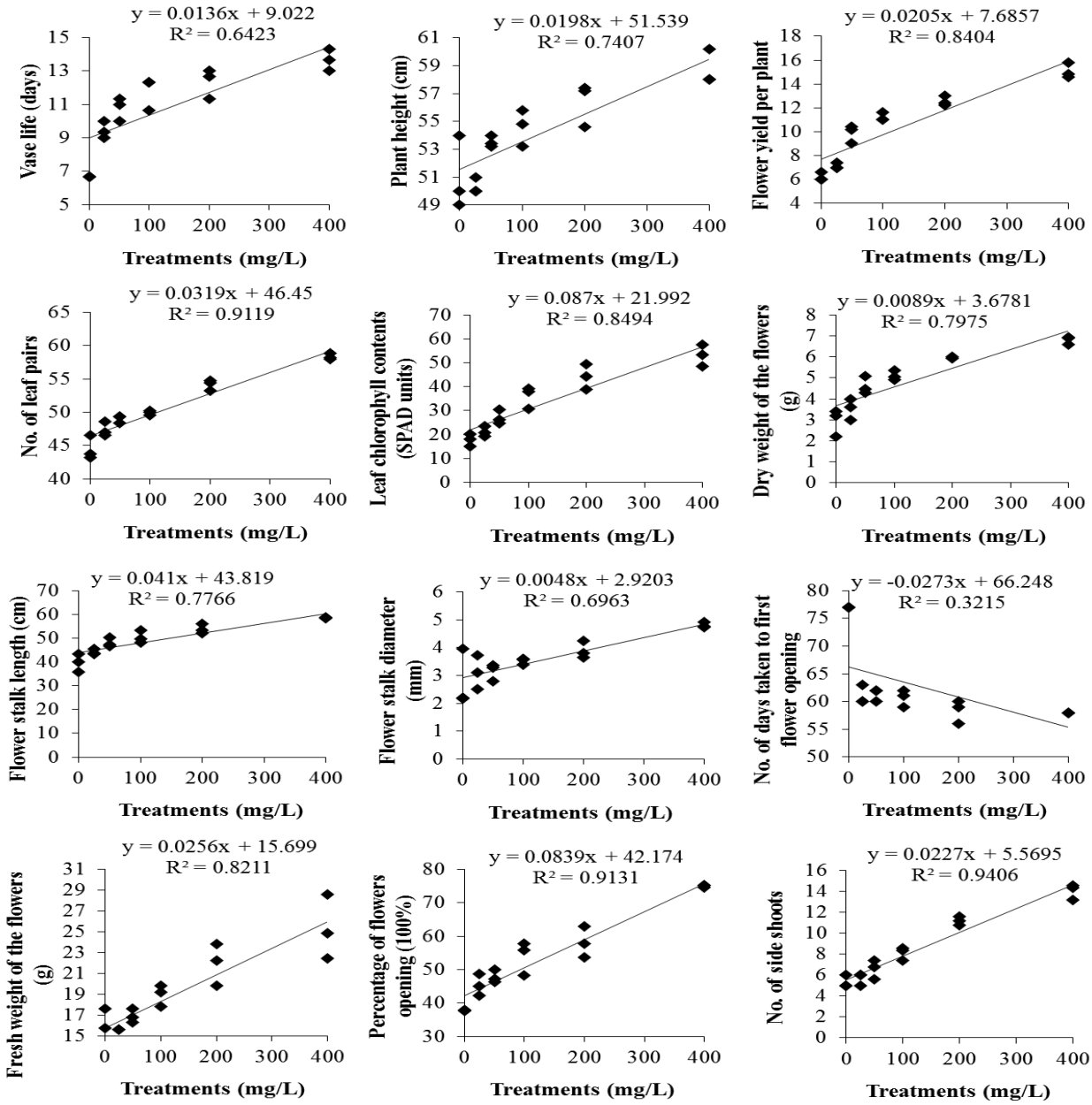

Fig. 2. Regression scatter plots of plant's physicochemical parameters as affected by different GA_3 treatments.

Table 4. Comparative analyses of antioxidants activity, membrane integrity, and ethylene synthesis among three flowering stages.

Antioxidants		Open brush bud (a)	Fully opened flower (b)	The onset of senescence (c)	Probability (*p*)-values		
					a *vs* **b**	**a** *vs* **c**	**b** *vs* **c**
SOD (U g^{-1} F. W)	Mean ± S.D	20.3 ± 2.8	12.6 ± 3	6.6 ± 2	<0.01	<0.01	<0.01
	Min – Max	16.3-24.5	7-18	3-9.7			
POD (U g^{-1} F. W)	Mean ± S.D	12.6 ± 3.1	8.1 ± 2	4.7 ± 2.3	<0.01	<0.01	<0.01
	Min – Max	6.3-16.5	2.9-10	6-7.2			
CAT (U g^{-1} F. W)	Mean ± S.D	2.4 ± 0.1	2.2 ± 0.28	1.4 ± 0.3	0.15	<0.01	<0.01
	Min – Max	2.2-2.5	1.2-2.4	0.7-1.7			

Min-Max = Minimum – Maximum values

Antioxidant enzymes activity: The foliar GA_3 spray (400 mg/L concentration) significantly influenced the SOD, POD, and CAT enzymes activities in and carnation cut flowers. We noted such outcomes in the course of different stages viz., open brush bud, and fully opened flower stage. GA_3 extends its stay in plastids increasing flower's longevity Tables 2-3 (Zhang, 2008), while antioxidant activities are reported in the higher state at open brush bud to fully opened flower stages, and decline at the onset of senescence. Previously, GA_3 is known to enhance senescence at a low level (Zhang, 2008). Senescence is often taken down by SOD and CAT activities in the carnation cut flowers (Cavaiuolo *et al*., 2013); because of the overproduction of hydrogen peroxide, that during oxidative stress causes imbalance and deprivation of NADPH. Such deprivation leads to the reduced synthesis of antioxidants such as CAT. Likewise, cell death may also occur following the perturbation of pro-apoptotic factors in mitochondria (Nel *et al*., 2006; Li *et al*., 2003), of which, a likely reason could be the catalysis of peroxide-free radicals regulating SOD and CAT genes. The synthesis of α-amylase and hydrolysis of starch contents into soluble carbohydrates thus enhances osmotic potential in flowers, maintaining the respiration process, and thereby keeping ethylene at its natural level (Asadi *et al*., 2014). In current results, the reduced activities of antioxidants at the senescence stage are attributable to the probable necrotic events. In present results, we evidenced that GA_3 treatments were beneficial for the integrity of the membrane, resulted in the betterment of cellular membrane as influenced by GA_3 mediated physiochemical improvements in the carnation plants. In general, the foliar spray strengthened the membrane integrity and enhanced vase life (Table 4).

Analysis of percentage-variance: The highest percentage of variance (~94 %) was observed in the development of side shoots in the plants (Fig. 2). Similarly, the GA_3 treatments also accounted for around 91 % of the variation in flower opening, significantly influencing the flower bloom. In past studies, early anthesis was associated with activation of florigen(s) via different pathways (Yu & Lin, 2006), including gibberellins. These are important for the activation of floral meristem identity gene LFY (LEAFY) to promote flowering (Duclos & Bjorkman, 2015). The increment in the flower weight and inflorescence succulence through cell wall tension remains a consequence of GA_3 mediation (Emami *et al*., 2011). At the gene level, gibberellin binds with growth suppressing DELLA proteins degrading them to initiate growth (Mutasa-Gottgens & Hedden, 2009). GA_3 causes the number and size of chloroplasts and ultra-structural morphogenesis of plastids to increase in number (Aftab *et al*., 2010), which explains that the observed chlorophyll contents, i.e., almost 84 percent of the variations were related to the increasing GA_3 concentration.

Conclusions

In conclusion, the foliar application of GA_3 grossly influenced the morphology, physicochemical, and floral traits in the carnation plant. The vase life of carnation cut flowers was prolonged, followed by the reduced ethylene synthesis and enhanced membrane integrity. Additionally, the augmentation of petal cells with antioxidant enzymes was also observed. The foliar spray of 400 mg/L GA_3 steadily improved the growth and vase quality of the carnation plant. A higher dosage of GA_3 was, therefore, influential on the morpho-physiological features and vase life attributes of carnation cut flowers.

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