

Biochemical changes associated with flower development in mini-potted carnation

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Abstract:

The present investigation was undertaken to determine the biochemical changes associated with flower development during the flowering season in the potted carnation cultivars of 'Lilac on purple' and 'Pink'. Ethylene production and the 1-aminocyclopropane-1-carboxylic acid (ACC) content of the flowers were observed to increase up to stage 5 (during the five stage of flowering) in both cultivars. Peroxidase (POD) and superoxide dismutase (SOD) showed three distinct phases: a low activity during the initial stages of flowering, a sustained activity during the mid-developmental stages, and accelerated activity at the full bloom. A significantly high activity of POD was observed in both cultivars during the fully open flower stage. The catalase (CAT) activity in 'Lilac on purple' was high during the 4th or 5th stages. However, no significant differences in CAT were observed in 'Pink'. H₂O₂ content increased with age, with the highest observed at the last stage. Finally, the sugar (Sucrose, Glucose, and Fructose) content in the tissues of the petals increased during the flower opening stage. The findings suggest that the regulation of flower development in mini-potted carnation may be linked to the antioxidant metabolism and the sugar status of the petal tissues.

Keywords: Fructose, Ethylene, Glucose, Peroxidase, Superoxide dismutase, Sucrose.

Introduction:

Carnations (*Dianthus caryophyllus* L.) have long been grown as a cut flower while their presentation as a potted plant is more recent and follows the development of dwarf species (Banon *et al.*, 2002). The developing flowers act as a sink and the rate of carbohydrate flow from the source to the sink depends on the rates of the activity of such enzymes as invertase. The developmental process is characterized by several phases including differentiation, cell division, cell enlargement, and senescence (i.e., near death). Much of the petal growth is associated with the result of cell enlargement (Sood *et al.*, 2006). The phyto-hormone ethylene is the primary regulator of floral senescence in a wide range of plant genera (Woltering and van Doorn, 1988). In sensitive species, petal senescence is associated with an increase in endogenous ethylene production while exposure to exogenous ethylene greatly accelerates the process (Macnisha, 2010). Petal senescence has been shown to be genetically programmed and involves degradation of proteins, lipids, carbohydrates, and nucleic acids (Eason *et al.*, 2002; Wagstaff *et al.*, 2002; Hoeberichts *et al.*, 2005; Zhou *et al.*, 2005; Eason, 2006; Price *et al.*, 2008; van Doorn and Woltering, 2008; Shibuya *et al.*, 2009). Petal senescence has been found to be accompanied by an

increase in the activity of catabolic enzymes, ion leakage, and nuclear fragmentation. This is all directed towards mobilization of nutrients from petals to other parts of the plant such as the developing ovary (Halevy and Mayak, 1979; Xu and Hanson, 2000; van der Kop, *et al.* 2003; Zhou *et al.*, 2005; Rogers, 2006; van Doorn and Woltering, 2008). In most cases, the flower is the organ with the shortest longevity varying greatly among different species (Halevy and Mayak, 1981). In *Narcissus tazetta* 'Kashmir Local', the sugar (reducing and total) content increases during the flower development to decline thereafter during senescence (Gul *et al.*, 2015). The soluble carbohydrates showed an increase in *Ranunculus asiaticus* petal tissues during the process of floral development from bud to fully open bloom, after which a declining trend was found during senescence (Shahri and Tahir, 2012). In *Dianthus chinensis* soluble proteins and sugar fractions reportedly increased with flower opening and showed a decrease as the senescence progressed (Ahmad Dar *et al.*, 2014). The present investigation was conducted to study the changes in potted carnation, 'Lilac on purple' and 'Pink', occurring during their flower development. The knowledge thus gained can be ultimately exploited to improve the postharvest performance of this flower.

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Material and Methods:

Cuttings of potted carnation (*Dianthus caryophyllus* L.) 'Lilac on purple' and 'Pink' cultivars were procured from a commercial grower in Pakdasht, Varamin, Iran. The cuttings 10 cm in height were placed in boxes filled with perlite. Root formation took about four to five weeks at 18 to 20 °C. The rooted cuttings were transplanted into plastic pots (1.5 L) already filled with a mixture of peat and perlite (3:1, v/v) and placed in a greenhouse with temperatures ranging from 20 to 25/10 to 15°C (day/night) and a relative humidity ranging from 50 to 60%. Flowers of both cultivars were collected during the flowering stage.

Stages of flower development were determined according to the scale of Goszczyńska and Rudnicki (1983) as follows (Fig. 1):

Stage 1. Tight buds, slightly visible petal color.

Stage 2. Petals partially open.

Stage 3. Flowers open to 1/3 of the final size.

Stage 4. Flowers open to 2/3 of the final size.

Stage 5. Fully open flowers with most of the petals horizontally oriented.

Measurement of ethylene production: One flower at each stage of flower development was cut directly below the calyx and placed in a closed jar (250 ml) at 23 °C to measure the ethylene produced. After two hours, 1 cm³ of the gaseous mixture of each glass was injected in a gas chromatograph (Shimadzu Gas Chromatograph) equipped with an activated alumina column fitted in a flame ionization detector. Nitrogen was used as the carrier gas (Yakimova and Woltering, 1997). The amount of ethylene was reported as nL g⁻¹FW h⁻¹.

Extraction and analysis of ACC: In 4 ml of 5% sulfosalicylic acid (SSA) solution, 2 g of crushed and frozen tissue (petal) was homogenized and centrifuged for 10 min at 3,090 × g in a pre-cooled centrifuge at 4 °C. ACC was assayed essentially as described by Bulens *et al.* (2011). Briefly, 0.4 ml of 10 mM HgCl₂ was added to 1.4 ml of the extract in a 9 ml vial and immediately sealed with a serum cap. Approximately 0.2 ml of the NaOH/NaOCl (2:1, v/v) mixture was injected into the vial through the serum cap. The mixture was vortexed for 5 seconds and allowed to react for 4 min on melting ice. The sample was vortexed again for 5 seconds to release all the ethylene into the vial headspace. Then, 1 cm³ of the gas sample was removed for ethylene determination by gas chromatography (GC). ACC content was calculated from the ethylene produced according to the method described in Bulens *et al.* (2011).

Peroxidase (POD; EC 1.11.1.7): For peroxidase assay, petals (100 mg FW) were crushed in a phosphate buffer (0.1 M, pH=7) containing 15% (w/w) PVPP, 2 mM EDTA, and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 10,000 × g for 20 minutes and the supernatant was collected for POD assay. Peroxidase activity was determined following o-dianisidine oxidation in the presence of H₂O₂ at 470 nm (Aebi, 1983). The protein extract was assayed using

the method described in Bradford (1976). All the enzyme activities and protein concentrations were quantified using a spectrophotometer (6405 UV/Vis, Jenway, England) at appropriate wavelengths.

Superoxide dismutase (SOD, EC 1.15.1.1): SOD activity was assayed using the procedure described in Beauchamp and Fridovich (1971). Briefly, the reaction mixture was prepared by mixing 0.1 mM nitrobluetetrazolium, 0.1 mM EDTA, and 50 μM xanthine and xanthine oxidase in 50 mM potassium phosphate buffer (pH=7.8). One unit of SOD was defined as the amount of enzyme that inhibits by 50% the control rate (0.025 units of absorbance at 550 nm min⁻¹) (McCord and Fridovich, 1969).

Catalase (CAT; EC 1.11.1.6): Catalase activity was measured using the Aebi (1984) method with slight modifications. Briefly, the activity was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 15 mM H₂O₂, up to 100 μl of the homogenate (7 mg protein ml⁻¹) with 0.2% (v/v) Triton X-100 in 50 mM potassium phosphate buffer (pH=7) and a requisite volume of the serum sample. The molar extinction coefficient of 43.6 M cm⁻¹ was used to determine catalase activity. The specific activity was calculated and expressed as μmoles/min/mg of the total protein.

Determination of sugar (Sucrose, Glucose, and Fructose) content: Each dried petal sample was extracted with 2 mL of 80% (v/v) ethanol at 80 °C for 20 minutes. The homogenates were centrifuged at 15,000×g for 10 minutes to give the ethanol-soluble and ethanol-insoluble fractions. The ethanol soluble fractions were pooled and evaporated to dryness using a concentrator. The mixed standard, or sample (20 μL), was measured using high performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan). A refractive index detector (RID-10A; Shimadzu) was used to quantify sugar content following separation with a Shim-pack SCR-101C column (Shimadzu) at 80 °C (Pharr and Sox, 1984; Wang *et al.*, 1999).

Experimental design and statistical analysis: This experiment was conducted in a completely randomized design with four replications in a factorial arrangement. Results were analyzed using SAS (version 6.12, SAS Institute Inc., Cary, NC, USA) software. Mean comparisons to identify significant differences between treatments were performed using the Duncan's test.

Results:

Ethylene production and ACC content: Analysis of variance revealed that cultivar, developmental stages, and their interactions had significant effects on ethylene production and ACC content of the flowers, which increased until stage 5 in both cultivars (Table 1). Ethylene production and ACC in 'Pink' were significantly greater than those in 'Lilac on purple' (Fig. 2A, B).

Antioxidant Metabolism: Analysis of variance revealed that cultivar, developmental stages, and their



Figure 1. Stages of flower development in potted carnation 'Lilac on purple' (Right) and 'Pink' (Left).

Table 1. Variance analysis of the studied characteristics in potted carnation during different developmental stages of flowers

s.o.v.	df	Ethylene	ACC	POD	CAT	SOD	Glucose	Fructose	Sucrose
Cultivar (A)	1	113.90**	15.62**	19.46**	0.10**	92.11**	4.22**	0.19 ^{ns}	0.12 ^{ns}
Stage (B)	4	156.06**	9.86**	59.86**	0.26**	126.03**	24.06**	81.85**	26.36**
A*B	4	8.50**	1.94**	3.28	0.07**	3.48**	0.16 ^{ns}	0.18 ^{ns}	0.03 ^{ns}
Error	27	0.89	0.13	0.52	0.001	0.27	0.53	0.56	0.07
C.V (%)	-	18.68	25.59	23.70	7.43	8.56	12.50	5.20	6.35

ns, * and **: Non-significant and significant at the 5 and 1 percentage probability levels, respectively.

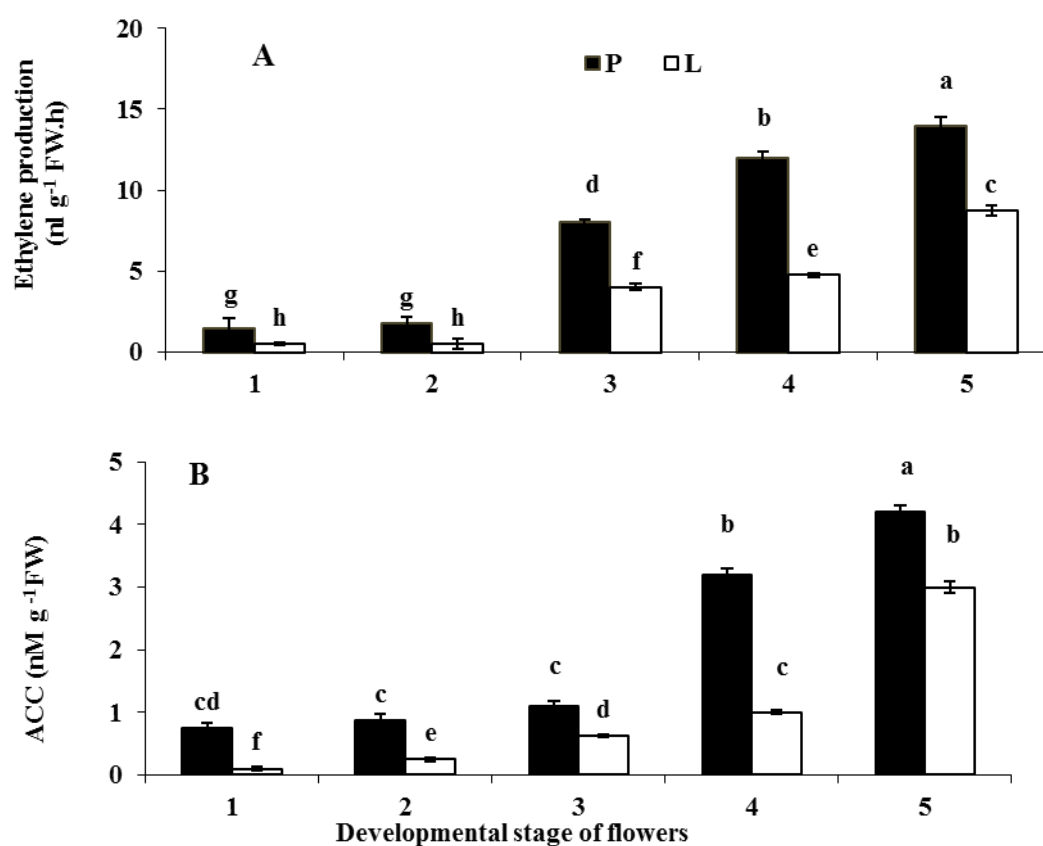


Figure 2. Changes in ethylene production (A) and ACC content (B) in the petals of potted carnation 'Lilac on purple' (□) and 'Pink' (■) during the different developmental stages of flowers. Different letters above the bars indicate significant differences at $p < 0.05$.

interactions had significant effects on the enzyme activity (Table 1). POD and SOD showed three distinct phases: a low activity during the initial stages of flower growth, constant levels of activity during the mid-

developmental stages, and high levels of activity during the full bloom (Fig. 3A, C). Significantly higher activities of peroxidase (POD) were observed in both cultivars during the fully open flower stage (Fig. 3A).

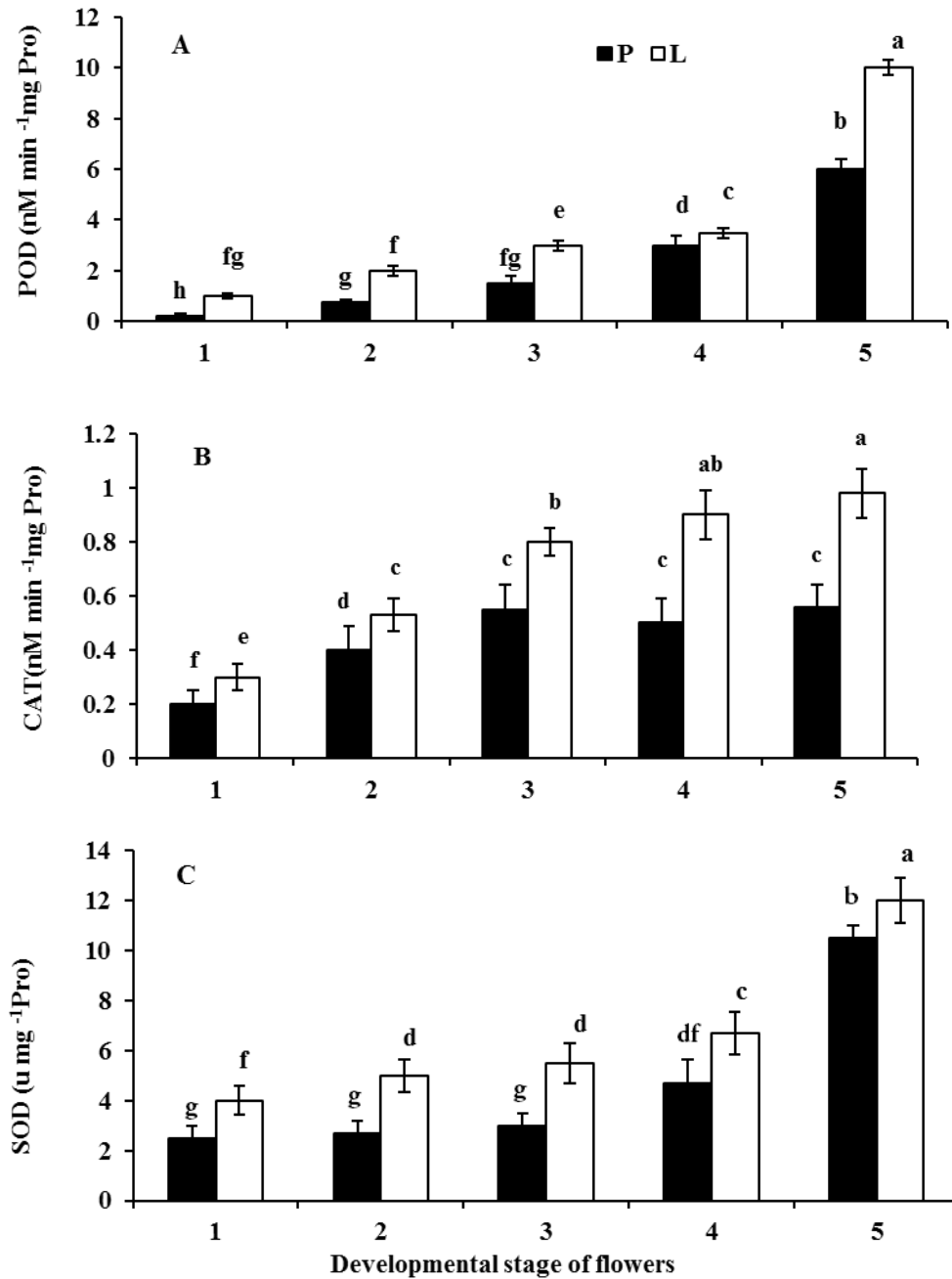


Figure 3. Changes in the levels of POD (A), CAT (B), and SOD (C) activities in petals of potted carnation 'Lilac on purple' (□) and 'Pink' (■) during the different developmental stages of the flowers. Different letters above the bars show significant differences at $p < 0.05$.

The CAT activity in 'Lilac on purple' was high during the stages 4 or 5. However, no significant differences in CAT activity were observed among the stages 3, 4, and 5 in 'Pink' (Fig. 3B). SOD activity increased significantly in both cultivars during the full bloom stage. H_2O_2 content increased with age, the highest observed at the final stage of sampling (Fig. 4).

Soluble Sugars Content: Flowers at all development stages contained three soluble sugars; namely, fructose with the highest concentration, glucose, and sucrose. Glucose and fructose levels of petals increased significantly as the flowers grew.

However, sucrose levels did not exhibit the same rapid increase but either remained constant or decreased during flower development (Table 2).

Discussion:

Petal growth is closely associated with cell enlargement (Sood *et al.*, 2006). Its senescence has been shown to be genetically programmed and involves degradation of proteins, lipids, carbohydrates, and nucleic acids (Zhou *et al.*, 2005; Eason, 2006; Price *et al.*, 2008; van Doorn and Woltering, 2008; Shibuya *et al.*, 2009). Ethylene is the primary regulator of floral senescence in a wide

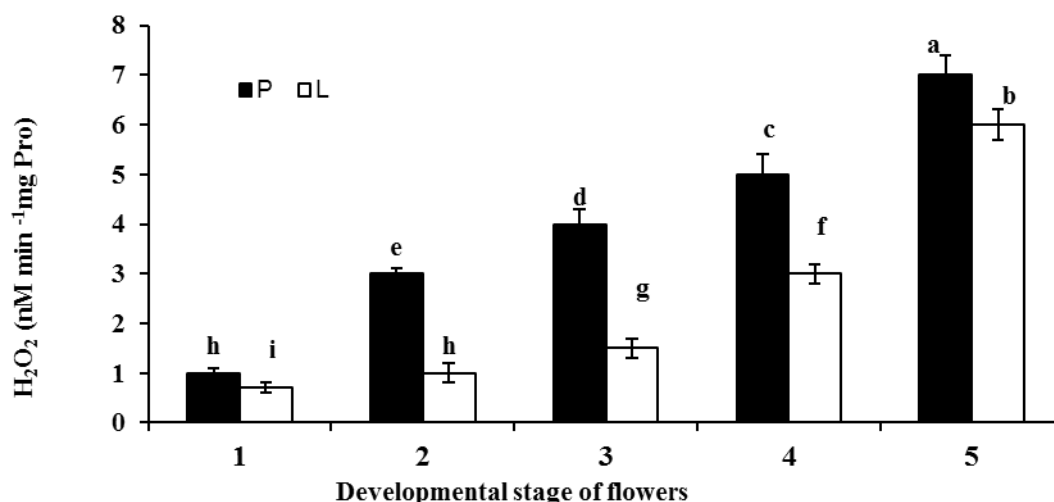


Figure 4. Changes in H₂O₂ content in the petals of potted carnation 'Lilac on purple' (□) and 'Pink' (■) during the different developmental stages of flowers. Different letters above the bars show significant differences at $p < 0.05$.

range of plant genera (Woltering and van Doorn, 1988). In this study, ethylene production and ACC content of the flowers were observed to increase until stage 5 in both cultivars. Increasing ACC content of the petals coincided closely with ethylene production in flowers. Petal senescence is associated with an increase in endogenous ethylene production; the process is, however, greatly accelerated as a result of exposure to exogenous ethylene (Macnisha, 2010). In this experiment, SOD activity was observed to increase significantly during the full bloom stage in both 'Pink' and 'Lilac on purple' cultivars. Sood et al. (2006) reported a highly significant antioxidant enzyme activity in *Rose damascene*. They claimed that the activity is even significantly higher during the early and full bloom stages.

A high level of H₂O₂ was observed during stage 5 in both cultivars investigated in the present study (Fig. 4). This was attributed to the fact that plants possess a well-defined enzymatic antioxidant defense system for protection against such reactive oxygen species (ROS) as H₂O₂, OH⁻, and O₂⁻ (Mates, 2000). Larrigaudiere et al. (2004) demonstrated that ethylene was involved in ROS pathways. During both the full bloom stage and the senescence, free radicals are overproduced, which may lead to damages and, thereby, to cell death. The low H₂O₂ content until stage 4 may be due to the lower levels of ethylene produced and the scavenging of H₂O₂ by SOD and POD enzymes. Hence, it may be concluded that although there were high antioxidant enzyme activities to remove ROS, they were not enough to protect the cells from being damaged. The capacity of the anti-oxidative defense system is enhanced at the onset of senescence but the imbalance between ROS production and antioxidant defense ultimately leads to oxidative damage (Macnisha, 2010). Among the different ROS, only H₂O₂ is relatively stable and capable of penetrating through the plasma membrane as

an uncharged molecule. In addition to being a toxicant, H₂O₂, has been regarded as a signaling molecule and a regulator of the expression of certain genes in the cell. Moreover, it acts as signal triggering a transduction pathway towards plant cell death. This was observed in experiments with soybean cell cultures in which a short pulse of H₂O₂ was sufficient to activate a hypersensitive cell death mechanism (Levine et al., 1994). Increasing peroxidase, superoxide dismutase, and catalase activities observed in the present study might reflect a similar process of oxidative stress in which these enzyme activities are implicated as part of the antioxidant response against H₂O₂.

The flowers at all stages of development exhibited three soluble sugars; namely, fructose (with the highest concentration), glucose, and sucrose. All plant parts depend on carbohydrates for their growth as they provide energy and the building blocks for growth processes (Ranwala and Miller, 1998.). Flower maturation and senescence have been found to be accompanied by a decline in the total soluble carbohydrate content in various flowers such as carnations, *Hemerocallis*, *Iris*, and *rose* (Nichols, 1973; Paulin and Jamain, 1982; Gulzar et al., 2005; Reid, 2005). Our findings clearly demonstrated that the opening of potted carnation flowers depended drastically on the import of carbohydrates. Shahri et al. (2011) showed sugars and soluble proteins in the petal tissues of *Helleborus orientalis* Lam. cv. *Olympicus* increased during flower opening but declined thereafter during the senescence. In another study, Shahri and Tahir (2012) found that the soluble carbohydrates in the petal tissues of *Ranunculus asiaticus* increased during the whole floral development from budding to the fully open bloom, followed by a declining trend during the senescence. In *Narcissus tazetta* 'Kashmir Local', the sugar (both reducing and total) content reportedly increased during flower development but declined

during the senescence (Gulet *et al.*, 2015). It may, thus, be concluded that the great deal of antioxidant enzyme

activity going on in plants to remove ROS is not enough to protect the cells against their likely damages.

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