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LONGEVITY AND SOME METABOLIC EVENTS IN POST-HARVEST SPRAY-CARNATION (*D. CARYOPHYLLUS F. SPRAY,* HORT.) FLOWERS

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Summary. Senescence and stresses have been documented to promote ethylene synthesis in ethylene-sensitive flower such as carnations. Thus the inhibition of ethylene evolution might lead to activation of other metabolic reactions. Present experiments were undertaken with cut at bud stage spray-carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Regina and cv. Naslada. Tested cultivars are a new breeding result at Institute of Floriculture, Sofia. After harvest treatments with AOA and sucrose were applied using AOA as senescence retarding agent. The goal was to trace how proline content and α-amylase were affected when ethylene synthesis was inhibited. Considerable extension of vase-life (about 128% over the control) and bud opening to fully open flowers were established in response to AOA and AOA+sucrose treatments.

A stimulation α-amylase activity was noticed at the beginning of post-harvest petal growth. In response to AOA treatment the activity of α-amylase and the content of free proline remained on a lower level which indicated less exhibited stress reaction and this was associated with a retardation of senescence processes. The studied metabolic events showed a specificity of cultivar behaviour.

Key words: α-amylase, senescence, spray-carnation, proline

Abbreviations: AOA – α-amino-oxiacetic acid

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Introduction

Each step in the life span of post-harvest flowers is associated with a number of coordinated biochemical, physiological, hormonal and structural changes that are strongly modulated by the fluctuations of environmental factors and stressors of biotic and abiotic origin. In general, the senescence of ethylene-sensitive flowers, such as carnations, is associated with a loss of membrane integrity, climacteric rise of respiration and enchanced ethylene synthesis (Mayak and Faragher, 1986; Larsen et al., 1993; Tang et al., 1994). AOA has been found to effectively delay petal wilting of carnations through an inhibition of ethylene biosynthesis on ACC synthase level (van Doorn and Woltering, 1991). There is ample evidence indicating that changes in the activity of several enzymes functioning during plant development play an important role during organ senescence (Salunkhe, 1990). It has been demonstrated that active degradation of starch occurred more intensively in senescing and stressed tissues where an enhanced induction of α-amylase was observed (Koizuka et al., 1995; Yakimova, 1997). Recently it has been suggested that in stress situations cells require more sugars to fulfil the energy and carbon needs for the defensive response to stresses (Koizuka et al., 1995). Since the cut flowers suffer from an energy deficiency, and are susceptible to different stresses, the demand for hexoses in petals might be satisfied partially by the hydrolysis of starch. Moreover, according to Hammond (1982) and Tirosh and Mayak (1988) the activity of α-amylase plays an important role in the mechanism of petal opening and regulates the appearance of senescence syndrome.

The post-harvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amount of metabolic sugars are factors that affect the rate of senescence. Keeping the flower in vase solutions containing sucrose has been shown to extend their vase-life (Ho and Nichols, 1977).

In water- and otherwise stressed plants it has been reported that the amount of free proline increased rapidly and a role of proline in cell defence reaction has been suggested (Hsiao, 1973). Treatments which retard senescence have been shown to inhibit proline accumulation while agents that promote senescence promote proline accumulation (Wang and Kao, 1983). It has been established that proline being involved in the increase of cell resistance to water loss stabilising membrane phospholipide structures prevented electrolyte leakage (Mayak and Faragher, 1986). Moreover, proline has been suggested to act as a nontoxic osmotic solute and to have enzyme protective function (Rudolph et al., 1986).

Aim of the present study was to investigate the changes in α-amylase activity and the content of free proline in petals of cut spray-carnation flowers and to follow the dynamics of these two biochemical stress indicators excluding the ethylene-dependant events by applying AOA as ethylene inhibiting substance.

Materials and Methods

Plant material and treatments

The experiments were undertaken with cut spray-carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Regina and cv. Naslada. Tested carnation cultivars are a new breeding result in the Institute of Floriculture, Sofia and their special characteristics are high ornamentality and a resistance to *Fusarium oxysporum f. dianthi* (Atanassova and Buchvarova, 1995). Flowers were grown in the greenhouses following standard cultivation technique. The inflorescences were harvested at stage 2 of apical flower bud development, recut and the end of stems were dipped in solutions for blossoming and testing their vase-life. Stages of flower development were determined according to the scale of Gosczcynska and Rudnicki (1983) as follows: stage 1 – tight buds, petal colour invisible; stage 2 – "paint brush", 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, most of the petals are horizontally oriented. The flowers were kept in solutions at air temperature 21°C, RH 60% and light intensity 15 $\mu mol.m^{-2}.s^{-1}$ and 12 h photoperiod.

The continuos treatment was provided with water solutions of AOA (α-aminooxiacetic acid) and 4% sucrose and with a combination of these two ingredients. Water control was used for comparison. Longevity (vase-life) was determined at the wilting of more than 1/3 of the flowers in the inflorescences.

Enzyme assay

The activity of α-amylase (EC 3.2.1.1.) was estimated according to the modified method of Plummer (1988). The extract was prepared from 0.1 g of petals with K-phosphate buffer (pH 7.4), centrifuged at 90 g and the supernatant was used for determination of the enzyme activity. The reaction mixture contained substrate 1% starch and was incubated at 37 °C for 3 min. The method is based on the interaction of 3,5-dinitrosalicylic acid with reducing sugars over boiling water and the amount of the resulting substance was measured colorimetrically. α-Amylase activity was expressed in mg maltose.g^{-1} FW.

Proline content

Proline was extracted and its concentration determined following the method of Bates et al. (1973). Samples from petals were homogenised in 3% (w/v) sulfosalicylic acid and centrifuged. The supernatant fluid was treated with acetic acid and acid-ninhydrin, boiled for 1h and the absorbance was measured at 520 nm. Proline content was expressed in $\mu mol.g^{-1}$ FW.

Activity of α-amylase and proline content in intact flower buds are presented as values on day 0.

The data were processed statistically by Student's T-criteria at $p \leq 0.05$.

Results

Vase-life study

The vase-life and the degree of flower development of studied spray carnation cultivars are presented in Table 1. Results showed that the keepability of cv. Regina

Table 1. Effect of AOA and sucrose on the post-harvest behaviour of cut spray-carnation (*D. caryophyllus f. spray*, Hort.) flowers

Treatment	Vase-life (days)		Degree of bud opening at the wilting (scale)	
	cv. Regina	cv. Naslada	cv. Regina	cv. Naslada
Control – water	4.4±0.36	4.2±0.14	3	3
AOA 250 mg/l	11.9±1.12	12.3±0.87	5	5
AOA 250 mg/l + sucrose 4%	11.7±0.97	12.7±1.07	5	5
Sucrose 4%	6.6±0.17	7.1±0.21	4	4

The degree of bud opening is estimated according to the scale of Gosczcynska and Rudnicki (1983)
± = standard deviation from duplicated measurements from each replication (n = 5).

and cv. Naslada increased remarkably over control flowers (held in water) when the treatment was provided with holding solutions, containing AOA. The addition of sucrose to AOA did not affect vase-life bud development as compared to single AOA application. Control flowers exhibited a short vase-life and did not open. In the variant where sucrose was singly used only slight extension of the longevity was observed. The flowers in these experiments were harvested at bud stage. Because the development and growth of petals is carbohydrate-dependent the concentration of sucrose was determined high enough to allow bud growth and flower opening. Nevertheless, flowers did not develop sufficiently to reach commercial phase. Comparing these results to those with AOA treatment most probably in sucrose treated and control flowers a higher ethylene production had occurred thus preventing further flower growth. In general, sucrose has been found to be a factor that suppresses ethylene evolution (Paulin, 1986). Similar pattern of slow developmental rate and bad expressed senescence retardation was found in our earlier experiments with spray-carnations kept in sucrose solution (Yakimova, 1997). Since ethylene production was not measured we could only suggest that single sucrose treatment might not be effective for the studied spray-carnation cultivars. In addition, other data concerning vase-life and metabolic events in these flowers are not available yet.

Activity of α-amylase

The dynamics of α-amylase activity in both studied cultivars (cv. Regina and cv. Naslada) showed an increase on day 4 at all tested holding solutions in comparison to day 0 (Fig. 1 and 2). The augmentation of enzyme activity on day 4 was slightly

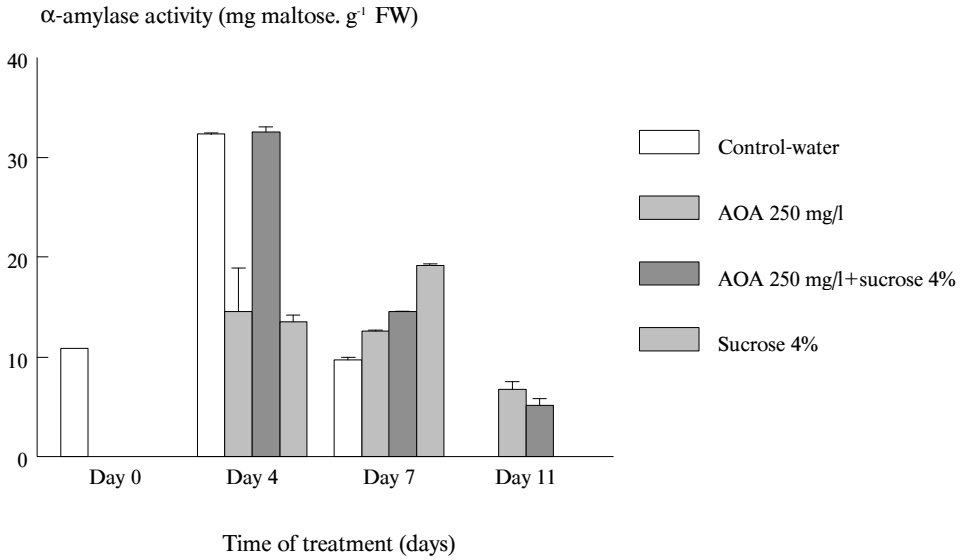

Fig. 1. Effect of AOA and sucrose on α-amylase activity in petals of cut spray carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Regina. Error bars represent SEM (n=5).

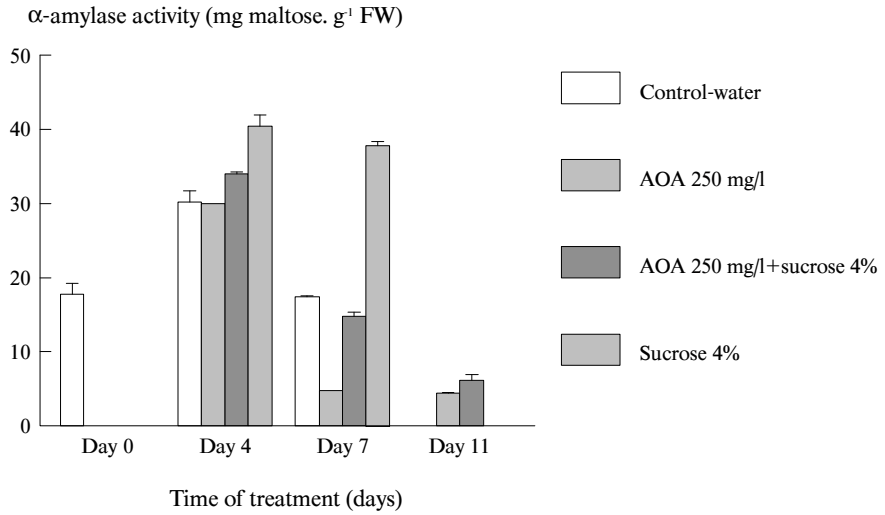

Fig. 2. Effect of AOA and sucrose on α-amylase activity in petals of cut spray carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Naslada. Error bars represent SEM (n=5).

pronounced in AOA treated cv. Regina flowers and did not change noticeably on day 7. In controls and solutions consisting of AOA+sucrose enzyme activity decreased toward the end of vase-life. An exception was observed in case of sucrose application to cv. Regina where a rise of α-amylase activity on day 7 was noticed. For cv. Naslada AOA treatment resulted in sharp inhibition of amylase activity on day 7 and similar level was monitored at wilting (day 11). The latest cultivar expressed high amylase activity on day 7 in the variant containing only sucrose.

Content of free proline

Proline content in petals of cv. Regina and cv. Naslada was obviously enhanced on day 7 in response to sucrose treatment (Fig. 3 and 4). Keeping the flowers of both cultivars in AOA+sucrose solution caused proline accumulation on day 11, being better expressed in cv. Regina. Proline content of control flowers was not considerably affected. Single AOA application did not influence remarkably the proline amount of the two studied cultivars.

Discussion

Senescence and stresses have been documented to promote ethylene synthesis (Hall, 1995). Thus the inhibition of ethylene evolution might lead to activation of other metabolic reactions in ethylene sensitive flowers. In the present experiments we used AOA as senescence retarding agent. The goal was to trace how proline content and α-amylase were affected when ethylene synthesis was inhibited.

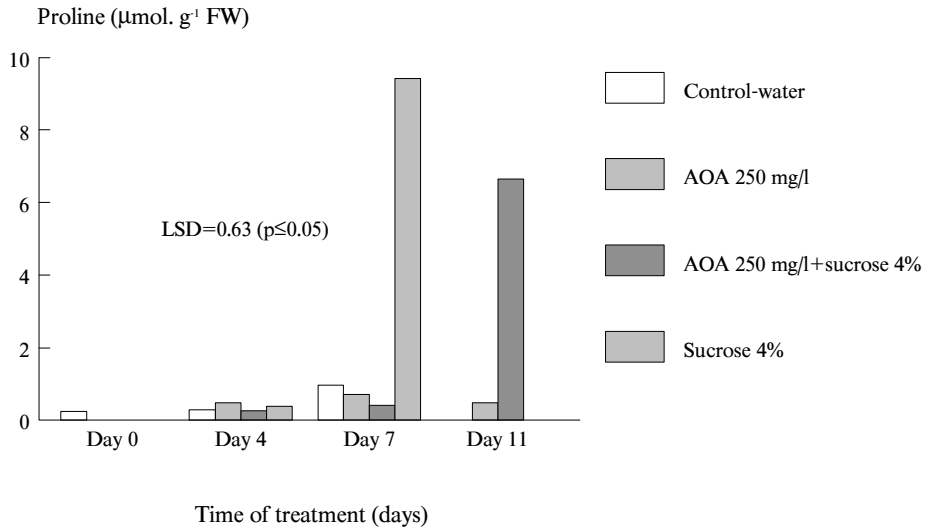

Fig. 3. Effect of AOA and sucrose on free proline content in petals of cut carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Regina.

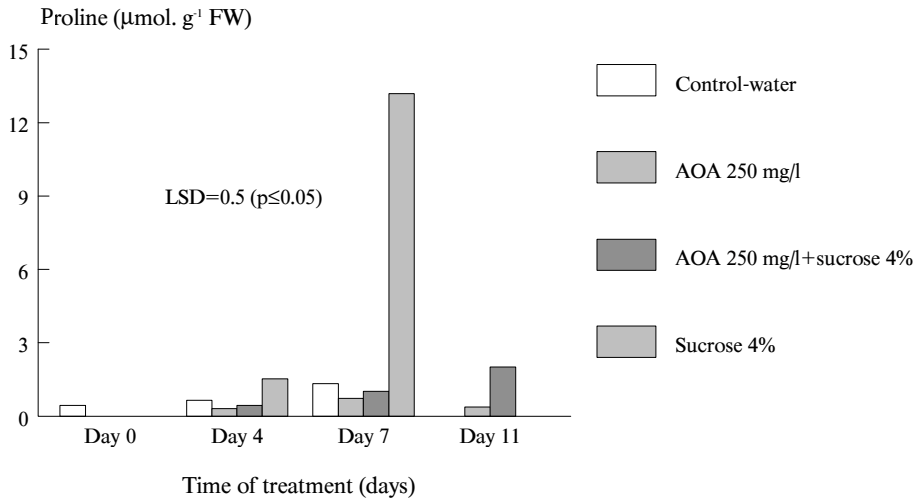

Fig. 4. Effect of AOA and sucrose on free proline content in petals of cut carnation (*D. caryophyllus f. spray*, Hort.) flowers, cv. Naslada.

After harvest the flowers are exposed to multiplied stress situations including mechanical, water, nutritional, temperature, chemical and pathogenic stressors. Regarding the view that amylolitic enzymes are stress-inducible (Dreier et al., 1995) and the content of free proline could be used as a stress marker (Karamanos, 1995) our results indicate that when ethylene production is suppressed by AOA petal tissues pronounced metabolic stress responds later and that results in extended longevity.

Petal growth contributes to flower opening and is a result of cell enlargement which needs substrates for energy, for a synthesis of membrane constituents and for a regulation of cell's osmotic potential. The enhancement of amylase activity measured on day 4 in the studied cultivars independently of the type of treatment coincided with our previous findings for cut carnation and rose flowers (Yakimova, 1997) where a stimulation of α-amylase occurred at the beginning of post-harvest growth of petals. The need of intensive starch degradation might be a possible explanation for the induction of α-amylase activity accompanying bud opening. Observed stimulation of α-amylase activity could also be part of an enhancement of defence mechanisms, leading to an increase in energy supply under stress conditions (Koizuka et al., 1995).

Lower α-amylase activity and lower proline amount were found in pre-senescence phase in response to AOA treatment and this was associated with retarded appearance of the senescence syndrome. We combined AOA with sucrose because the addition of sugars in vase solutions is essential for good flower development (Paulin, 1986). Sucrose feeding of cut spray carnations caused an acceleration of enzyme activity and proline accumulation in the petals at the end of vase-life. The development of buds when treated with sucrose solution was suppressed which might be due to growth

of some micro-organisms. This contributed to the increase of amylase activity and proline content established in the late senescence phase.

In contrast to our previous studies with standard carnations, in control flowers of the here studied spray carnations, cv. Regina and cv. Naslada (kept in water) proline content did not change remarkably. Similar reaction was found in our experiments with cut dahlias where proline showed certain changes in cv. Gelb Hirshgewaih, but not in cv. Otelo (Yakimova, 1997). To improve understanding of this phenomena further investigations are needed. They may provide a clue to better evaluation of the role of α-amylase and proline in post-harvest flower senescence.

Conclusions

Treatments with AOA or AOA+sucrose effectively retarded the longevity of cut spray-carnation flowers, cv. Regina and cv. Naslada. The same substances affected positively bud growth and allowed the flowers to reach fully open stage. It was established that in case of suppressed ethylene synthesis by AOA application, the activity of α-amylase and the level of proline remained lower, a fact indicating less pronounced stress-reaction and in accordance with delayed flower wilting. An acceleration of α-amylase activity, despite the treatment used, was found at the beginning of the post-harvest petal growth.

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