BIOLOGIA PLANTARUM 50 (4): 581-585, 2006

Changes in abscisic acid and flower pigments during floral senescence of petunia

A. FERRANTE*[1], P. VERNIERI**, F. TOGNONI** and G. SERRA***

*Department of Crop Science, University of Milan, Via Celoria 2, Milan, I-20133, Italy**
*Department of Crop Biology, University of Pisa, Pisa, I-56124, Italy***
*Sant'Anna School of Advanced Studies, Pisa, I- 56127, Italy****

Abstract

The present work was focused on abscisic acid (ABA) changes in three differently coloured petunias during flower development and senescence. The ABA content was studied in correlation with changes of flower pigments and other phytohormones. The variations of anthocyanins and endogenous hormones were induced by treatments with 1 or 2 mM amino-oxyacetic acid (AOA), 50, 100 μM thidiazuron (TDZ) and 50 μM 6-benzyladenine (BA). ABA content decreased during bud development and increased during senescence. The AOA reduced the anthocyanins content and avoided ABA increase, while the cytokinins (BA and TDZ) did not significantly affected anthocyanin contents but increased ABA content. TDZ doubled the ABA content compared to the control. However, the treatments did not affected flower life, confirming the secondary role of ABA during flower senescence.

Additional key words: anthocyanins, amino-oxyacetic acid, benzyladenine, carotenoids, cytokinins, *Petunia* × *hybrida*, thidiazuron.

Introduction

The plant hormone abscisic acid (ABA) influences numerous aspects of plant growth and development including embryo maturation, seed dormancy, fruit ripening, and water balance in response to environmental stresses (Zeevaart and Creelman 1988, Pandey *et al.* 2003/4, Purty *et al.* 2005). Exogenous applications of ABA accelerate the symptoms of flower senescence in carnation, rose and daylily flowers (Mayak and Halevy 1972, Mayak and Dilley 1976, Panavas *et al.* 1998). Endogenous content of ABA increased during senescence in several flowers (Panavas *et al.* 1998, Hunter *et al.* 2004) and this may be due to watersoaking or conversion of carotenoids to ABA (Eze *et al.* 1986, Milborrow 2001). In some flowers, ABA causes senescence through ethylene as inhibitors of ethylene production or action, preventing the response (Mayak and Dilley 1976, Nowak and Veen 1982, Wilhelmová *et al.* 2005). In other flowers, *e.g.*, daylilies, ABA presumably induces senescence independently of ethylene action, as the senescence of the flower is known to be ethylene independent (Panavas *et al.* 1998).

The interrelationship between ABA and other flower components such as carotenoids and pigments has been recently studied. In many organs, the interaction between ABA and anthocyanins has been clearly demonstrated. Exogenous applications of ABA affect pigments biosynthesis in many plants, inducing anthocyanins accumulation in strawberry fruits (Jiang and Joyce 2003), in cut snapdragon flowers and grapes (Sang *et al.* 1992, Jeong *et al.* 2004).

The aim of this work was to study the changes of flower pigments and ABA during flower development and senescence. Variations of flower pigments were induced using chemical treatments known to interact with anthocyanins accumulation.

Materials and methods

Petunia (*Petunia* × *hybrida* L.) flowers were grown in the greenhouse under natural conditions (April-August), at 28/18°C day/night temperatures. Three cultivars having different colour were used for the experiment: white

Received 15 December 2004, *accepted* 10 June 2005.
Abbreviations: ABA - abscisic acid; AOA - amino-oxyacetic acid; BA - benzyladenine; f.m. - fresh mass; TDZ - thidiazuron.
[1] Corresponding author; fax: (+39) 02 50316575, e-mail: antonio.ferrante@unimi.it

(cv. Primetime White), pink (cv. Dreams Appleblossom) and blue (cv. Ultra Blue). All flowers were harvested at fully opening stage, no later than 11:00.

Flowers were treated by placing their pedicels in the solution containing distilled water (control), 1 or 2 mM amino-oxyacetic acid (AOA, *Sigma*, Milan, Italy), 50 or 100 μM thidiazuron (TDZ, *Duchefa*, Milan, Italy), 50 μM benzylaminopurine (BA, *Sigma*). The effect of treatments was evaluated in postharvest room under the following conditions: photon flux density 15 μmol m^{-2} s^{-1}, 12-h photoperiod, 60 % RH and 20 °C temperature. The flower life was determined by daily observation and was considered ended at first symptom of senescence (wilting).

Petal samples (80 - 100 mg f.m.) were collected, weighed, immediately frozen in liquid nitrogen and stored at -20 °C until needed. Samples were extracted with distilled water (water: tissue, ratio 10:1) for 16 h at 4 °C in the dark. Quantitative analysis was performed on crude aqueous extracts using a solid-phase radioimmunoassay based on a monoclonal antibody (DBPA1) raised against free (S)-ABA, as described previously (Vernieri *et al*. 1991).

We investigated the presence of competitive interferences in crude aqueous extracts by HPLC fractionation using an LDC (*Shimadzu LC9*, Kyoto, Japan) instrument equipped with a UV absorbance detector operating at 254 nm. A column (∅ 15 cm × 6.35 mm) packed with *LiChrosorb RP 18* (*Merck*, Germany), 10 μm was used. The solvent flow was 1 cm^3 min^{-1}. The column was eluted as follows: 30 % methanol in water (0.05 M acetic acid) for 6 min; a linear gradient 30 - 50 % methanol for 20 min; 50 % methanol for 6 min; a linear gradient 50 - 100 % methanol for 15 min. Fractions of 2 cm^3 were collected, evaporated under vacuum, and the residue resuspended in 75 mM PBS buffer (pH 7). Each fraction was assayed in triplicate by immunoassay (RIA). The results showed the absence of significant competitive interferences, most of the immunoreactivity being located in the ABA fraction (data not shown).

Noncompetitive interferences were evaluated by internal standardization experiments. Aliquots of crude aqueous extract were added to standards, and the ABA quantities recovered were plotted against the amount of ABA added, in order to check the parallelism of the lines obtained. Results indicate the absence of noncompetitive interferences (data not shown).

Total carotenoids were extracted using 99.9 % methanol as solvent. Samples were kept in dark cold room at 4 °C for 24 h. Quantitative carotenoids determinations were carried out immediately after extraction. Absorbance was measured at 470 nm. Carotenoid concentrations were calculated by Lichtenthaler's (1987) formula. Anthocyanin contents were determined spectrophotometrically. Samples of the frozen tissue (100 mg) were ground in pre-chilled mortar and were extracted into methanolic HCl (1 %). Samples were incubated overnight at 4 °C in darkness. The concentration of cyanidin-3-glucoside equivalents was determined spectrophotometrically at 535 nm.

The data are reported in figures and tables as means with standard errors (SE). The data were subjected to one-way or two-way analysis of variance and the differences among treatments were analyzed by Bonferroni post-test ($P < 0.05$). Each treatment was composed of 6 replicate flowers.

Results

The three selected *Petunia* × *hybrida* cultivars had different flower life ranging from 3 to 6 d. The longest flower life was observed in white and blue petunias, while the shortest was observed in the pink petunia (Table 1). The blue petunia showed highest ABA and anthocyanin contents, while the highest content of carotenoids was observed in pink petunia. The white petunia had lowest amounts of ABA, carotenoids and anthocyanins.

Table 1. Flower life [d], ABA, carotenoid and anthocyanin contents [ng g^{-1}(f.m.)] in the petunia cultivars (blue, pink and white) determined at the fully open stage. Data were subjected to one-way analysis of variance and differences among cultivars were determined by Bonferroni post-test. Different letters denote significant differences at $P < 0.05$.

Parameters	Blue	Pink	White
Flower life	5.00± 0.45a	3.40± 0.25b	4.60±0.40a
ABA	36.74± 3.82a	26.29± 0.84b	14.60±0.50c
Carotenoids	1.18± 0.39a	1.40± 0.34a	0.27±0.09b
Anthocyanins	631.21±89.41a	269.14±17.43b	3.83±1.55c

Changes of ABA and pigment contents were also studied during the flower development stages. The ABA concentration was high at the bud stage and before the bud developed to flower, decreased during flower opening and reached the lowest value at the fully open stage. Than, it increased again during flower senescence (Fig. 1*A*). The ABA changes during flower development showed the same trend in all cultivars. The ABA content was correlated with flower colour at all stages. The ABA content in blue cultivars was highest in all stages considered, intermediate values were observed in pink petunias and lower values were found in white petunias (Fig. 1*A*).

Flower pigments, carotenoids and anthocyanins, had the same trend from the bud stage to the complete flower senescence (Fig. 1*B,C*). The higher values were found at bud stage and decreased during flower opening and slightly increased again at last stage, during flower senescence.

Treatments with AOA and cytokinins were performed to study the ABA changes induced by variations of the colour and the internal hormones equilibrium. These treatments were performed on pink petunias that had intermediate values of flower pigments and ABA

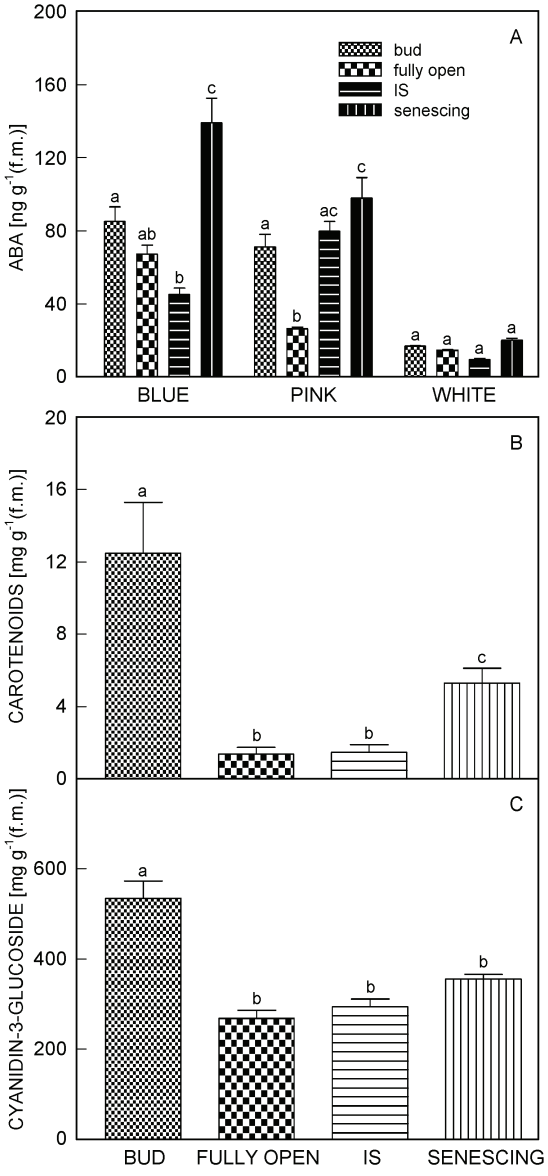

Fig. 1. *A* - Endogenous ABA content in corollas of three petunia cultivars placed in distilled water during their development (bud, fully open, IS - initial senescence, senescing); *B* - Total carotenoid content of pink petunia (cv. Dreams Appleblossom) flowers placed in distilled water during development; *C* - Anthocyanins content (expressed as cyanidin-3-glucoside equivalents) of pink petunia (cv. Dreams Appleblossom) flowers placed in distilled water during development. Values are the means ± SE of 6 flowers randomly harvested from the greenhouse. Data were subjected to one-way analysis of variance and differences among treatments were determined by Bonferroni post-test. Different letters denote significant differences at $P < 0.05$.

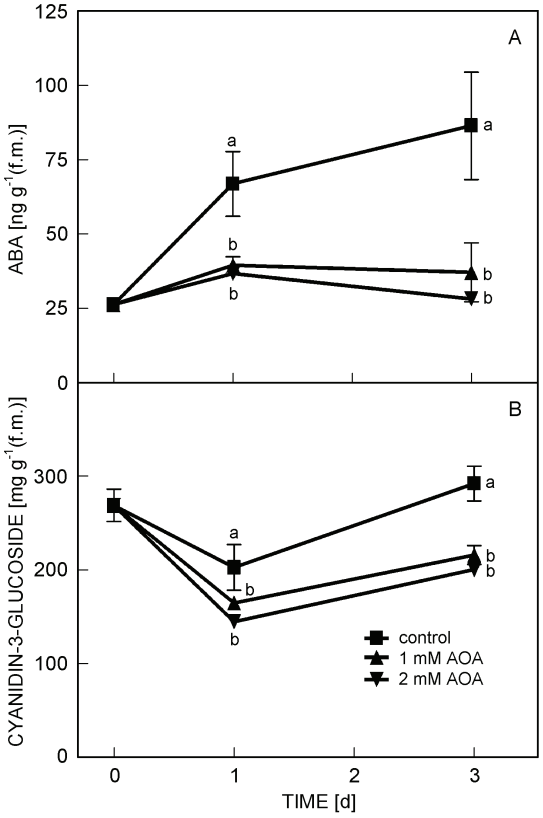

Fig. 2. Endogenous ABA content (*A*) and anthocyanins content (expressed as cyanidin-3-glucoside equivalents) (*B*) in pink petunia (cv. Dreams Appleblossom) flowers treated with distilled water (control), 1 mM or 2 mM AOA. Values are the means ± SE of 6 flowers randomly harvested from the petunia grown in greenhouse. Different letters denote significant differences at $P < 0.05$.

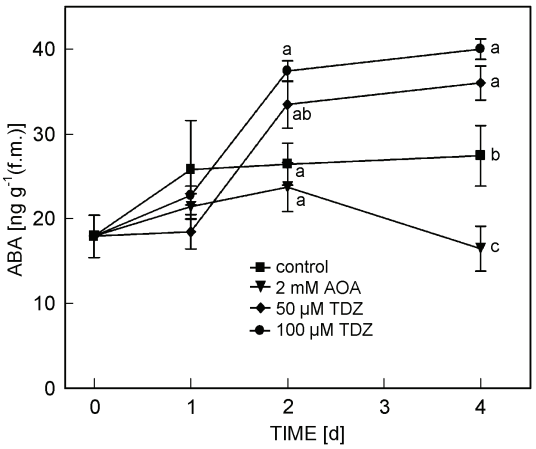

Fig. 3. Endogenous ABA content in pink petunia (cv. Dreams Appleblossom) flowers treated with distilled water (control), 2 mM AOA, 50 or 100 µM TDZ. Values are the means ± SE of 6 flowers randomly harvested from the petunia grown in greenhouse. Different letters denote significant differences at $P < 0.05$.

concentration. Applications of 1 mM or 2 mM AOA avoided the ABA increase during flower senescence; the values were constant during the whole flower life (Fig. 2*A*), keeping the endogenous ABA concentration at the same level of fully open flowers. The AOA also extended the flower life of 2 - 3 d compared with the untreated flowers, in fact the control flowers showed symptoms of senescence after 3 d, while the AOA treated flower had a vase life of 5 - 6 d. The petunia flowers treated with AOA underwent discoloration and the anthocyanins content slightly decreased (Fig. 2*B*). Applications of cytokinins, such as BA or TDZ, did not significantly affect neither colour change nor flower longevity, but increased the ABA concentrations (Fig. 3). The ABA concentration in flowers treated with 50 or 100 μM TDZ doubled the concentration during the 4 d of vase life. In fact, ABA at the beginning of experiment was 16 ng g^{-1}(f.m.) and increased up to 40 ng g^{-1}(f.m.) when flowers reached the initial senescence stage (Fig. 3). The treatments did not affect the carotenoids concentrations in petunias (data not shown).

Discussion

The ABA content during flower development has a well-defined trend that is common in many plant species such as squash flowers, four o'clock flowers, daylily and daffodil (Panavas *et al*. 1998, Hunter *et al*. 2004). The flower pigments have a similar trend and may interfere with ABA concentration (Table 1). The interaction between ABA and anthocyanins was also already demonstrated in other plant organs. The applications of ABA increased anthocyanins accumulation in flowers, fruits and seeds (Sang *et al*. 1992, Jiang and Joyce 2003, Jeong *et al*. 2004). Moreover, the inhibition of ABA biosynthesis reduced anthocyanins accumulation during maize kernel development (Kao *et al*. 1996).

The AOA was used for reduction the anthocyanins accumulation. The ability of AOA to affect the anthocyanins biosynthesis was demonstrated in buckwheat hypocotyls through the inhibition of phenylpropanoid pathway. The application of 0.5 mM AOA reduced anthocyanins production by 87 % (Amrhein 1979). Petals discoloration was also observed on cut rose flowers treated with AOA. Treatments with AOA inhibited ABA increase and induced corolla discoloration of pink petunias. This result may confirm the inter-relationship between anthocyanins and ABA. The longer flower life induced by AOA might be attributed to ethylene inhibition. In fact, AOA is also an ethylene biosynthesis inhibitor and is commonly used as postharvest treatment for preserving cut flowers. The AOA precisely inhibits the ACC synthase that converts the S-adenosylmethionine to 1-aminocyclopropane-1-carboxylate (ACC). In petunias sensitive to ethylene, the AOA treatments delayed flower senescence (Singh *et al*. 1992).

Treatments with BA or TDZ were performed for induction of anthocyanins accumulation. The effect of cytokinins on flavonoids pathway and anthocyanins accumulation was well demonstrated in *Arabidopsis* plants (Deikman and Hammer 1999, Wade *et al*. 2003). Treatments with BA applied to *Arabidopsis* increased the mRNA accumulation of chalcone synthase, a key enzyme of anthocyanins biosynthesis, as soon as after 2 h (Deikman and Hammer 1999).

The applications of cytokinins increased the ABA biosynthesis, but did not affect the anthocyanins accumulation in petunia flowers. Moreover, the increase of ABA induced by cytokinins did not accelerate the senescence. Analogous results were observed in transgenic petunia that overproduces cytokinins. They had lower amount of ABA and corollas senescence was delayed compared to wild type flowers (Chang *et al*. 2003).

The increase of endogenous ABA in petunia flowers during flower senescence does not seem to be correlated with the flower life. These results were confirmed by comparison of the flower life and ABA content of the three petunias used and by the AOA treatments that lowered ABA and anthocyanins content without affecting the flower life.

The AOA, contrarily to cytokinins, avoided ABA accumulation in petunia flowers. These results were in according with previous experiments that showed as ethylene inhibitors have been able to inhibit ABA increase in two different varieties of tomato during fruit ripening (Martínez-Madrid *et al*. 1996).

In conclusion, treatments with cytokinins induced ABA accumulation but did not act as senescence enhancer, while AOA treatments prolonged the flower life. These results also suggest that treatments with commercial compounds containing AOA may be used for extending the flowering stage of petunias.

References

Amrhein, N.: Novel inhibition of phenylpropanoid metabolism in plants. - In: Luckner, M., Schreiber, L. (ed.): Regulation of Secondary Products and Plant Hormone Metabolism. Pp. 173-182. Pergamon Press, Oxford 1979.

Chang, H., Jones, M.L., Banowetz, G.M., Clark, D.G.: Overproduction of cytokinins in petunia flowers transformed with PSAG12-IPT delays corolla senescence and decreases sensitivity to ethylene. - Plant Physiol. **132**: 2174-2183, 2003.

Deikman, J., Hammer, P.E.: Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. - Plant Physiol. **108**: 47-57, 1999.

Eze, J.M.O., Mayak, S., Thompson, J.E., Dumbroff, E.B.: Senescence in cut carnation flowers: temporal and physiological relationships among water status, ethylene, abscisic acid and membrane permeability. - Physiol. Plant. **68**: 323-328, 1986.

Hunter, D.A., Ferrante, A., Vernieri, P., Reid, M.S.: Role of abscisic acid in perianth senescence of daffodil. - Physiol. Plant. **121**: 313-321, 2004.

Jeong, S.T., Goto-Yamamoto, N., Kobayashi, S., Esaka, M.: Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. - Plant Sci. **167**: 247-252, 2004.

Jiang, Y.-M., Joyce, D.C.: ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. - Plant Growth Regul. **39**: 171-174, 2003.

Kao, C.-Y., Cocciolone, S.M., Vasil, I.K., McCarty, D.R.: Localization and interaction of the cis-acting elements for abscisic acid, VIVIPAROUS1, and light activation of the C1 gene of maize. - Plant Cell **8**: 1171-1179, 1996.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic membranes. - Methods Enzymol. **148**: 350-382, 1987.

Martínez-Madrid, M.C., Serrano, M., Riquelme, F., Romojaro, F.: Polyamines, abscisic acid and ethylene production in tomato fruit. - Phytochemistry **43**: 323-326, 1996.

Mayak, S., Dilley, D.R.: Regulation of senescence in carnation (*Dianthus caryophyllus*). Effect of abscisic acid and carbon dioxide in ethylene production. - Plant Physiol. **58**: 663-665, 1976.

Mayak, S., Halevy, A.H.: Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. - Plant Physiol. **50**: 341-346, 1972.

Milborrow, B.V.: The pathway of biosynthesis of abscisic acid in vascular plants: a review of the present state of knowledge of ABA biosynthesis. - J. exp. Bot. **359**: 1145-1164, 2001.

Nowak, J., Veen, H.: Effects of silver thiosulfate on abscisic acid content in cut carnations as related to flower senescence. - J. Plant Growth Regul. **1**: 153-159, 1982.

Panavas, T., Walker, E.L., Rubinstein, B.: Possible involvement of abscisic acid in senescence of daylily petals. - J. exp. Bot. **49**: 1987-1997, 1998.

Pandey, D.M., Goswami, C.L., Kumar, B., Physiological effects of plant hormones in cotton under drought. - Biol. Plant. **47**: 535-540, 2003/4.

Purty, R.S., Agrawal, V., Gupta, S.C.: Induction of a novel boiling stable protein in response to desiccation and ABA treatments in *Sesbania sesban* var. *bicolour* leaves. - Biol. Plant. **49**: 137-140, 2005.

Sang, C.K., Kim, H.Y., Yun, H.S.: Effects of light, sucrose, and growth regulators on the coloration of cut snapdragon flower. III. Effects of various artificial light and growth regulator treatments. - J. Soc. hort. Sci. **33**: 79-86, 1992.

Singh, A., Evensen, K.B., Kao, T.H.: Ethylene synthesis and floral senescence following compatible and incompatible pollinations in *Petunia inflata*. - Plant Physiol. **99**: 38-45, 1992.

Vernieri, P., Pardossi, A., Tognoni, F.: Influence of chilling and drought on water relations and abscisic-acid accumulation in bean. - Aust. J. Plant Physiol. **18**: 25-35, 1991.

Wade, H.K. Sohal, A.K. Jenkins, G.I.: *Arabidopsis* ICX1 is a negative regulator of several pathways regulating flavonoid biosynthesis genes. - Plant Physiol. **131**: 707-715, 2003.

Wilhelmová, N., Procházková, D., Macháčková, I., Vágner, M., Srbová, M., Wilhelm, J.: The role of cytokinins and ethylene in bean cotyledon senescence. The effect of free radicals. - Biol. Plant. **48**: 523-529, 2004.

Zeevaart, J.A.D., Creelman, R.A.: Metabolism and physiology of abscisic acid. - Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 439-473, 1988.