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Regulation of ABA Levels in Senescing Petals of Rose Flowers

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Abstract. During the vase life of a rose flower, changes in the levels of abscisic acid (ABA) were observed: a decrease during the first 3 days, followed by a steady state at a low level, and finally a sharp increase in late senescence. Feeding [2-^{14}C]ABA to isolated petals showed that metabolism was very active despite the age of the flower, oxidation processes increased with age, whereas conjugation decreased but the level of nonmetabolized ABA remained stable. When the isolated petal was subjected to water stress, whatever its age, the ABA level increased. Hydrolysis of ABA-GE was not involved in this phenomenon. Thus, ABA synthesis occurred in the isolated petal; it could be directly correlated to the decrease in water potential. However, the ABA increase in isolated petals was limited. Moreover, on the rose tree, increases in ABA levels were not correlated to water potential changes. ABA levels seemed, therefore, mainly regulated by changes in import from leaves and other parts of the flower.

As early as 1972, Mayak and Halevy showed that endogenous levels of abscisic acid (ABA) increased in rose petals as the flower senesced. Moreover, higher ABA levels were found in short-lived cultivars compared to long-lived cultivars (Halevy and Mayak 1975, Mayak et al. 1972). In a stomataless system (leafless flower shoots) or in leafy shoots held in darkness when all stomates are closed, ABA enhanced aging of the flowers and some biochemical processes associated with it (Halevy et al. 1974). These results indicate that ABA participates in the endogenous regulation of senescence processes in rose flowers (Halevy and Mayak 1981).

However, little is known about the regulatory mechanisms which control ABA levels in relation to senescence. Synthesis, transport, and breakdown may be involved. In order to define the respective contribution of each of these processes, we compared endogenous ABA levels, during natural senescence or during senescence induced by a water stress, of isolated petals, or of petals maintained on the cut flower in the presence or absence of leaves, or on the whole plant. At the same time isolated petals were fed with [2-^{14}C]ABA in order to determine changes in uptake and breakdown of ABA during senescence.

Materials and Methods

Plant Material

Roses (Rosa hyb.) cv. Royalty were cut in a greenhouse of Sélection Meilland (Antibes) and were brought to the laboratory after 24 h at 4°C. Flowers were kept in water at 20°C for 2, 6, 9, and 13 days. After preliminary experiments showed heterogeneity between petals according to their position, the three external petals were discarded and measurements were performed only on the five remaining petals (4–8).

All experiments were repeated two or three times and the results obtained for the various experiments were similar. Therefore, the results presented here are taken from one experiment of each series.

Dehydration Treatment in Controlled Conditions

For the drying treatment, isolated petals were placed in a desiccator over activated Actigel at 20°C for various periods of time (2, 4, 8, 16, and 24 h).

Petal–Water Status

The petal–water potential was measured with a Wescor HR-33 T dew-point microvoltmeter after equilibration for 3 h at 23°C.

In some cases, petal–water status was expressed by water deficit. Ten discs of petals were weighed (FW). They were saturated by floating on distilled water and were weighed again

(FTW). After drying for 24 h at 100°C, they were weighed again (DW) and water deficit was calculated by the following formula:

$$\text{Water deficit} = \frac{\text{FTW} - \text{FW}}{\text{FTW} - \text{DW}} \times 100$$

A good correlation between water potential and water deficit could be established.

ABA Extraction Procedure

Ten petals or petal discs were ground in a mortar with chilled 80% methanol containing 2,6-di-t-butyl-4-methyl phenol as an antioxidant (100 mg L^{-1}). The homogenate was stirred for 2 h at 4°C and centrifuged for 10 min at 2000 *g*. The pellet was reextracted twice with the same volume of cold 80% methanol. The supernatants were collected and evaporated under reduced pressure at 40°C. An ether-soluble fraction was prepared from this acidic (pH 3.0) aqueous extract. The remaining aqueous phase was then hydrolyzed at 60°C at pH 11.0 for 30 min and, after acidification, extracted three times with diethyl ether. The aqueous phase was then evaporated to dryness and the residue was dissolved in methanol.

Endogenous ABA Determination by Radioimmunoassay

ABA was quantified by radioimmunoassay (RIA) performed as described earlier (Le Page-Degivry et al. 1984). Anti-ABA antibodies were obtained by immunization of rabbits with (+)ABA–human serum albumin conjugate obtained via a mixed anhydride reaction. The tracer was the iodinated (^{125}I) derivative of the conjugate obtained by coupling (+)ABA to the peptide glycyl-L-tyrosin. Radioimmunological incubations were performed by equilibrium dialysis.

A study of the cross-reactivities of structurally related compounds showed the specific binding of (+)ABA, when the acid function was free, esterified, or linked by amide linkage. The conversion of the carboxyl group of ABA into an amide inducing an increase in sensitivity of the free (+)ABA estimation, allowed ABA values in an extract to be calculated by differential measurement before and after amidation (Le Page-Degivry et al. 1984). Each value was the average of results obtained from four different dilutions of the extract (two replicates for each).

Incubation with [^{14}C]ABA

Incubations were performed either with whole isolated petals, the bases of which were immersed for 1 cm in an ABA solution, or with discs of 8 mm diameter cut from the petals and floated on the ABA solution. Radioactive ABA (specific activity 947 MBq $mmol^{-1}$) was used at 5×10^{-5} M.

During the incubation, which was either 24 h or of 3 days duration, petals were maintained in darkness.

[^{14}C]ABA Metabolism Analysis

The three different fractions obtained after extraction, two diethyl ether acid phases containing respectively the free acids and those released by mild alkaline hydrolysis and the remaining aqueous phase, were analyzed. An aliquot (1/20) was separated before chromatography and directly counted in order to calculate total uptake of radioactivity and the distribution between free acids and conjugates.

The free acid fractions were chromatographed on Merck precoated silicagel F254 plates and developed in toluene:ethyl acetate:methanol:acetic acid (50:30:7:4 vol/vol/vol/vol). The chromatogram was divided into half R_f zones, and the radioactivity was counted in a dioxane-based scintillation fluid using a SL 32 Intertechnique Liquid Scintillation Spectrometer.

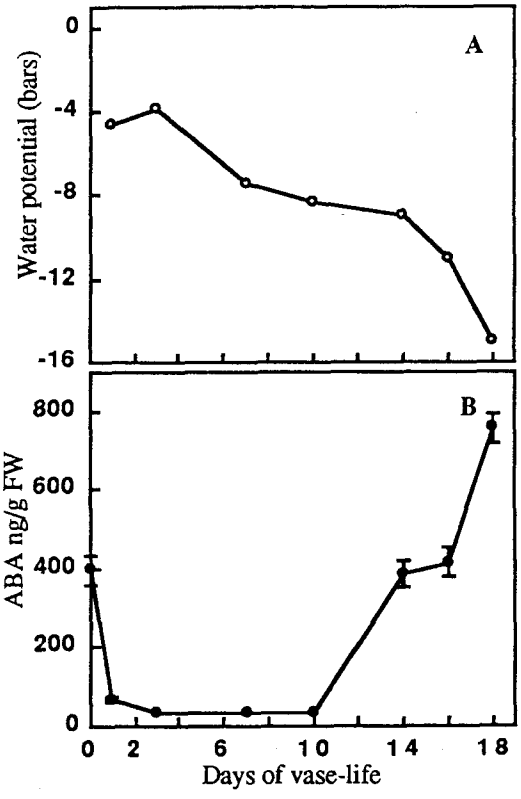

Fig. 1. With time of flower vase life, changes in (**A**) water potential and (**B**) ABA levels of petals.

Results

Changes in Endogenous ABA Content of Petals During Flower Senescence in a Vase

Endogenous ABA was measured in petals removed directly from a rose tree at harvest day (t_0) and in petals isolated after different times of subsequent vase life in water at 20°C.

Observed changes in water potential during vase life are shown in Fig. 1A. After a slight increase corresponding to a rapid uptake of water during the first 3 days, water potential progressively decreased to −15 bars in late senescing flowers. Changes in ABA content can be observed in Fig. 1B. The ABA level was high in rose petals sampled from the rose

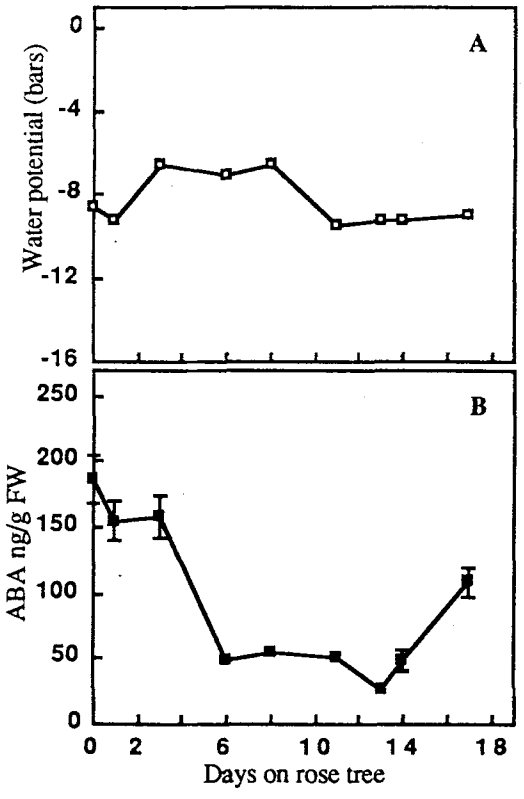

Fig. 2. With time of development and senescence on the rose tree, changes in water potential of petals (**A**) and their ABA levels (**B**).

tree but sharply decreased during the first day. Thereafter, the ABA level decreased to a low value for 1 week. A sharp increase occurred only at the end of vase life in the senescing flower.

Changes in Endogenous ABA Content of Petals During Flower Senescence on Rose Tree

At harvest, some flowers remained on the rose tree and were tagged during their development and senescence. Water potential of the petals changed little, fluctuating around −7.5 bars (±1.5) (Fig. 2A). ABA levels (Fig. 2B) remained high for 3 days; thereafter, the level decreased first sharply and then more slowly. It finally increased in petals ready to drop on the 14th and 17th days.

Changes in Endogenous ABA Content of Petals During Fast Senescence upon Water Starvation of Cut Flowers

In all cases, dehydration was performed by keeping 1-day-old cut flowers out of water during 22 h in the laboratory (i.e., at 20°C and at 60% relative humidity). Three different conditions were compared: either whole cut flowers were dehydrated in the presence of five leaves; either all leaves were removed from the stem before dehydratation; or isolated petals themselves were subjected to water stress.

Table 1. Changes in water deficit, ABA, and ABA-GE levels in petals of rose flowers according to dehydration conditions.

Dehydration conditions	Water deficit (%)	ABA (ng/g FW)	ABA-GE (ng/g FW)
Beginning of the experiment	15	125 ± 10	25 ± 3
Whole flowers dehydrated during 22 h			
With leaves	63	570 ± 50	1000 ± 100
Without leaves	55	315 ± 30	110 ± 10
Isolated petals	64	225 ± 20	36 ± 4

Changes in the water deficit and in ABA and ABA-GE content of petals after treatments are shown in Table 1. A significant increase in ABA content was already observed in isolated petals; however, this increase was higher when other parts of the flower were present and even higher when the leaves were present. ABA-GE did not increase significantly in isolated petals. It increased fourfold when the whole flower was maintained on the stem and even more in the presence of leaves.

Changes in Endogenous ABA Content of Isolated Petals Under Controlled Water Stress

Petals isolated from flowers of two different ages were subjected to water stress in a desiccator over activated Actigel. For petals isolated from 1-day-old cut flowers, water potential (initially around −5 bars) decreased progressively during their dehydration over Actigel and reached −18 bars after 24 h (Fig. 3A). ABA content (Fig. 3B) decreased rapidly during the first 4 h of treatment and then progressively increased. During this second step, the increase in ABA could be correlated to the decrease of water potential (Fig. 4a). When dehydration was performed at a lower water potential (Fig. 4b), the increase in ABA was greater. When petals were isolated from 8-day-old cut flowers, at the beginning of the treatment, water potentials (around −9 bars) were lower than in young flowers. During dehydration over Actigel, water potential (Fig. 5A) decreased very slowly during the first 24 h and sharply fell afterward, reaching −30 bars after 48 h. For flowers kept 8 days in a vase, the ABA level in the petal was low; it did not sig-

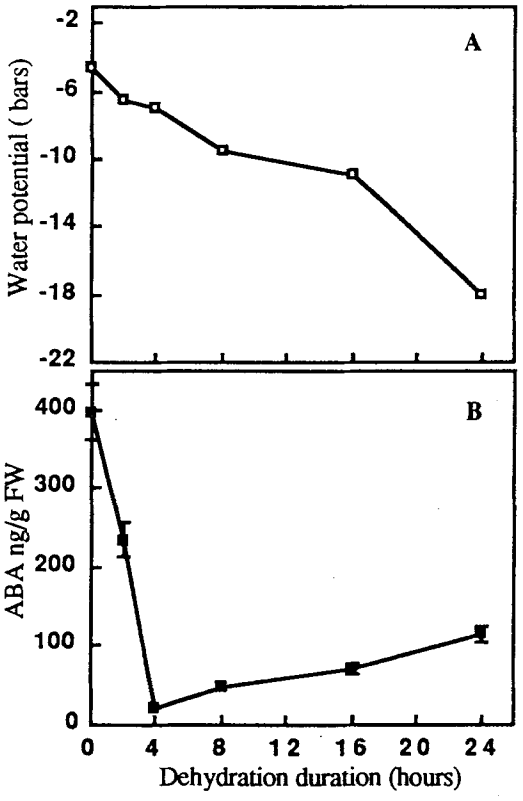

Fig. 3. With duration of dehydration on activated Actigel of isolated 1-day-old petals, changes in water potential (A) and ABA level (B).

nificantly increase during the first 24 h of dehydration but sharply increased, thereafter, reaching 300 ng/g FW after 48 h (Fig. 5B). When the increase in ABA was correlated to the decrease in water potential, the slope of the regression line (Fig. 4c) was the same as that observed for petals isolated from 1-day-old cut flowers.

Changes in [^{14}C]ABA Uptake by Petals and Petal Discs During Vase Life Flower Senescence

When petal discs were incubated in [^{14}C]ABA (Fig. 6A) the uptake of radioactivity after 24 h was very high in discs prepared from recently cut flowers. It sharply decreased between the third and seventh day of vase life and more slowly thereafter.

When whole petals isolated from flowers of different ages were incubated with their bases immersed in the solution of [^{14}C]ABA, the uptake of radioactivity measured after 24 h (Fig. 6B) appeared much reduced from that at the beginning of the experiment. It also decreased with the age of the flower but to a lesser extent than with petal discs.

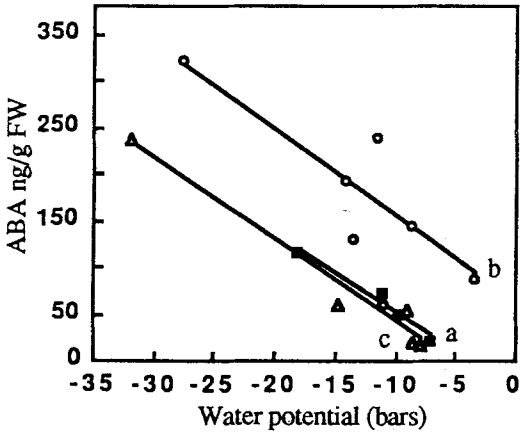

Fig. 4. Correlation between ABA level and water potential during dehydration of isolated petals on activated Actigel. a (■) and b (○): 1-day-old petals; c (△): 8-day-old petals. (a: $y = -8.31x - 30.92$, $r = 0.95$; b: $y = -9.14x + 65.07$, $r = 0.98$; c: $y = -8.73x - 45.33$, $r = 0.98$).

Changes in [^{14}C]ABA Metabolism of Petals During Flower Senescence

Table 2 compares the ABA metabolism of petals isolated at different ages. As the petals aged, the percentage of conjugates (ester and alkali nonhydrolyzable conjugates) decreased. This decrease could be observed after 1 day, as well as after 3 days of uptake.

Thin-layer chromatography on silica gel allowed the nonmetabolized ABA to be distinguished from its oxidation products: phaseic acid (PA) and dihydrophaseic acid (DPA). The percentage of oxidation products increased with the age of the petals (Table 3).

However, whatever the age of the petals, nonmetabolized ABA remained at the same level (45 ± 3% of the total uptake).

Discussion

Changes in the levels of ABA were observed in petals of cut rose flowers during their vase life: a decrease during the first 3 days, followed by a steady state at a low level, and finally by a sharp increase when senescence became clearly visible. The first step was reported by Borochov et al. (1976). The ABA content of petals in cut cv. Super Star roses, which were placed in water, decreased for the first 3 days following cutting. A similar decrease occurred in isolated petals within the first 4 h of water stress. This response was also observed in petals from flowers maintained on the rose tree but only on day 6. This decrease was thus observed despite

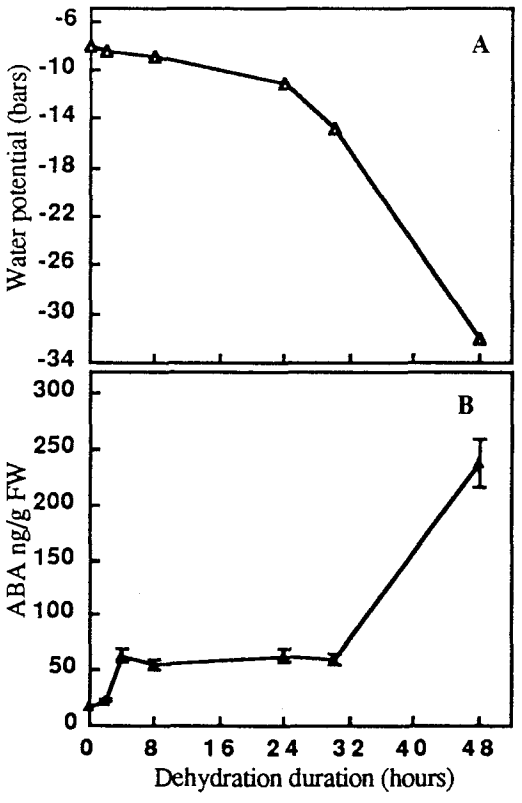

Fig. 5. With duration of dehydration on activated Actigel of isolated 8-day-old petal, changes in water potential (A) and ABA level (B).

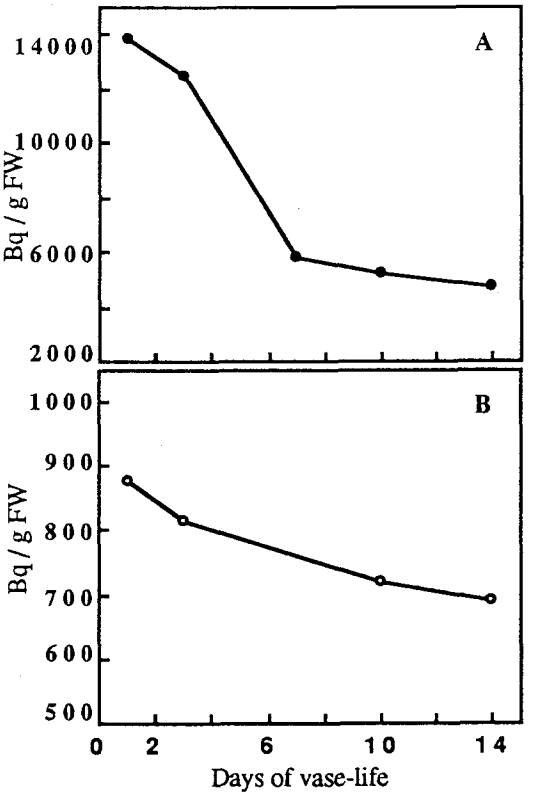

Fig. 6. With time of flower vase-life, changes in [^{14}C]ABA uptake over 24 h by (A) discs of petals (floated on ABA solution) or by (B) intact petals (the base of which was immersed in ABA solution).

conditions of petal conservation. It was not associated with harvest but only accelerated by harvest.

Vardi and Mayak (1989) suggested that the capacity to metabolize ABA to inactive forms is greater in young flowers than in older ones of Petunia. The feeding of [2-^{14}C]ABA to isolated rose petals in the present study showed very active metabolism. Whatever the age of the petals, the quantity of non-metabolized ABA after 24 h remained about the same. Only a shift in the type of metabolism was observed. Oxidation processes increased with the age of the petal, whereas conjugation decreased. ABA levels were, therefore, mainly regulated by changes in synthesis or import.

Water stress has been shown to accelerate senescence and to reduce longevity of cut flowers (Borochov and Mayak 1984, Borochov et al. 1976, Mayak et al. 1985). Following water stress, the free ABA level increased in young isolated petals. This rise was not accompanied by a change in the level of ABA-GE, which remained low. This conjugate was not a potential source of free ABA and in situ synthesis in petals therefore occurred. The increase in ABA could thus be directly correlated to the decreases in water potential. Such a correlation has often been reported for leaves (Wright 1977, Zabadal 1974), but has not yet been demonstrated for isolated petals. In primary leaves of bean, Eze et al. (1981) noted that the capacity to synthesize ABA was highest in the young expanding tissue. A similar conclusion was suggested by Eze et al. (1986) in carnation flowers where an ethylene treatment induced the highest increase in ABA concentration in the youngest flowers. Our results showed an equal capacity of ABA synthesis for 1- and 8-day-old isolated petals. However, the decrease in water potential was slowest in the oldest petals and the increase in ABA content appeared later. The increase in ABA observed during the final stage of flower senescence could be considered simply as a response to changes in water status.

In cut carnation flowers placed in deionized water (Eze et al. 1986) marked changes in water status of the petal tissue, including losses in water potential, also preceded the rise in ABA. However, these authors, like Borochov et al. (1976) and Nowak and

Table 2. Percentage (relative to total uptake) of free acids and conjugates among [^{14}C]ABA metabolites after 1 or 3 days of uptake by isolated petals.

	After 1 day uptake			After 3 days uptake	
Vase life (days)	2	4	6	2	8
Free acids	54%	65%	79%	24%	48%
Esters	18% } 46%	12% } 35%	8% } 21%	32% } 76%	20% } 52%
Alkali nonhydrolyzable conjugates	28%	23%	13%	44%	32%

Table 3. Percentage (relative to total free acids) of nonmetabolized [^{14}C]ABA and its oxidation products after 24 h uptake by isolated petals (R_f: DPA 0.3; PA 0.5; ABA 0.65).

Vase life (days)	1	7	14
ABA	81%	74%	66%
PA	5% } 17%	6% } 25%	5% } 31%
DPA	12%	19%	26%

Veen (1982) submitted the whole flower with its stem to water stress. Our results showed that the ABA increase in isolated petals was always limited even for a very low water potential (300 ng/g for −27 bars). This increase was greater, even for higher water potential, if water stress was applied to the whole flower in the presence or absence of leaves. ABA-GE also considerably increased in these cases. This conjugate was not a source of ABA, but it appeared as an end product of metabolism which accumulated during senescence. Bray and Zeevaart (1985) showed that in leaves, ABA-GE was accumulated as an end product in vacuoles; such a localization is also likely for petals. Thus, a significant part of ABA detected in attached petals must come from other parts of the flower or from the leaves. Indeed even though uptake of [2-^{14}C]ABA decreased with time, its import was still possible in 14-day-old petals. On the rose tree, increases in ABA levels were observed independently from significant water potential changes. This appeared to be the result of an increase in the import associated with changes occurring in the whole plant or in the flower itself.

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