

Abscisic Acid Content of Senescing Petals on Cut Rose Flowers As Affected by Sucrose and Water Stress¹

Received for publication December 30, 1975 and in revised form April 12, 1976

AMIHUD BOROHOV, TSIPORA TIROSH, AND ABRAHAM H. HALEVY

The Hebrew University of Jerusalem, Department of Ornamental Horticulture, Rehovot, Israel

ABSTRACT

Leafless cut Superstar roses (*Rosa hyb.*) were kept in a 1% sucrose solution. During the first few days of treatment, the abscisic acid content and the water deficit in the petals was higher in treated flowers than in controls kept in water. Later and up to the termination of the flower's life, ABA content and water deficit values were lower in petals of sucrose-treated flowers than in controls. Water stress treatments resulted in higher water deficit values and higher ABA content of petals. An 8-day sucrose treatment following temporary water stress improved the quality of flowers and reduced the level of ABA in the petals. We conclude that the effect which sucrose has on the ABA content of rose petals is at least partly due to its effect on changes in water deficit in the petals. This happens in spite of the fact that rose petals have no stomata, and therefore, ABA is not involved in regulating water balance via the stomata.

Abscisic acid has been found in many plant tissues, including rose petals (10). The role attributed to ABA in petals is that of regulating the process of senescence. Endogenous levels of ABA rise as petals senesce, and exogenous treatment with ABA shortens the life of cut roses, thereby enhancing changes associated with senescence, such as changes in petal color and a decrease in the protein content (3, 7, 10). Sucrose is the main transport form of the products of photosynthesis. When flowers are cut, they are severed from their sucrose supply and this no doubt is one of the reasons for imperfect development and the shorter life span of the cut flower (17). Sucrose supplied exogenously, promotes normal development of the cut flower, lengthens its life span, diminishes the change in petal color, and reduces proteolytic breakdown (1, 3, 16).

As pointed out above, sucrose and ABA have opposite effects on senescence of rose petals. The effects are nullified when flowers are treated with a combination of the two substances (3). This fact does not prove antagonism at one site of action.

The objective of the present study was to investigate further the interrelationship between sugar and ABA. The specific question posed was, "How do sucrose treatments which delay senescence affect the endogenous levels of ABA in the flower?"

MATERIALS AND METHODS

Plant Material and Environmental Conditions. Roses (*Rosa hyb.*) Superstar (*Tropicana*) were cut in a commercial greenhouse and were brought to the laboratory within 1 hr. The

flower stems were graded to a uniform length of 40 cm and before placing in 500-ml vases containing the treatment solutions, all leaves were removed from the stem. The flowers were kept under controlled conditions of 20 ± 1 C, $55 \pm 10\%$ relative humidity, and continuous illumination from cool white fluorescent light at a light energy flux density of $650 \mu\text{w}/\text{cm}^2$. The base of the flower stalk was recut every other day and the solutions in the vases was brought up to a volume of 500 ml. The environment was not changed in experiments involving water stress, but flowers were removed from the solutions for specified periods of times (Fig. 3, Table I). At the end of the stress treatment the stems were recut. Some of the flowers which had been subjected to water stress conditions for 4 hr were given a recovery treatment. This consisted of wrapping the flower bud with wet blotting paper after the flower had been returned to its vase containing the treatment solution. This treatment raised the humidity around the flower to 90 to 100%. All treatment solutions contained $300 \mu\text{g}/\text{ml}$ $\text{Al}_2(\text{SO}_4)_3 \cdot 16 \text{H}_2\text{O}$ to prevent development of bacteria.

Extraction and Determination of ABA in Petals. Extraction, separation, and identification methods were adapted from those described in previous reports (10, 15, 18, 19). Details of certain changes follow.

Extraction and Purification. Each sample consisted of all the petals from five flowers. Samples were weighed (12 to 30 g fresh weight) and blended in 80% methanol (10 ml/g) for 2 min in a Waring Blender. The resulting suspension was shaken for 18 hr at 4 C in the dark. After filtering, the solids were further extracted by shaking in the dark for 1 hr at 4 C with 3 ml/g 100% methanol. The combined methanol fractions were evaporated down to the aqueous phase at 38 C under vacuum. After centrifugation for 20 min at 500g, the supernatant was brought to pH 8.3 with NH_4OH and washed three times with methylene chloride (v/v) which was then discarded. After acidifying the aqueous phase to pH 3 with HCl, it was washed six times with methylene chloride (v/v), after which the aqueous phase was discarded. The methylene chloride fraction was evaporated to dryness at 38 C under vacuum.

Preparative TLC. Samples were dissolved in 1 ml methylene chloride and these were loaded as a band on glass plates (20 × 20 cm) coated with a 0.25-mm layer of silica gel (Kieselgel HF₂₅₄, Merck) activated at 120 C for 1 hr. The plates were developed in 1-propanol-1-butanol- NH_4OH - H_2O (6:2:1:2, v/v). After drying at room temperatures, R_F zone 5 to 8 was scraped off and eluted with 30 ml 100% methanol. After evaporation to dryness at 38 C under vacuum, the residue was redissolved in 1 ml methylene chloride and again loaded as described above. The solvent system used this time was benzene-ethyl acetate-acetic acid (40:5:2, v/v). After drying at room temperature, R_F zone 1 to 3 was scraped off and eluted with 30 ml 100% methanol which was then evaporated at 38 C under vacuum. Samples were methylated with diazomethane prepared from diazald (Aldrich Chemical Co.).

¹ This research was supported by the Pealstein-Dautoff Fund in Horticultural-Floral Science at the Hebrew University in the name of H. Roth. We are grateful to the donors for their generous help.

GLC. A 1- μ l aliquot of the sample was injected into a Packard 7400 gas chromatograph using a glass column (183 cm long \times 3.2 mm i.d.), packed with 5% QF-1 on gas-chrom Q, 60 to 80 mesh. The column temperature was 210 C, injection and detector temperatures were 215 C and 195 C, respectively. Nitrogen gas flow at a rate of 60 ml/min served as a carrier. An electron capture detector was used with radioactive tritium foil.

Qualitative and Quantitative Determination of ABA. ABA was identified by the method described by Mayak and Halevy (10), by locating the ABA band on TLC plates under UV illumination, and by the appearance of an additional peak on the chromatogram after irradiating the sample with UV. This peak was identified as methylated trans-ABA by comparing its retention time with that of an authentic sample. ABA was determined quantitatively by calculating the area of the peak and comparing it with peaks of standard quantities of authentic (\pm)-ABA (F. Hoffman La Roche) ranging from 50 to 750 pg/injection.

Water Deficit in Petals. Two 12-mm discs from each of three petals were weighed (fresh weight). They were saturated by floating on distilled H₂O for 2 hr and were weighed again (FTW). After drying for 48 hr at 80 C the discs were weighed again (DW) and water deficit was calculated according to Barrs (2) by the following formula:

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

All experiments described above were conducted 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.

Qualitative Definition of Petal Color. Petals 6, 7, and 8 (counted from the outside inward) were sampled from every flower. They were examined with the aid of a Gardner XL-10 color difference meter as previously described (3). Color quality is expressed by the values of the *b* axis and the lower the value, the bluer is the color of the sample, which indicates an increase in the pH of the cell and thus senescence (3).

RESULTS AND DISCUSSION

The opposite effect which sucrose and ABA have on cut roses has been explained in a previous report (3) to be due to the enhancement of metabolic processes under the influence of ABA which also hasten senescence of the petals. An exogenous supply of general metabolite substrate such as sucrose reduces or even nullifies the effect of ABA (3). A similar explanation was given by Goldthwaite (5) in his study using *Rumex* leaves. In the present study we tried to examine the possibility that an exogenous supply of sucrose reduces the level of endogenous ABA in rose petals and, therefore, brings about the opposite effect.

The ABA content of petals in cut roses which were placed in water fell for the first 3 days after cutting. From the 4th day onward, ABA levels rose (Fig. 1). In contrast, the ABA content of petals in cut flowers placed in 1% sucrose, rose on the 1st day after cutting and remained higher than in control flowers, for the 2nd and 3rd day. From the 4th day after cutting, for as long as the flowers lived, the ABA level in treated flowers was lower than in flowers kept in water.

Changes in the water balance of plant organs are probably the main factor affecting the ABA content of these organs. As the water balance deteriorates, the endogenous level of ABA rises; whereas, when water balance improves ABA levels fall (8, 9, 15). The high ABA levels found in sucrose-treated flowers for the first few days after cutting, raised the question of whether they are a result of impeded water relations due to the osmotic potential of the sucrose solution. This point was examined in the following experiment (Fig. 2).

From the time of cutting and placing flowers in the vase, the

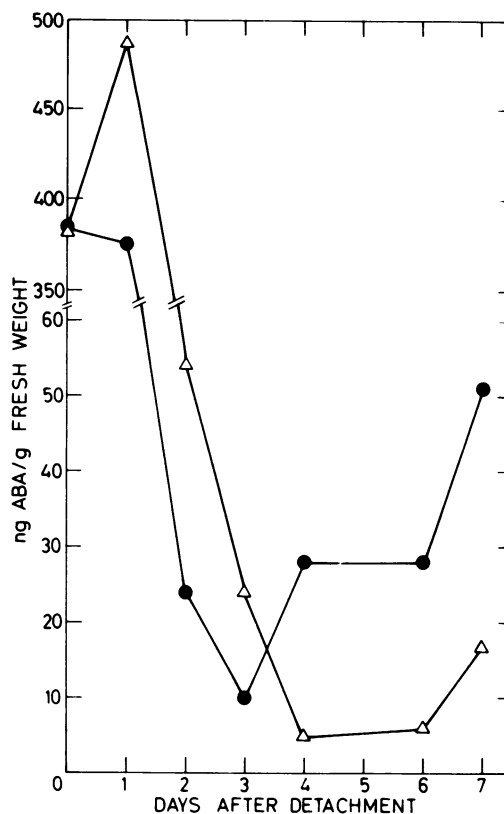


FIG. 1. Change in ABA content of petals on cut Superstar roses kept in water (●) or in 1% sucrose (Δ). Values are means of three replications each of petals from five flowers.

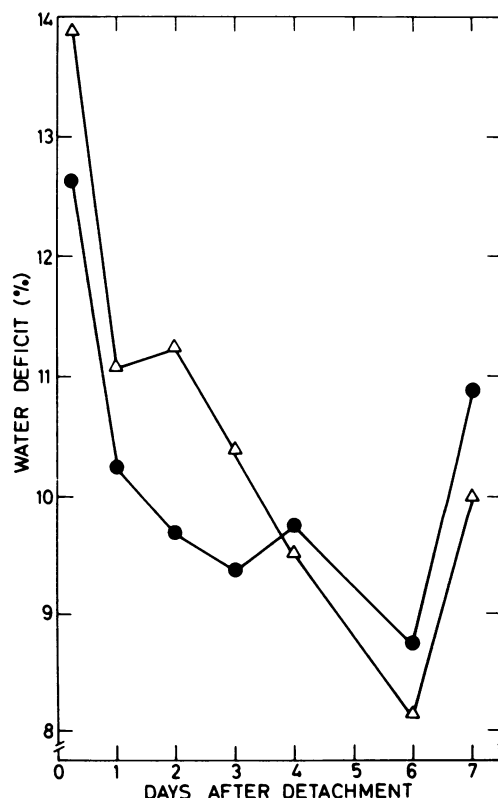


FIG. 2. Changes in water deficit (%) of petals on cut Superstar roses under the influence of water (●) or 1% sucrose (Δ) treatments. Values are means of six replications.

water deficit of the petals fell for the first 3 days (Fig. 2). The water deficit values found in flowers kept in sucrose were higher than those for flowers in water. However, from the 4th day onward, water deficit was lower in flowers kept in sucrose, *i.e.* the petals were more turgid than those of control flowers. The differences between control and sucrose-treated flowers are significantly different ($P = 0.05$) at all days except at days 4 and 6.

Placing stems of gladiolus and chrysanthemum cut flowers in high concentrations of sucrose (5 to 30%) temporarily impairs their water balance (4, 6). To our great surprise the very low concentration of sucrose (1% = 0.03 M) used in this study caused a significant increase in water deficit of rose petals which lasted a number of days but which later on was reversed. It seems that the eventual improvement in water potential in sucrose-treated flowers as compared with controls is due to the uptake of sugar by the flower and the subsequent increase in osmotic potential of the petal tissue, as has been previously found for gladiolus and chrysanthemum flowers (6). Comparison between results presented in Figures 1 and 2 (in spite of the fact that these results were obtained in two different experiments) indicates that there is a correspondence between changes in water deficit and changes in the ABA content of the petals.

Application of ABA induces the closure of stomata (14). Most reports dealing with the effect of water stress on ABA content of plants concern organs which bear stomata (12). However, when the effect of water stress was examined in tissues lacking stomata, little or no effect on endogenous levels of ABA was found (12, 13).

Rose petals have no stomata (11, 20). In contrast to most other studies with cut roses, we conducted the present investigation with flowers whose leaves were removed. We could, therefore, compare water stress in petals and its effect on ABA content, with the effects generally known for green leaf tissues. Water stress lasting only 4 hr increased water deficit (Fig. 3B) and the ABA content (Fig. 3A) of petals, respectively. A further 18 hr of water stress considerably increased both the water deficit and ABA content. Recovery treatments which reduced water deficit also reduced ABA to levels comparable with those found prior to water stress. A high positive correlation ($r^2 = +0.9980$) was found between the water deficit and the ABA content of the petals. These conditions are similar to those found in leaves bearing stomata (8, 9, 15), although in our case, ABA cannot be affecting the water balance in the same manner and thus cannot play a similar role in the adaptation of the tissue to stress conditions (7).

From the previous experiment we learned that sucrose treatment reduced water deficit as flower development progressed. The question which now arose was whether sucrose treatment can cause the recovery of flowers which had been temporarily subjected to water stress.

Cut flowers were either put into water or were subjected to 7.5 hr of water stress. At the end of the stress treatment some of the flowers were placed in water while others were placed in sucrose solution. Some of the flowers originally placed in water were also transferred to sucrose. After 8 days flowers were sampled, color quality, fresh weight, and ABA content of the petals were determined. Results presented in Table I show that even 8 days after exposure to temporary water stress, the petals of stressed flowers were bluer, their fresh weight was lower, and their ABA content was higher than in water control flowers. Sucrose treatment after stress improved petal color, increased fresh weight and reduced the ABA content to values comparable with those obtained for the water control. The treatment did not nullify the damaging effect of earlier water stress if compared with the sucrose control.

The prolonged effect which the temporary water stress has on flower quality can be explained by the effects which ABA has on enhancing the appearance of senescence symptoms (3). The

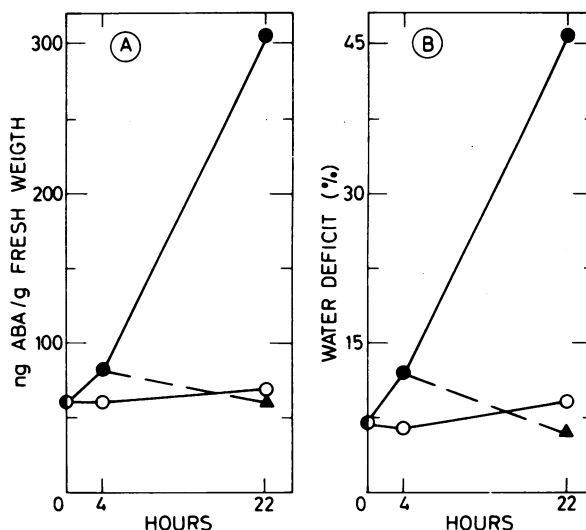


FIG. 3. The effect of water stress and recovery treatment on ABA content (A) and water deficit (B) of petals on cut Superstar roses. ○, control (flowers in water); ●, water stress (flowers out of water); ●--▲, recovery treatment (flowers after water stress in water and 90 to 100% relative humidity). Values are means of three replications and of petals from five flowers.

Table I. Effect of 1% Sucrose Treatment on Color, Dry Weight, and ABA Content of Petals on Cut Rose Flowers (*cv. Superstar*) Which Had Been Subjected to 7.5 Hr Water Stress

Data were recorded 8 days after water stress occurred. Values of color and fresh weight are the means of 15 replications. Values for ABA content are the means of 3 replications each consisting of five flowers.

Treatment	Color quality ⁽¹⁾	Flower fresh wt. g	ABA content ng/g fresh wt.
Water control	9.2 b (2)	7.87 c	56.7 b
Sucrose control	14.0 c	9.40 d	21.9 c
Water stress followed by water	6.5 a	7.04 ab	187.2 a
Water stress followed by sucrose	9.2 b	8.80 bc	36.4 b

¹ Expressed as *b* axis values obtained on a Gardner XL-10 color difference meter. Lower values represent bluer color.

² Values followed by different letters are significant at the 5% level (MRT).

apparent effect of recovery found following sucrose treatment may be ascribed either to the antagonistic metabolic effect which sucrose has on ABA (3) or to the improved water relations induced at a later stage of flower development (Fig. 2). These results corroborate those of Paulin (16) who found that temporary water stress enhances senescence in Iris flowers.

Changes in petal color quality following the various treatments (Table I) indicate that these treatments affect the flower metabolism. The enhanced blue color of the petals follows subtle changes in pH which are in turn caused by enhanced catabolism and protein breakdown (3, 16).

LITERATURE CITED

- AARTS, J. F. T. 1957. Over de houdbaarheid van snijbloemen. Meded. Landbouwhogeschool Wageningen 57: 1-62.
- BARRS, H. O. 1968. Determination of water deficits in plant tissues. In T. T. Kozlowski ed., *Water Deficits and Plant Growth*. Academic Press, New York, pp. 235-368.
- BOROHOV, A., S. MAYAK, AND A. H. HALEVY. 1976. Combined effects of abscisic acids and sucrose on growth and senescence of rose flowers. *Physiol. Plant.* 36: 221-224.
- BRAVDO, B., S. MAYAK, AND Y. GAVRIELI. 1974. Sucrose and water uptake from concentrated sucrose solutions by gladiolus shoots and the effect of these treatments on floret life. *Can. J. Bot.* 52: 1271-1281.
- GOLDTHWAITE, J. 1974. Energy metabolism of *Rumex* leaf tissue in the presence of senescence-regulating hormones and sucrose. *Plant Physiol.* 54: 399-403.
- HALEVY, A. H. AND S. MAYAK. 1974. Improvement of cut flowers quality, opening and longevity by preshipment treatments. *Acta Hort.* 43: 335-347.
- HALEVY, A. H., S. MAYAK, T. TIBOSH, H. SPIEGELSTEIN, AND A. M. KOFRANEK. 1974.

- Opposing effects of abscisic acid on senescence of rose flowers. *Plant Cell Physiol.* 15: 813-821.
8. HIRON, R. W. P. AND S. T. C. WRIGHT. 1973. The role of endogenous abscisic acid in the response of plants to stress. *J. Exp. Bot.* 29: 769-781.
 9. LOVEYS, B. R. AND P. E. KUEDEMANN. 1973. Rapid changes in abscisic acid-like inhibitors following alterations in vine leaf water potential. *Physiol. Plant.* 28: 476-479.
 10. MAYAK, S. AND A. H. HALEVY. 1972. Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. *Plant Physiol.* 50: 341-346.
 11. MAYAK, S. AND A. H. HALEVY. 1974. The action of kinetin in improving the water balance and delaying senescence processes of cut rose flowers. *Physiol. Plant.* 32: 330-336.
 12. MILLBORROW, B. V. 1974. The chemistry and physiology of abscisic acid. *Annu. Rev. Plant Physiol.* 25: 259-307.
 13. MILLBORROW, B. V. AND D. R. ROBINSON. 1973. Factors affecting the biosynthesis of abscisic acid. *J. Exp. Bot.* 24: 537-548.
 14. MITTELHEUSER, C. J. AND K. VAN STEVENINCK. 1969. Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. *Nature* 22: 281-282.
 15. MIZRAHI, Y., A. BLUMENFELD, S. BITNER, AND A. E. RICHMOND. 1971. Abscisic acid and cytokinin contents of leaves in relation to salinity and relative humidity. *Plant Physiol.* 48: 752-755.
 16. PAULIN, M. A. 1975. Effects of watering following a drought period on nitrogen metabolism of cut *Iris germanica* flowers. *Acta Hort.* 41: 13-20.
 17. ROGERS, M. N. 1973. An historical and critical review of post-harvest physiology research on cut flowers. *Hort. Sci.* 8: 189-198.
 18. RUDICH, J., A. H. HALEVY, AND N. KEDAR. 1972. The level of phytohormones in monoecious and gynoecious cucumbers as affected by photoperiod and Ethephon. *Plant Physiol.* 50: 585-590.
 19. SEELEY, S. D. 1971. Electron capture gas chromatography of plant hormones with special reference to abscisic acid in apple bud dormancy. Ph.D. thesis. Cornell University, Ithaca.
 20. STUBBS, J. M. AND M. J. O. FRANCIS. 1971. Electron microscopical studies of rose petal cells during flower maturation. *Planta Med.* 20: 211-218.