ROLE OF FLOWER BUDS IN FLOWER BUD ABSCISSION IN *HIBISCUS*

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Abstract

Plants of *Hibiscus rosa-sinensis* L. 'Nairobi' kept in darkness showed abundant shedding of their flower buds after 4-6 days, due to abscission at an abscission layer in the pedicels about 3 mm below the bud. Abscission was accompanied by an increased evolution of ethylene by the flower buds as well as an increased ACC and MACC content. Removal of flower buds induced abscission of the remaining pedicels under white light conditions, but delayed dark-induced abscission. In disbudded plants, time curves of cumulative abscission in darkness and under white light conditions were the same. Pedicel explants showed no abscission unless the flower bud was attached to the pedicel. Exogenous ACC induced abscission in pedicel explants. IAA drastically reduced ACC-induced pedicel abscission of explants. The results indicate that the flower bud is as well a source of an abscission-promoting-substance as an abscission-inhibiting-substance, by which the flower bud has a dualistic role in pedicel abscission in *Hibiscus*.

Additional index words
Ethylene, auxin, ACC, darkness, postharvest handling, quality

Abbreviations
ACC = 1-aminocyclopropane-1-carboxylic acid; IAA = indole-3-acetic acid; MACC = 1-(malonylamino)-cyclopropane-1-carboxylic acid; NAA = α-naphtaleneacetic acid; STS = silver thiosulphate

1. Introduction

As with many of the popular flowering potted plants (Cameron and Reid, 1983), *Hibiscus* plants are likely to lose flower buds during postharvest handling (Thaxton et al., 1988). In cut flower crops, shattering (loss of petals or florets) is a common problem in a wide range of products (Reid and Goszczyńska, 1985). A great part of postharvest handling exists of storage and/or transport in darkness. Dark storage of *Hibiscus* induces abscission of the flower buds (Force et al., 1988; Van Lieburg et al., 1990). Bud abscission in *Lilium* has been associated with increased sensitivity to and a subsequent rise in biosynthesis of ethylene by the flower buds following dark treatment (Van Meeteren and De Proft, 1982). A silver thiosulphate (STS) spray reduces abscission in *Hibiscus* plants stored in the dark (Thaxton et al., 1988). This implies also a role for ethylene in dark-induced flower bud shedding in *Hibiscus*, since STS is thought to inhibit abscission by blocking ethylene action (Veen and Van de Geijn, 1978; Veen and Kwakkenbos, 1983).

The mechanism of abscission of plant parts has been studied most extensively with leaves (Beyer and Morgan, 1971). In the widely accepted model for the hormonal mechanism of leaf abscission the process is seen as an event that is repressed by auxin and activated by ethylene (Sexton and Roberts, 1982; Osborne, 1989). Removal of the leaf blade, which means taking

away the auxin source, enhances abscission at the abscission zone in the remaining petiole.

Dark-induced flower bud shedding in *Hibiscus* occurs in the pedicel about 3 mm from the flower bud. The present work was conducted to determine the role of the flower bud itself in dark-induced flower bud shedding due to pedicel abscission in *Hibiscus*, and to test the involvement of ethylene biosynthesis.

2. Material and methods

2.1. Plantmaterial.

All experiments were carried out with *Hibiscus rosa-sinensis* L. 'Nairobi'. Rooted cuttings were planted from July till October in 14-cm plastic pots containing a commercial potting soil. Plants were grown in a greenhouse with setpoints of 20°C (day) and 19°C (night) temperatures. From October till April supplementary light was given using high pressure sodium lamps for 16 hours/day (50-60 $\mu mol.s^{-1}.m^{-2}$ PPF). About 2 months before the plants were used in the various experiments, terminal meristems were removed to promote lateral growth. Chloromequat (2-chloroethyl trimethylammonium chloride) was applied at 117 $mg.liter^{-1}$ as a foliar spray 3 times with 2 week intervals, starting 1 week after pinching. Plants were grown for a total of 5-7 months. Experiments were carried out from January till April.

In all experiments plants were used with at least 3 flower buds between 5 and 10 mm. As the number of mature buds influence the number of abscissing buds (Force et al., 1988), in experiments with intact plants, all flower buds from which the inner sepals started to open and buds in more advanced developmental stages, were removed at the start of the experiment.

2.2. Exposure to dark or white light conditions.

Dark or white light conditions were imposed by placing plants in a controlled environment chamber at 20°C and 70% to 80% RH. Dark conditions existed of complete darkness, white light conditions of artificial light 12 hours/day, Philips TLD 32W/84HF, 14 $\mu mol.s^{-1}.m^{-2}$ PPF.

2.3. Ethylene, ACC and MACC measurements.

For measurements on flower buds, buds with a length of about 10 mm were used. After the desired exposure time, buds were cut from the plants above the abscission zones in the pedicels, and placed in glass vials (24 ml) with their cut ends in wet 'Oasis', at 20°C. Vials were closed after 30 min; after 90 min of accumulation ethylene concentration in 1-ml air samples were measured by gas chromatography. After gas sampling buds were frozen in liquid nitrogen for ACC and MACC measurements. ACC and MACC measurements were carried out according to Lizada and Yang (1979) using combined lyophilized material of 5 buds, extracted three times with 80% methanol, evaporated to dryness *in vacuo* and resuspended in water.

2.4. Application of ACC and IAA to pedicel explants.

Pedicel explants were taken from flower buds with a length of about 10 mm. Pedicels were cut 10 mm below the abscission zone; buds were removed at the insertion place of the sepals on the pedicel (about 3 mm above abscission zone). Pedicels were placed in glass vials, lined with wet filter paper, with their proximal ends in 1% water-agar blocks. At the distal ends agar blocks were supplied with 1% water, $5*10^{-4}$ M IAA, 10^{-3} M ACC or $5*10^{-4}$ M IAA + 10^{-3} M ACC. After 30 min vials were closed and ethylene measurements carried out as with flower buds.

3. Results

3.1 Intact plants.

Hibiscus plants kept in the dark started to show flower bud shedding after 3 days of darkness (Figure 1) due to pedicel abscission; 50 % of the buds was shed after 6 days. Plants kept under white light had no abscission at all. Flower buds from plants exposed to darkness showed an increase in their ethylene evolution, and ACC- and MACC content with exposure time (Figure 2). This increase was absent in the light.

When the flower buds were removed from plants under white light conditions, abscission at the abscission layer of the remaining pedicels started 5 days after the disbudding; 50 % of the pedicels of these plants was abscised after 7-8 days. When plants without buds were placed in darkness, they showed about the same time curve of cumulative abscission as plants without buds kept in the light (Figure 1).

3.2 Explants.

Explants existing of a pedicel and the attached flower bud, placed in darkness showed 50 % abscission after 4 days (Table 1). Pedicels without buds, however, showed no abscission at all. The ethylene evolution rate of these isolated pedicels was at a constant low level (Figure 3). A small increase in ethylene evolution rate resulted from application of IAA to the pedicels. Pedicels with applicated IAA, also showed no abscission (data not shown). Application of ACC increased ethylene evolution by the pedicels 8 times within a few hours. After 70 hours all pedicels with exogenous ACC showed abscission (Figure 3). Simultaneous application of IAA and ACC resulted in the same enhancement of ethylene evolution as ACC alone. ACC-induced abscission however, was drastically reduced by IAA.

4. Discussion

Wien et al. (1989) demonstrated with bell pepper that disbudding of plants induces abscission of the remaining flower pedicels. Also in our investigation with *Hibiscus*, removal of the flower buds induced abscission in the remaining pedicels in plants placed under white light conditions (Figure 1). These observations are similar to those reported using explants excised from leaf abscission zones. The conclusion of such studies is that the organ distal to the abscission zone is the source of an abscission inhibitor (Beyer, 1975). The polar auxin transport inhibitor TIBA caused abscission in pepper flower pedicels when applied to the flowers or to the pedicel between flower and abscission zone (Wien et al., 1992). Abscission of disbudded pedicels was completely prevented by infusion of NAA (Wien et al., 1989). ACC-induced abscission in isolated pedicels of *Hibiscus* was reduced by IAA (Figure 3), as was ethylene-induced abscission in pedicels of tomato (Roberts et al., 1984). It is very likely that flower tissues are the source of auxin acting as an abscission inhibitor, confirming the suggestion of Hänisch ten Cate et al. (1975), based on Begonia explants, that reproductive structure abscission has many similarities with leaf abscission.

Flower bud shedding in *Hibiscus* plants can be induced by darkness, especially in less developed flower buds (<30 mm) (Force et al., 1988). Bud removal of plants in the dark delayed the dark-induced pedicel abscission (Figure 1), suggesting that in darkness the flower bud (in specific developmental stages) is a source of an abscission-promoting-substance. This was confirmed by the finding that isolated pedicels on water showed only abscission when a bud was attached to the pedicel (Table 1). As in lilies (Van Meeteren and De Proft, 1982) dark-induced flower bud shedding in *Hibiscus* is accompanied by an increase in ethylene evolution by the flower buds (Figure 2) and abscission can be prevented by blocking ethylene action by STS (Thaxton et al., 1988). Isolated pedicels produced no ethylene and showed no abscission unless ACC was applied (Figure 3). It is very likely that in darkness, at specific developmental stages, the flower bud is a source of ACC and/or ethylene as abscission-promoting-substance, by which the flower bud has a dualistic effect on pedicel abscission.

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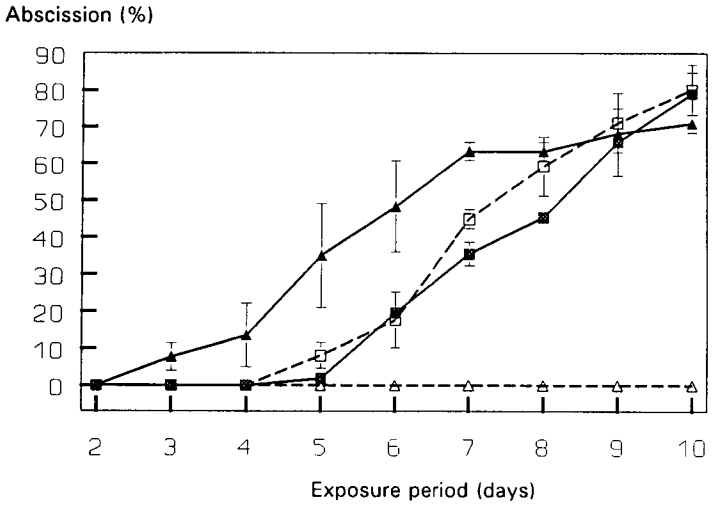

Figure 1. Cumulative pedicel abscission from plants placed in light (broken lines) or darkness (solid lines). Flower buds were either removed from the plants at the beginning of the exposure period (squares) or attached to the pedicels (triangles). Vertical bars represent SE of the mean of 9 plants per treatment.

Table 1. Abscission of explants existing of a disbudded pedicel or a pedicel with the flower bud attached to it. Explants taken from the plants and placed with their proximal ends in water at time 0. Number of explants was 16 per treatment.

Time (days)	Abscission (%)	
	Pedicel	Pedicel + bud
4	0	50
5	0	100

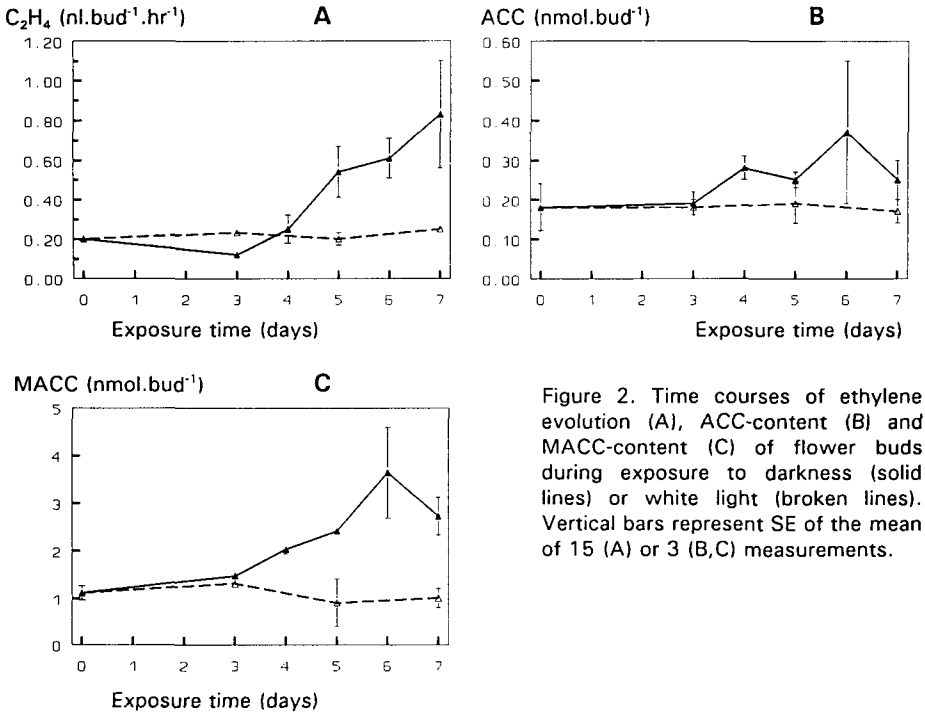

Figure 2. Time courses of ethylene evolution (A), ACC-content (B) and MACC-content (C) of flower buds during exposure to darkness (solid lines) or white light (broken lines). Vertical bars represent SE of the mean of 15 (A) or 3 (B,C) measurements.

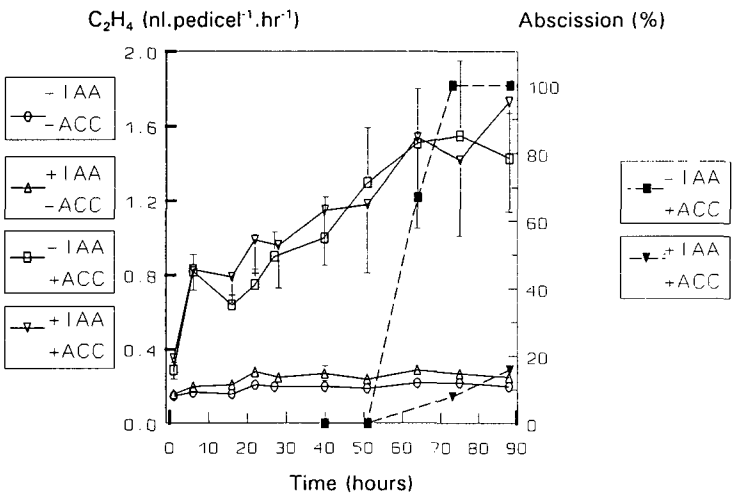

Figure 3. Ethylene evolution (solid lines) and abscission (broken lines) of pedicel explants without flower buds, after application of IAA ($5*10^{-4}$ M) and/or ACC (10^{-3} M) at Time 0. Twelve explants were used for each treatment.