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Role of abscisic acid in perianth senescence of daffodil (*Narcissus pseudonarcissus* 'Dutch Master')

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Daffodil (*Narcissus pseudonarcissus* L. 'Dutch Master') flowers detached at the base of their ovaries and held with their cut ends in 10–100 μ*M* abscisic acid (ABA) senesced prematurely. Symptoms of the ABA treatment included water-soaking of the tepals and early collapse of the corona. No water-soaking was seen in tepals of flowers held in water. Instead, the tepals of these flowers dried. The ABA content increased in tepals of the potted flowers as they senesced. The rise in tepal ABA content coincided with the appearance of visual signs of senescence. When the flowers were cut and placed in water, a treatment that accelerated their senescence, the increase in ABA occurred earlier. Exogenously applied ABA enhanced the premature accumulation of senescence-associated transcripts in the tepals. Their ABA-mediated induction was not prevented when the flowers were pre-treated with 1-methylcyclopropene, an inhibitor of ethylene action, indicating that ABA induced the transcripts independently of ethylene. The transcripts accumulated in opened control flowers before the rise in endogenous ABA. Attempts to extend floral longevity by using putative inhibitors of ABA biosynthesis [tungstate, fluridone (applied as Sonar®) and 1,1-dimethyl-4-(phenylsulphonyl)semicarbazide (DPSS)] were unsuccessful. However, inclusion of 100 μ*M* gibberellic acid (GA_3) in the vase solution reduced the senescence-inducing effects of 50 μ*M* ABA suggesting a possible mechanism for in-vivo control of senescence.

Introduction

Increasing evidence suggests that abscisic acid (ABA) is a natural regulator of perianth senescence in flowers. The hormone is present in higher amounts in naturally senescing petals (Wei et al. 2003), and in petals senescing prematurely in response to water stress (Panavas et al. 1998), or an alteration in light quality (Garello et al. 1995). When applied exogenously, ABA has been shown to accelerate the senescence of a number of flowers (Mayak and Halevy 1972, Mayak and Dilley 1976, Panavas et al. 1998). It does so by inducing many of the same molecular and biochemical events that occur during normal senescence. In daylilies, exogenously applied ABA causes a loss of differential membrane permeability, an increase in lipid peroxidation, induction of proteinase and ribonuclease activities, and induces a senescence-like mRNA profile in pre-senescent petal tissue (Panavas et al. 1998).

The senescence caused by ABA is mediated through ethylene in carnation flowers and certain cultivars of miniature roses as exogenously applied ABA does not cause their senescence when they are pre-treated with inhibitors of ethylene action (Mayak and Dilley 1976, Ronen and Mayak 1981, Muller et al. 1999). In daylilies, ABA presumably induces senescence independently of ethylene because daylily senescence is ethylene insensitive (Lay-Yee et al. 1992).

The timing of ABA accumulation in petals suggests that the hormone co-ordinates the early events in the senescence signal transduction pathway in some flowers, whereas in others it affects only the latter stages of senescence, perhaps serving to drive the process to completion. In daylilies, the ABA content of the petals increases before the increases in activities of hydrolytic

Abbreviations – ABA, abscisic acid; DPSS, 1,1-dimethyl-4-(phenylsulphonyl)semicarbazide; GA_3, gibberellic acid; 1-MCP, 1-methylcyclopropene.

enzymes and well before the flowers have opened (Panavas et al. 1998), whereas in roses, the ABA content increases comparatively late in the petals, 2 days after the surge in ethylene production (Mayak and Halevy 1972).

Some researchers have proposed that the ABA produced in the carnation gynoecium determines the longevity of the carnation flower (Shibuya et al. 2000), because the hormone accumulates in the gynoecium well before ethylene production increases and senescence is evident (Nowak and Veen 1982, Onoue et al. 2000). Certainly, it has been shown that removal of the gynoecium from the carnation flower inhibits the increase in floral ethylene production, delays petal senescence, and prevents exogenous ABA from accelerating senescence (Shibuya et al. 2000). However, because it has also been shown that ethylene action is required for the increase in ABA to occur in the gynoecium (Nowak and Veen 1982), the role of gynoecium-derived ABA in directing floral senescence is still not clear.

The importance of ABA in floral senescence might be determined if its synthesis or action in tissue could be reduced. No inhibitors specific to ABA biosynthesis are known, but chemicals such as fluridone, tungstate, and 1,1-dimethyl-4- (phenylsulphonyl)semicarbazide (DPSS) have been successfully used by researchers to inhibit ABA production in plant tissues. Fluridone inhibits ABA synthesis by inhibiting phytoene desaturase, an enzyme involved in biosynthesis of C40 carotenoids from which ABA is derived. Tungstate inhibits molybdenum-requiring enzymes, including ABA aldehyde oxidase that catalyse the last step in ABA biogenesis. These chemicals have been found to inhibit ABA accumulation in cocoa flowers (Aneja et al. 1999) and avocado mesocarp (Cowan et al. 1999), respectively. The mode of action of DPSS is unknown, although it was reported to reduce the ABA content in carnation flowers and substantially extend flower life (Onoue et al. 2000).

In the study reported here we examined the role of ABA in floral senescence of daffodils. We previously showed that potted 'Dutch Master' daffodils are short-lived flowers whose display life is around 8 days at 20°C and is reduced to about 5 days when the flowers were detached and placed with their pedicels in water (Hunter et al. 2002). We have also found, like others, that the detached flowers last longer when they are held in solutions containing gibberellic acid (Sultan and Farooq 1999, Ichimura and Goto 2000) a known antagonist of ABA action (Rodriguez et al. 1987, Weiss et al. 1995) and die prematurely when they are exposed to ethylene (Hunter et al. 2004). We hypothesized that stress-induced ABA might be the cause of the accelerated senescence of the cut flower.

Materials and methods

Plant material

Bulbs of 'Dutch Master' daffodils obtained from Oregon Bulb and Perennial Farms Inc., Sandy, OR, were placed in pots (four to five per pot) containing a soil mix consisting of 50% perlite, 20.5% fir bark, 17% peat moss, and 12.5% sand (v/v). The bulbs were pre-cooled at 7°C for 15 weeks to induce floral bud formation and break dormancy. When the leaves were approximately 1–2 cm above the soil surface the pots were transferred to the greenhouse with 11 h days (23°C)/13 h nights (17°C). Upon bud break (splitting of spathe to reveal the yellow perianth) the pots were moved to the post-harvest laboratory and illuminated at 15 $\mu mol\, m^{-2}\, s^{-1}$ PAR from Cool White fluorescent lamps with 12 h days (20 ± 2°C, 55% relative humidity)/12 h nights (20 ± 2°C, 55% relative humidity) to standardize conditions during opening, maturation and senescence. Each flower was tagged when it opened (at least five of the tepals perpendicular to the corona).

Carnation flowers (*Dianthus caryophyllus* L. 'Jun') cut at commercial maturity were transported from W. F. Suyeyasu Wholesale (Fremont, CA) to the Department of Environmental Horticulture, University of California, Davis. The flowers were stored overnight at 4°C in deionized water (water) and used the next day after their stems had been recut to 30 cm.

Senescence description in daffodils

The perianth of the 'Dutch Master' daffodil flower consists of an outer whorl of three sepals, an inner whorl of three petals and a trumpet-like paracorolla (corona). The sepals and petals were indistinguishable from each other and therefore were termed tepals. The timing and characteristics of perianth senescence in the attached and detached daffodil flowers were as follows: The first visible signs of perianth senescence were a discoloration at the tepal tips (onset) and often an inrolling of the tepal margins. The tepals dried as they discoloured and the discoloration (drying) spread inward to envelop the majority of each tepal (when approximately 1 cm from the tip inwards discoloured, designated 50% senescent). Following this, the corona lost turgidity and collapsed (corona collapse).

Plant treatments

Experiments were conducted either on newly opened whole detached daffodil flowers, their isolated complete perianths, or individual tepals. The flowers were detached by peeling back the spathe tissue and severing the exposed pedicel tissue about 1 cm below the base of the ovary. The intact perianth tissue (tepals plus corona) was isolated from the ovary by excising the tissue where the corona was attached to the ovary, and the individual tepals were isolated from the perianth tissue by excising the tepals where they were adjoined to the corona.

The detached flowers or their isolated plant parts were treated with various chemicals by placing their cut ends in small vials containing the chemical dissolved to the

appropriate concentration in deionized water (water). The chemicals used were abscisic acid (ABA) (Sigma, St Louis, MO), silver thiosulphate (STS, prepared as described in Reid et al. 1980), gibberellic acid (GA_3, Sigma), sodium tungstate (Sigma), fluridone (as Sonar®: SePRO Corp, Carmel IN, USA) and DPSS (0.8 m*M* DPSS in 2.6 m*M* citrate, kindly provided by Dr Pawan Srivastava, Syndicate Sales, Kokomo, IN).

The effect of the ovary on senescence of daffodil tepals was examined in a number of ways. In the first experiment, three of the six tepals of each flower were removed (four flowers per treatment) and placed in water and their time to senescence compared with the senescence of the other three tepals that remained on the detached flower held in water. The experiment was repeated. In another experiment the complete perianths of four flowers were removed and placed in water and their rate of visual senescence compared with that of whole detached flowers.

The effect of 100 μ*M* ABA on loss of fresh weight of newly opened flowers and the influence of STS and 1-MCP on this loss was examined by placing flowers in triplicate into each of the treatments. Flowers were pre-treated with STS prior to placement into ABA by placing their cut ends in a 1-m*M* solution of STS for 1 h. Flowers were pre-treated with 1-MCP at 20°C for 6 h prior to placement into ABA by initially holding their cut ends in water and sealing the flowers in a glass chamber into which 500 nL l^{-1} 1-MCP gas was released from the calculated weight of bound 1-MCP (EthylBloc; BioTechnologies for Horticulture, Inc. Walterboro, NC). Each day the flowers in the different treatments were weighed and their extent of perianth senescence recorded as either 'onset', '50% senescent', or 'corona collapse'. The experiment was repeated again in triplicate, except for the STS-treatment which was not repeated. The ability of lower concentrations of ABA to accelerate perianth senescence of detached flowers was tested by placing duplicate flowers in either 0, 10 or 100 μ*M* ABA and recording the time to senescence symptoms.

The effect of DPSS on flower longevity was examined by holding the cut ends of five daffodil flowers in 0.8 m*M* DPSS/2.6 m*M* citrate for 0, 0.5, 3, or 24 h. Controls were held in 2.6 m*M* citrate or water for the same time. All the flowers were then transferred to water and their stage of perianth senescence recorded daily. On day four the tepals of a duplicate set of five flowers that had been pulsed for 0.5 h with DPSS were harvested and their ABA content determined. As a positive control, carnation 'Jun' stems (10 flowers per treatment) were also pulsed with DPSS for 0 or 0.5 h and the time in days for their flower petals to completely brown recorded.

The effect of sodium tungstate on daffodil flower longevity was tested on both detached flowers and individual tepals. For detached daffodil flowers, three flowers per treatment were held with their cut ends in 0, 10 or 100 μ*M* sodium tungstate and their extent of perianth senescence recorded daily. The effect on tepals was examined by placing the six tepals from each of three flowers into vials containing different concentrations (0, 1, 10, 100, 500, 1000 μ*M*) of sodium tungstate. Each day the extent of tepal discoloration was estimated as a percentage of the total area of the tepal. The experiment was repeated a further two times (three tepals per treatment) with 0, 10, and 100 μ*M* sodium tungstate.

The ability of Sonar® (fluridone) to affect daffodil longevity was examined in detached intact flower buds, immature tepals removed from the flower buds, open flowers, and intact perianths. In the first experiment, the spathes of three unopened buds were split vertically with a razor blade and the immature tepals removed and their cut ends placed into vials containing 0, 0.05, 0.5, 1 or 5 μl l^{-1} Sonar. Each flower had a tepal in each of the treatment regimes. The 0 and 5 μl l^{-1} Sonar treatments were repeated on further sets of immature tepals three times (six replicate tepals per time). In one of the experiments six tepals were also held in 20 μl l^{-1} Sonar. In other experiments, three replicates each of intact buds (cut about 1 cm below the spathe/scape junction), newly opened intact detached flowers, and their isolated whole perianths were placed in water or 5 μl l^{-1} Sonar and the extent of tepal discolouring and/or stage of senescence recorded daily.

The effectiveness of GA_3 at antagonizing the ability of ABA to induce senescence was examined in whole detached flowers, their isolated perianths, tepals and corona. In the first experiment, each of three flowers had its six tepals isolated and placed into each of the following six treatments: water, 1 μ*M* ABA, 10 μ*M* ABA, 1 μ*M* ABA/100 μ*M* GA_3 and 10 μ*M* ABA/100 μ*M* GA_3. In the next experiment, the cut ends of two whole detached flowers and the three isolated tepals from a third flower were placed in 10 μ*M* ABA and another set in 10 μ*M* ABA/100 μ*M* GA_3. In further experiments, the whole corona tissue was isolated and placed with its cut end in 10 μ*M* ABA or 10 μ*M* ABA/100 μ*M* GA_3 (one corona per treatment), and three isolated perianths were placed in 50 μ*M* ABA and three in 50 μ*M* ABA/100 μ*M* GA_3.

Ethylene measurement

Whole detached flowers held in water or ABA were placed in glass chambers ventilated at 0.68 l h^{-1} with dried CO_2- and ethylene-free air. The concentration of ethylene produced by the flowers was determined from a 3-ml sample of the air exiting the chambers using an HNU P-51 photoionization detector after passage through a 1-m activated alumina column fitted to a Carle AGC 111 gas chromatograph (Fullerton, CA). The carrier gas was He at 55 psi and the column temperature was 70°C.

RNA isolation and gel blot analysis

RNA was isolated from tepals harvested from detached flowers held in water or water containing 100 μ*M* ABA for 0, 6, 24 and 48 h. Some flowers were pre-treated with

Table 1. cDNA probes used as molecular indicators of the timing of senescence in daffodil tepals. All eight cDNA probes were previously shown to hybridize to transcripts that accumulated in the perianth of senescing attached daffodil flowers (Hunter et al. 2002).

SAG number	Putative function	cDNA probe (bp)	Accession
DAFSAG1	S1-type endonuclease	409	AF420010
DAFSAG2	cysteine protease	273	AF420011
DAFSAG3	vacuole processing enzyme	517	AF420012
DAFSAG4	auxin transport protein EIR1	406	AF420013
DAFSAG5	subtilisin	766	AF420014
DAFSAG6	putative protein	414	AF420015
DAFSAG7	nitrate transporter	488	AF420016
DAFSAG8	metallothionein-like protein II	434	AF420017

1-MCP before the 48 h ABA treatment. At the above times the tepals were excised, weighed, immediately frozen in liquid nitrogen and stored at −80°C until needed. Total RNA was isolated from the tepal tissue and 20 μg amounts from each sample analysed by northern analysis as described in Hunter et al. (2002). Replicate membranes were probed with one or more of the eight cDNA sequences (Table 1) that were previously shown to hybridize to mRNA transcripts that increased in abundance in the daffodil tepals during their natural senescence (Hunter et al. 2002). If the membranes were reused they were first stripped following the instructions of the Strip-EZ® DNA labelling kit (Ambion, Austin, TX). After the membranes had been probed with the cDNA sequences they were then probed with a rRNA sequence to confirm that each membrane lane contained equal amounts of RNA.

ABA analysis

Newly opened flowers were either left intact on the plant or detached and their cut ends placed in water. Tepals were then collected from the detached flowers after −1, 0, 1, 2, 3, 4, 5 and 6 days. An extra harvest at 8 days was carried out for the tepals of the attached flowers. The tepals were only collected from the bud tissue in the first experiment used for the detached flowers. The tepal samples were weighed, immediately frozen in liquid nitrogen and stored at −80°C until needed. Samples were then lyophilized and extracted with water (water : tissue ratio 10 : 1 v/w) for 16 h at 4°C in the dark. Quantitative ABA analysis was performed on crude aqueous extracts using a solid-phase radioimmunoassay based on a monoclonal antibody (DBPA1) raised against free (S)-ABA, as previously described (Vernieri et al. 1991).

Results

Effect of exogenously applied ABA on daffodil perianth senescence

The perianths of newly opened daffodil flowers that were detached at the base of their ovaries and placed with their cut ends in water became incipiently senescent (onset) at day 4, 50% senescent at day 5, and fully senescent (corona collapse) at day 6 (Fig. 1A). When the flowers were held continuously in 100 μ*M* ABA the perianths senesced earlier – the time to onset and 50% senescence occurred at day 3 and the corona collapsed at day 4. The ABA-treated flowers were more water-soaked than those held in water and showed an earlier and greater decline in the fresh weight of the flower (Fig. 1B).

A

	Flower treatments			
	control	ABA	1-MCP/ABA	STS/ABA
Onset	4± 0	3± 0	3± 0	3± 0
50% senescent	5± 0	3± 0	3± 0	3± 0
Corona collapse	6± 0	4± 0	4± 0	4± 0

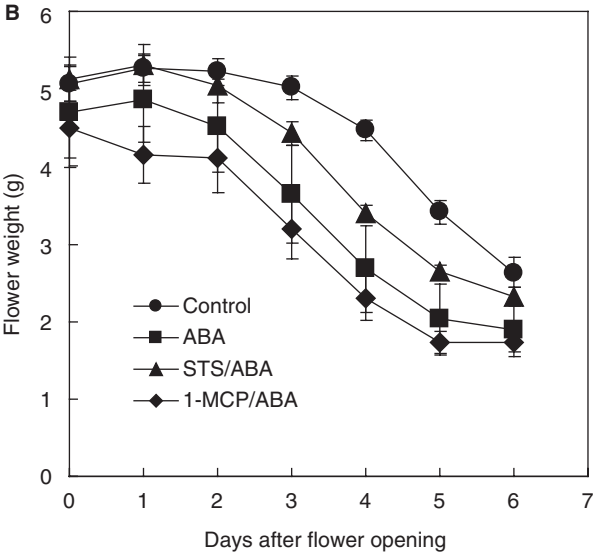

Fig. 1. Effect of ABA on longevity (A) and loss of fresh weight (B) of newly opened daffodil flowers and the influence of ethylene-action inhibitors on the ABA effect. Newly opened flowers were detached at the base of their ovaries and placed with their cut ends in water or 100 μ*M* ABA. Certain flowers were placed in water and pre-treated with 500 nl l^{-1} 1-MCP for 6 h or placed in 1 m*M* STS for 1 h before transfer to ABA. Each day their fresh weights and extent of senescence (time in whole days to 'onset', '50% senescent', 'corona collapse') were recorded. Values are means of three flowers ±SE.

Lower concentrations of ABA (10 μ*M* ABA), also accelerated the senescence of the perianth but the rate of senescence was less than that resulting from 100 μ*M* ABA (data not shown). Pre-treatment of the flowers with a concentration of STS and 1-MCP that successfully prevented exogenously applied ethylene from causing premature senescence of the flower (Hunter et al. 2004) did not prevent ABA from inducing the premature senescence of the flowers (Fig. 1A).

Changes in ABA content of the tepals of attached and detached daffodil flowers during maturation and senescence

The concentration of ABA in the tepals of the attached and detached daffodil flowers increased as the flowers senesced (Fig. 2). The increase occurred at day 3 in the tepals of the detached flowers (Fig. 2A), just prior to the appearance of visible signs of senescence. By day 6, the day when the coronas of the detached flowers collapsed, the tepal ABA concentration was about four times the level it had been in the newly opened flowers. Although the dry weight of the tepals declined as the ABA concentration increased, the decline in dry weight (approximately two-fold) was smaller than the increase in ABA concentration in the tissue indicating that the increase in ABA in the tepals also occurred on a per tepal basis.

The increase in tepal ABA content of the attached flowers did not occur until day 5, which was 2 days after the ABA levels had started to increase in the tepals of the detached flowers (Fig. 2B). This later rise in the ABA content of the tepals coincided with the later appearance of visual symptoms of senescence in the attached flowers. As in the detached flowers the concentration of ABA was highest (approximately four-fold) at the time the corona collapsed, which was the last time point examined. Again the decline in dry weight of the tepals (approximately 1.8-fold) was much less than the corresponding increase in ABA concentration indicating that the increase in ABA was also occurring on a per tepal basis.

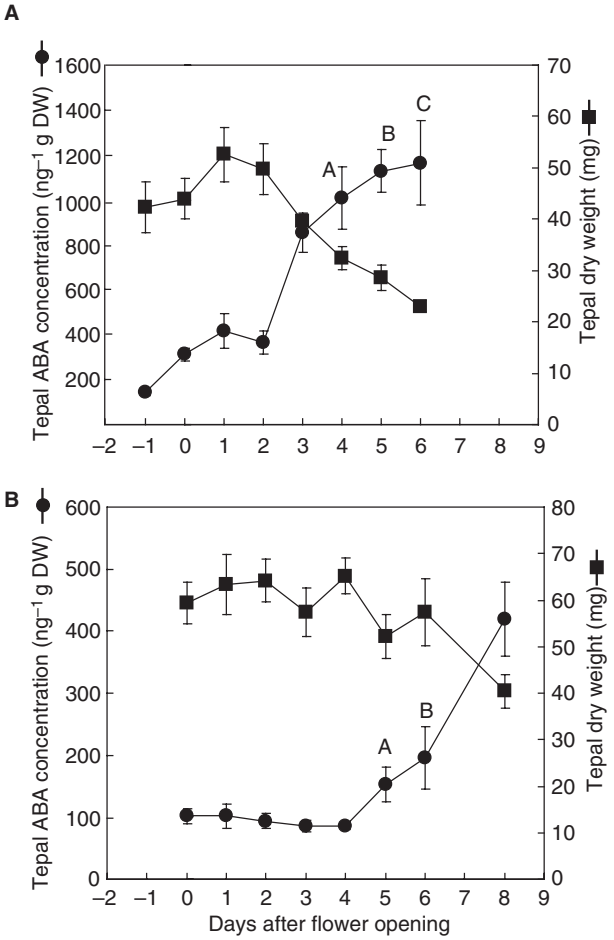

Fig. 2. ABA content of tepals of detached (A) and attached (B) flowers during maturation and senescence. On the specified days after flower opening the tepals of the attached flowers or the detached flowers held in water were collected, weighed, freeze-dried and analysed for ABA. The letters 'A', 'B', and 'C' specify stages of flower senescence that correspond to onset, 50% senescent, and corona collapse, respectively. Values are means of four to five flowers ±SE.

Senescence-associated gene expression in tepals of detached flowers held in water or 100 μM ABA.

The eight cDNA probes used to study changes in transcript accumulation in the tepals of detached flowers held in water or 100 μ*M* ABA over 2 days are listed in Table 1. The expression of the transcripts differed in the tepals over the 2 days (Fig. 3). Some transcripts, such as those encoding a putative metallothionein like protein II, putative protein and cysteine protease, were easily detected in the tepals of the newly opened flowers, whereas others, such as those encoding an S1-type endonuclease, subtilisin and nitrate transporter, were barely detectable. All eight transcripts accumulated in the tepals of the control flowers well before the onset of discoloration and the rise in ABA content (Figs 1 and 2A). The transcripts differed in the rate at which they accumulated. Some, such as those encoding a cysteine protease, and subtilisin, accumulated comparatively quickly, whereas others, such as the S1-type endonuclease and the nitrate transporter, took 2 days to increase noticeably. Certain transcripts such as those encoding a metallothionein-like protein II and an auxin transport protein increased comparatively little in the tepals of the control flowers over the first 2 days.

All eight transcripts accumulated earlier in the tepals when ABA was included in the vase solution, increasing within 6–24 h. The transcripts that increased most in response to the ABA treatment, such as cysteine protease, S1-type endonuclease, and putative protein, were also the transcripts that increased the most in the tepals of flowers held in water.

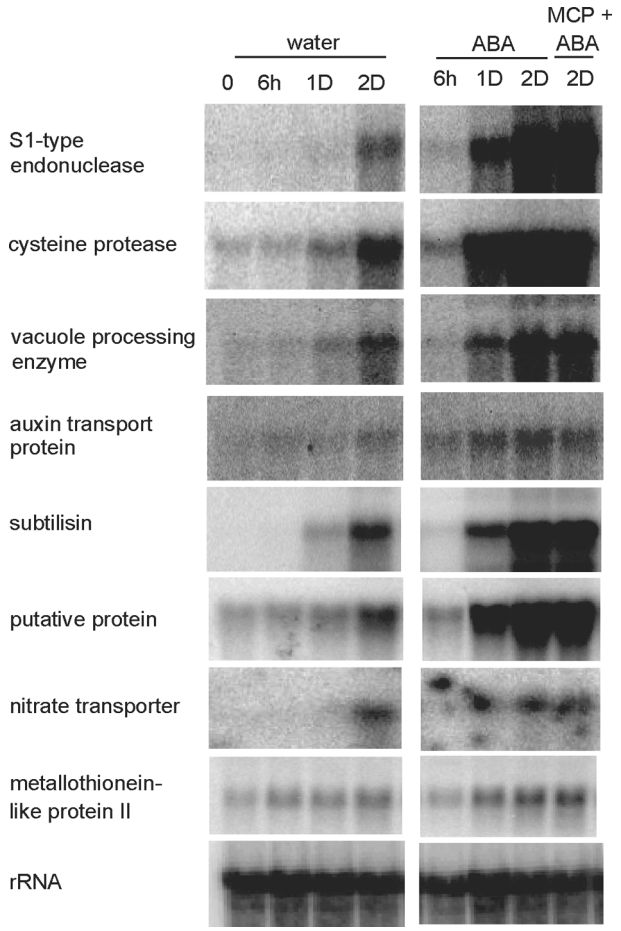

Fig. 3. Effect of exogenously applied ABA on senescence-associated gene expression in daffodil tepals. Newly opened flowers were detached just below the ovary and placed with their cut ends in water or 100 μ*M* ABA. Certain flowers were placed in water and pre-treated with 500 nl l^{-1} 1-MCP for 6 h before transfer to ABA. Total RNA was isolated from their tepals harvested at 0 h, 6 h, 1 day (D) and 2D. Twenty micrograms of RNA from each sample was analysed by northern analysis using the eight cDNA sequences listed in Table 1 as probes. Blots were probed finally with a rRNA sequence to confirm that equal quantities of RNA from each sample had been analysed. Results of a representative rRNA hybridization are shown.

The detached flowers did not produce increased amounts of ethylene when their cut ends were held in 100 μ*M* ABA treatment (data not shown), nor did pre-treatment of the flowers with 500 nL l^{-1} 1-MCP for 6 h prevent ABA from enhancing the accumulation of the transcripts (Fig. 3).

Effect of putative ABA biosynthetic inhibitors and ABA antagonists on flower longevity

The longevity of daffodil flowers was not extended when they were treated with the putative inhibitors of ABA biosynthesis; DPSS, fluridone, and tungstate. When newly opened detached daffodil flowers were placed with their cut ends in 0.8 m*M* DPSS/2.6 m*M* citrate for 30 min and then transferred to water, the flowers did not last longer than the controls (Table 2). However, this same treatment substantially increased the longevity of cut carnation flowers (Table 3). When the daffodil flowers were treated for longer times (3 and 24 h) the cut ends of the flowers discoloured and collapsed. The 24 h pulse was particularly deleterious causing the premature collapse of the corona within 4 days (data not shown). The 30 min pre-treatment of the daffodil flowers with DPSS also did not substantially lower the amount of ABA produced by the flowers (Table 2).

Placing isolated tepal and perianth tissue in water did not alter their rate of senescence from that seen in the detached flower (data not shown). Therefore in certain experiments when tissue was limiting flowers were split into their six tepals and the tepals spread over the various treatments under study. Immature tepals placed in 0.05, 0.5, 1, 5 and 20 μl l^{-1} fluridone (Sonar) did not last longer than those held in water, although fluridone was able to inhibit the yellowing of the tepals that occurred as they developed. Fluridone had no effect when it was supplied at 5 μl l^{-1} to detached buds, whole detached flowers or their isolated perianths (data not shown). Similarly, when tungstate was supplied at 1, 10, or 100 μ*M* it had no effect on the longevity of isolated tepals. At 500 and 1000 μ*M* tungstate accelerated the discoloration of the tepals (data not shown), while, detached flowers senesced earlier when their cut ends were held in 100 μ*M* tungstate (data not shown).

Gibberellic acid was effective at inhibiting the ability of ABA to induce senescence of isolated tepals (Fig. 4). The hormone also inhibited ABA from causing senescence of

Table 2. Effect of DPSS on ABA accumulation and perianth longevity in daffodil and on longevity of carnation flowers. Daffodils and carnations were placed with their cut ends in either water, 2.6 m*M* citrate, or 0.8 m*M* DPSS/2.6 m*M* citrate for 30 min and then transferred to water for the remainder of the experiment. The longevity of daffodils was recorded as time in whole days to corona collapse. Values are the mean and standard error of five replicates. The ABA content of the daffodil tepals was recorded on a duplicate set of five flowers harvested at day 4 (D4). Carnation longevity was recorded as the time to 100% browning of the petals. Values are the mean and standard error of 10 replicates.

	Daffodil 'Dutch Master'		Carnation 'Jun'
	Days to corona collapse	ABA content at D4 (ng g^{-1} DW)	Time to 100% browned petals
Water	5.2 ± 0.2	510 ± 32.9	10.6 ± 0.5
Citrate	5.6 ± 0.3	464 ± 79.8	
Citrate/DPSS	5.6 ± 0.3	430 ± 34.6	23.6 ± 1.5

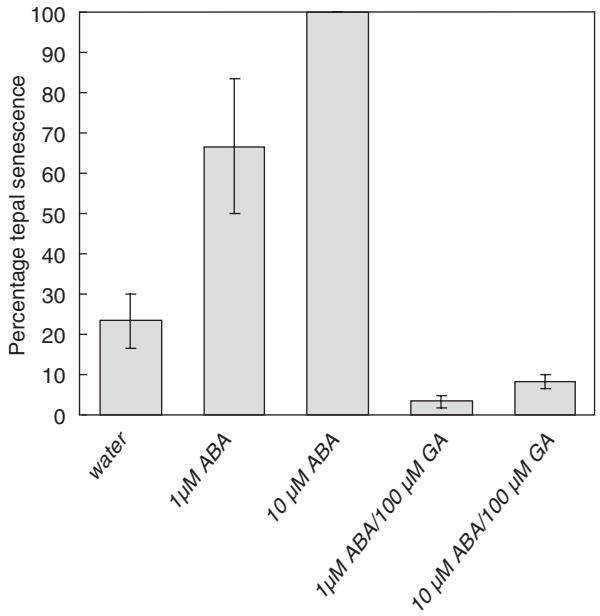

Fig. 4. Antagonism of ABA-induced tepal senescence by GA_3. The six tepals of each newly opened daffodil flower were excised and the cut end of each tepal placed into one of the following treatments: 1 μ*M* ABA, 10 μ*M* ABA, 1 μ*M* ABA/100 μ*M* GA_3, 10 μ*M* ABA/100 μ*M* GA_3. On day 4 the percentage discoloration was estimated. Values are means of three flowers ±SE.

detached flowers and their isolated perianths, although the GA_3/ABA-treated whole flowers and isolated perianths still senesced more quickly than those held only in water.

Discussion

Our results support the view that ABA is a natural regulator of flower senescence. The hormone accumulated in the tepals of the attached 'Dutch Master' flowers as they senesced, and when applied exogenously, the hormone induced both the accumulation of transcripts associated with natural senescence of the flower and the premature senescence of the flower.

ABA accumulated in the tepals in a biphasic manner. It initially increased as the flowers opened and then later as they senesced. The role of this early increase in ABA is not clear. It may have a regulatory role early in flower development which is unrelated to senescence. We know that the early increase in endogenous ABA does not noticeably induce the expression of the senescence-associated genes within the first 24 h of the attached flowers being opened (Hunter et al. 2002) whereas exogenously applied ABA induces accumulation of the transcripts within 6–24 h (Fig. 3). However, it must also be noted that the concentration of the ABA applied (100 μM) was that which has previously been shown to maximally induce an ABA-responsive promoter (Gampala et al. 2001) and so if the physiological levels in the flower are lower it may take longer than 24 h to induce the transcripts.

We attempted to inhibit ABA production in the tepals of the bud by treating with fluridone. Whether we placed intact immature buds or immature tepals in fluridone (Sonar^{TM}) we did not see any effect on tepal or flower longevity although in some instances tepal yellowing was inhibited suggesting that carotenoid synthesis was inhibited. Panavas et al. (1998) also attempted to inhibit ABA synthesis in daylily petals by placing them in 1 mM fluridone and like us they saw no effect on longevity. They also measured ABA levels after fluridone application and did not see any effect on ABA levels concluding that the high levels of carotenoids present in the petals were probably sufficient to provide ample substrate for ABA synthesis.

We found that the second rise in ABA in the tepals of the flowers was tightly linked to the onset of tepal discoloration. Levels of the hormone were higher in tepals of detached flowers at three days and symptoms of discoloration occurred between three to four days. Other researchers have also shown a close association between petal senescence and petal ABA increases (Nowak and Veen 1982, Hanley and Bramlage 1989, Onoue et al. 2000). In some flowers such as carnations, petal ABA increases have been shown to either precede or be coincident with the onset of wilting indicating that ABA could be either important in the early events of the petal senescence program or be produced later as a result of the program. In our study, we used expression of senescence-associated genes to more closely define the relationship between tepal ABA levels and the senescence program. Our results suggest that the rise in ABA occurs after the induction of the senescence-associated genes indicating that in daffodil 'Dutch Master' flowers ABA most likely increases as a consequence of the cellular stresses that occur during senescence and likely functions to help drive the process to completion than trigger it.

The tepal ABA values of the newly opened detached daffodil flowers were higher than the tepal ABA values of the newly opened attached flowers when it would be expected that they should be the same since the physiology of the flowers at that collection point should be the same. We put this down to the variation inherent in measuring ABA from a set of tissue collected at a different harvest time. In our experience in measuring ABA levels this sort of variation is not uncommon.

In some flowers such as carnations and the 'Bronze' miniature rose ABA induces floral senescence indirectly through its effect on increasing ethylene production and perhaps ethylene sensitivity as inhibitors of ethylene synthesis or action prevent the ABA response (Ronen and Mayak 1981; Muller et al. 1999). By contrast, in 'Dutch Master' daffodils, ABA appears to cause floral senescence independently of ethylene as the flowers do not synthesize increased amounts of ethylene following exposure to ABA (data not shown) and pretreatment of them with 1-MCP or STS did not prevent exogenously applied ABA from accelerating their senescence (Fig. 1) or from inducing expression of eight of their

senescence-associated genes (Fig. 3). The inability of 1-MCP and STS at preventing ABA from accelerating senescence was not because we used inadequate concentrations of 1-MCP and STS or treatment times since we have demonstrated that these same treatments were effective at preventing the ethylene-induced premature senescence of the flower, and in the case of 1-MCP, the ethylene-induced accumulation of these same eight transcripts (Hunter et al. 2004). The ineffectiveness of 1-MCP and STS was also not likely to be due to the use of an ABA concentration (100 μM) that was too high as Muller et al. (1999) previously demonstrated that treatment of 'Bronze' miniature roses with 200 $nL\,l^{-1}$ 1-MCP for 6 h was able to inhibit the premature senescence of the rose flowers when their cut ends were subsequently placed in 1 mM ABA. We conclude therefore that ABA is able to cause daffodil flower senescence independently of ethylene action.

It is likely that the significance of ABA in floral senescence will only become clear when ABA biosynthesis or action can be specifically inhibited in floral tissue. Although chemicals that have been successfully used in other tissue systems to inhibit ABA synthesis did not extend daffodil floral longevity, it is possible that these compounds were not effective because of their deleterious effects on cellular integrity (unrelated to ABA) or because they did not alter ABA synthesis. Certainly DPSS did not reduce the ABA content of the daffodil tepals (Table 3).

We were able to show that GA_3 antagonizes ABA action in the daffodil perianth tissue (Fig. 4 and data not shown). It has been known for some time that these hormones can antagonize each others action (Nolan et al. 1987; Rodriguez et al. 1987), although there has been only one study that reported on this antagonism in flowers (Weiss et al. 1995). In this study, exogenously applied ABA was shown to prevent GA responses such as corolla pigmentation, elongation and up-regulation of a chalcone synthase gene in petunia. Our results suggest that the rate of daffodil perianth senescence will be altered by the relative concentrations of GA_3 and ABA in the perianth tissue.

Our study on daffodils adds to the number of flowers (carnations, roses, daylilies) that have been shown to senesce in response to the plant hormone ABA. We have also shown that ABA levels increase in the tepal tissue of the flower well after appearance of transcripts associated with the senescence program suggesting that ABA is produced as a consequence of the senescence-associated cellular stresses and functions to help drive the senescence program to completion. Unlike other ethylene sensitive flowers such as carnations and roses, daffodils senesce prematurely in response to ABA without synthesizing increased amounts of ethylene or requiring ethylene action. The ability of exogenous ABA to cause senescence could be inhibited by treatment of the flowers with GA_3, a hormone which extends the life of detached flowers suggesting that the effect of GA_3 could in part be due to it antagonizing the ability of endogenous ABA to cause senescence.

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