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The role of ascorbic acid in the control of flowering time and the onset of senescence

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Abstract

Ascorbic acid (AA) is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death, and responses to pathogens through a complex signal transduction network. The biological activity of AA is defined by its oxidation and subsequent regeneration into the reduced form. Some studies suggest that the total endogenous level of AA influences induction of flowering and senescence. Both processes require the co-ordinated regulation of gene expression, which is mediated by various phytohormones. For example, gibberellins and salicylic acid are known to promote flowering, but inhibit or retard senescence in *Arabidopsis*. Ethylene and abscisic acid accelerate senescence. Ascorbic acid serves as an important co-factor for the synthesis of some of these hormones. Therefore, it is assumed that AA affects phytohormone-mediated signalling processes during the transition from the vegetative to the reproductive phase and the final stage of development, senescence. This review summarizes recent reports that investigate the effect of AA on flowering time and the onset of senescence. An attempt was made to bring these findings in context with previously characterized flowering and senescence pathways and a model is proposed that may explain how AA influences flowering and senescence both under long- and short-day conditions in *Arabidopsis*.

Key words: *Arabidopsis*, ascorbic acid, flowering, senescence.

Introduction

The small antioxidant molecule vitamin C (L-ascorbic acid, AA) fulfils essential metabolic functions in the life of animals and plants. Some fungi can synthesize erythroascorbic acid, a vitamin C analogue with similar metabolic functions. Among prokaryotes, only cyanobacteria have been reported to have a small AA amount (Arrigoni and De Tullio, 2002).

L-Ascorbic acid serves as a co-factor for many enzymes (Arrigoni and De Tullio, 2000; De Tullio *et al.*, 1999) and it contributes to the detoxification of reactive oxygen species (ROS) (Smirnoff and Wheeler, 2000; Conklin, 2001; Conklin and Barth, 2004). This antioxidant activity of AA is associated with resistance to oxidative stress and longevity both in animals and plants. Furthermore, the endogenous level of AA has recently been suggested to be important in the regulation of developmental senescence and plant defence against pathogens (Pastori *et al.*, 2003; Barth *et al.*, 2004; Pavet *et al.*, 2005). Recent evidence (discussed below) suggests that it may also play a role in floral induction.

The endogenous level of AA is determined by both *de novo* AA biosynthesis and recycling of the oxidized forms of AA, monodehydroascorbate radical (MDA) and dehydroascorbate (DHA) via MDA reductase and DHA reductase, respectively (for a review see Conklin and Barth, 2004). Remarkable progress has been made in the understanding of the biosynthesis of AA. Plants synthesize AA via several distinct pathways including routes via L-galactose (Wheeler *et al.*, 1998) and L-gulose (Wolucka and Van Montagu, 2003). Genes encoding the biosynthetic enzymes of these pathways have been identified (Conklin

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Abbreviations: AA, L-ascorbic acid; ABA, abscisic acid; DHA, dehydroascorbate; GA, gibberellins; JA, jasmonic acid; MDA, monodehydroascorbate radical; PR, pathogenesis-related; ROS, reactive oxygen species; SA, salicylic acid; *SAG*s, senescence-associated genes; *SDG*s, senescence-down-regulated genes.

et al., 1999; Wolucka *et al.*, 2001; Gatzek *et al.*, 2002). Furthermore, two salvage pathways for AA biosynthesis involving D-galacturonic and D-glucuronic acid have been reported (Agius *et al.*, 2003; Nishikimi and Yagi, 1996). It has recently been shown that AA biosynthesis may be regulated by jasmonates (Sasaki-Sekimoto *et al.*, 2005; Wolucka *et al.*, 2005). The significance of this finding will be discussed below.

Due to the fact that AA also serves as an important co-factor in the biosynthesis of many plant hormones, including ethylene, gibberellic acid (GA), and abscisic acid (ABA), one has to assume that the endogenous level of AA will affect not only the biosynthesis, but also the levels and therefore the signalling of these molecules. This will have profound effects on the regulation of developmental processes including senescence, as both ethylene and ABA are known to promote (Nakashima *et al.*, 1997; Weaver *et al.*, 1998) senescence, whereas GA delays it (Goldwaithe, 1972; Manos and Goldwaithe, 1975). Salicylic acid (SA) and cytokinin are also involved in regulating the developmental senescence process. This review will highlight the recent advances in our understanding of how AA may regulate senescence and also floral induction via a network of phytohormone signalling pathways.

The flowering process and the involvement of ascorbic acid in floral induction

The transition from the vegetative to the reproductive and to the final developmental senescence phase is of vital importance for the survival of flowering plants. Flowering is controlled by the developmental age of the plant and environmental signals, including photoperiod, vernalization, light quality, and the availability of water and nutrients (Bernier, 1988; Bernier *et al.*, 1993). Significant progress has been made in understanding floral induction in the facultative long-day plant *Arabidopsis* (reviewed in Komeda, 2004). Four genetic pathways of flowering have been identified through the isolation of flowering time mutants (Fig. 1; reviewed in Komeda, 2004; Corbesier and Coupland, 2005). The strongly inductive long-day (or light-dependent) pathway that only operates in long-day conditions (e.g. 16/8 h light/dark) involves photoreceptors and the circadian clock. The gibberellin (GA) pathway is a more weakly inductive pathway and is essential in short-day conditions (e.g. 10/14 h light/dark). The autonomous pathway is required in both long and short photoperiods. The vernalization pathway requires cold temperatures for flower development. Floral induction causes the up-regulation of floral-meristem-identity genes such as *LEAFY* (Komeda, 2004; Corbesier and Coupland, 2005; Fig. 1).

Two independent lines of evidence suggest a role for AA in floral induction. When grown under long-day conditions (16 h photoperiod), AA-deficient *vtc1* mutants enter the flowering stage before the wild type (Conklin and Barth, 2004), suggestive of an inhibitory effect of AA on the timing of floral induction. In further support of the involvement of AA in floral induction, Mario De Tullio's laboratory has very recently shown that elevation of AA content in *Arabidopsis* via feeding with the AA precursor L-galactono-1,4-lactone leads to an average delay of 5 d in the time to flowering (Fig. 2). Furthermore, expression of a *LEAFY::GUS* transgene in the vegetative apex of *Arabidopsis* is significantly delayed in plants pretreated with L-galactono-1,4-lactone (Fig. 3). As gibberellins activate the *LEAFY* promoter (Blazquez *et al.*, 1998), these results suggest an antagonistic role for AA in *LEAFY* expression.

Interestingly, when *vtc1* is grown under short days, the mutant exhibits a delayed rather than an early flowering phenotype (Pavet *et al.*, 2005; Veljovic-Jovanovic *et al.*, 2001). This discrepancy could very likely be a result of the differing photoperiod and merits further investigation. At present, it can only be speculated as to why flowering is accelerated in *vtc1* under long days and delayed under short days. The delayed flowering phenotype may be explained

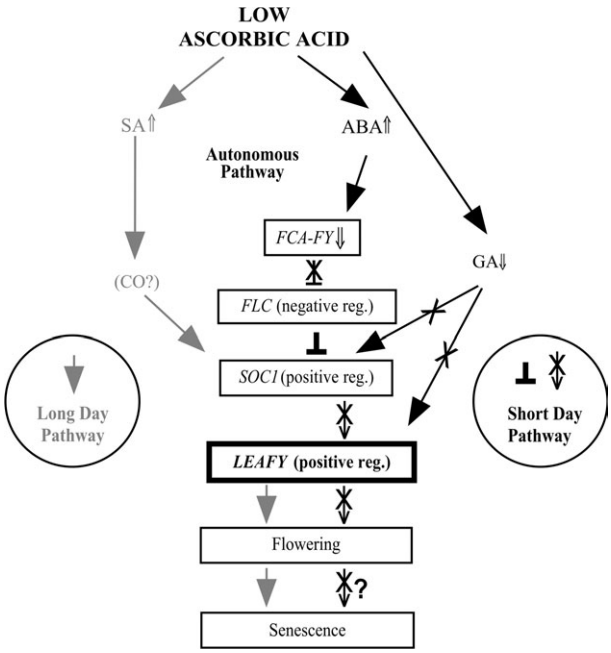

Fig. 1. A simplified diagram illustrating the hypothetical effects of low levels of ascorbic acid in regulating flowering time and senescence via three of the major genetic pathways of flowering in *Arabidopsis*. It is speculated that low levels of ascorbic acid affect the long-day pathway either through alterations in circadian rhythms/photoperiod and/or through elevated levels of SA, thus resulting in early flowering and senescence under long-day conditions. By contrast, ascorbic acid deficiency may inhibit the GA pathway, and therefore delay flowering and senescence under short days. Finally, high levels of ABA present under short days, may delay flowering and senescence via an inhibition of the autonomous pathway. *CO*, *CONSTANS*, *FCA-FY,* negative regulator of flowering repressor *FLC* (*FLOWERING LOCUS C*). *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1)*.

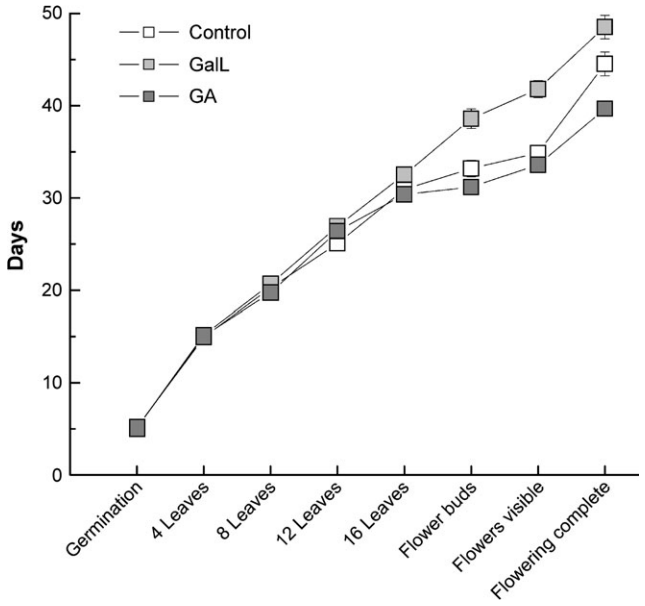

Fig. 2. Flowering is delayed by high levels of ascorbic acid and accelerated by gibberellin (M De Tullio, unpublished results). *Arabidopsis* plants (ecotype Columbia) grown under long days were sprayed every other day with either water (Control), 5 mM L-galactono-1,4-lactone (GalL), the direct precursor of ascorbic acid, or sprayed with 100 μM gibberellin (GA_3). Vegetative and reproductive development was followed and the days required to reach the growth stages indicated were determined. Mean values ±SE of at least 20 plants per treatment are shown. Similar results were obtained in two independent replicates. SE not shown where smaller than symbols.

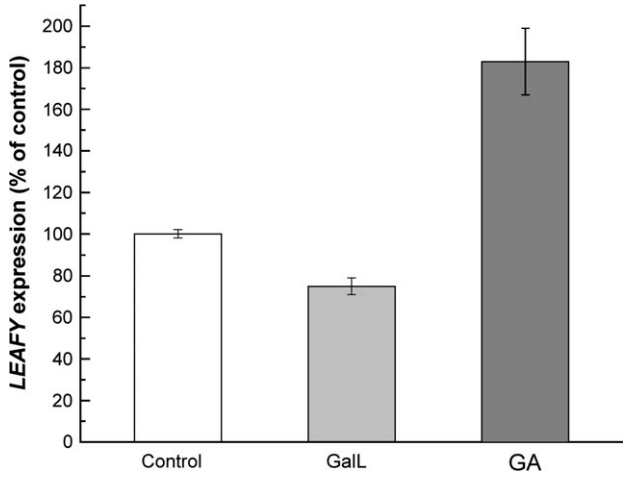

Fig. 3. Expression of *LEAFY* is delayed in the presence of high levels of ascorbic acid (M De Tullio, unpublished results). Plants of *Arabidopsis thaliana* ecotype Columbia transformed with a *LFY::GUS* construct (a kind gift of Professor Detlef Weigel, Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany) were treated every other day with either water (Control), 5 mM L-galactono-1,4-lactone (GalL), or 100 μM gibberellin (GA_3) for 4 weeks and the expression of the GUS fusion protein was quantified. Data are expressed as a percentage of GUS expression in controls. Mean values ±SE of 10 plants are depicted.

by a defect in the GA-mediated flowering pathway that induces flowering under short days through the induction of *LEAFY* (Wilson *et al.*, 1992). *Arabidopsis* harbours three GA 20-oxidase genes, *AtGA20ox1*, *AtGA20ox2*, and *AtGA20ox3* (Phillips *et al.*, 1995). *AtGA20ox1* is mainly expressed in the stem and inflorescence. GA20-oxidase, which is involved in GA biosynthesis but not in GA degradation, requires AA for its activity. Transgenic *Arabidopsis* expressing an antisense copy of *AtGA20ox1* display a delayed flowering phenotype, but only under short-day conditions (Coles *et al.*, 1999). The delayed flowering phenotype of *vtc1* under short-day conditions may, therefore, be explained by a possible deficiency in GA because of limiting GA20-oxidase activity. In addition, a second GA biosynthetic enzyme, gibberellin-3-β-hydroxylase, also requires AA as a co-factor, and a deficiency in this activity could further contribute to a possible GA deficiency in *vtc1*. Kiddle and Foyer have evidence suggesting that *vtc1* has a significant deficiency in GA4 (Kiddle, 2004; Foyer *et al.*, 2006), lending concrete support for this hypothesis. Finally, high levels of ABA in *vtc1*, presumably caused by the up-regulation of the AA-requiring ABA biosynthetic enzyme NCED dioxygenase (Pastori *et al.*, 2003), may contribute to the late-flowering phenotype under short days. This result seems somewhat surprising and contradictory, as the ABA biosynthetic pathway requires AA. However, Pastori *et al.* (2003) speculate that NCED transcripts are up-regulated in response to AA deficiency, perhaps as compensation for the decreased co-factor availability and to increase maximal catalytic capacity. Abscisic acid is known to act antagonistically to GA (in a non-competitive manner; Blazquez *et al.*, 1998; Pastori *et al.*, 2003). Thus, ABA may (indirectly) contribute to the down-regulation of *LEAFY*, and hence to the late-flowering phenotype. In summary, AA may play an important yet indirect role in floral induction because of its necessity for GA and ABA biosynthesis. Elevation of ABA under short days in *vtc1* might lead to an increase in FCA–ABA complexes and therefore disruption of FCA–FY complexes. FCA–FY is part of the autonomous pathway and acts to promote flowering by inhibition of floral inhibitor FLC mRNA accumulation. ABA delays flowering via its binding to FCA. In *vtc1*, this predicted decline in active FCA-FY due to elevated ABA could lead to an increase in FLC transcripts and therefore a delay in flowering (Razem *et al.*, 2006; Fig. 1).

A more difficult question to address is why AA deficiency causes early flowering under long days. The GA pathway does not play a role in the regulation of flowering under long days. It is possible that the strongly inductive light-dependent pathway and/or the autonomous pathway is/are altered in the AA-deficient mutant *vtc1*. However, the level of ABA under long day-grown *vtc1* has not been determined so invoking an involvement of ABA (and FCA-FY) would be pure supposition. However, recent experiments support a regulatory role of SA in flowering

(Martinez *et al.*, 2004). These authors' data suggest that SA may regulate flowering, as SA-deficient plants show a late-flowering phenotype both under short and long days. Using a mutant analysis, Martinez and co-workers were able to show that SA regulates flowering under long days in a CO-independent but photoperiod-dependent pathway. Under short-day conditions, SA may regulate flowering time in a photoperiod-independent but FLC-dependent pathway (through direct repression of FLC repression). Finally, Martinez *et al*. (2004) suggested a third FCA-independent pathway. Given these intriguing results, one might speculate that the high level of SA present in *vtc1* grown under long days (Barth *et al.*, 2004), promotes early flowering via the photoperiod yet CO-independent pathway regulated by SA (Fig. 1). Whether or not SA affects the delayed flowering phenotype of *vtc1* under short day conditions is not clear. Pastori *et al*. (2003) did not detect increased activity of PAL, an enzyme required for SA biosynthesis. Furthermore, the SA level in *vtc1* under short days has not yet been determined. Finally, regulation of flowering time in *vtc1* via the GA pathway (and ABA) may dominate over the other flowering induction pathways.

In summary, there is good evidence suggesting a role of AA in regulating the transition from the vegetative to the reproductive phase. However, how AA acts in the various pathways to control flowering requires further investigation.

The senescence process and the involvement of ascorbic acid in the regulation of senescence-associated genes

After flowering and during the production of seeds and fruits, plants undergo senescence. Senescence refers to the final steps leading to the death of the organism. It comprises a highly regulated series of cytological and biochemical events to co-ordinate the degradation of macromolecules and remobilization of nutrients from senescing tissue into reproductive and young organs as well as storage tissues. Leaf senescence may be induced/promoted by external and internal factors. Extreme temperature, drought, nutrient deficiency, ozone, insufficient light, darkness, and pathogen attack are some examples of external senescence-promoting factors (Buchanan-Wollaston, 1997; Noodén, 1988). The age of a plant and developmental stage of reproductive organs are internal factors that affect the onset of senescence.

During senescence, cells undergo highly co-ordinated changes in cell structure, metabolism, and gene expression. In the early stages of senescence, chlorophyll degrades and photosynthetic activity decreases due to a decrease in the expression of, for example, the small subunit of *RUBISCO* and chlorophyll *a*/*b*-binding (*CAB*) genes. These genes are referred to as senescence-down-regulated genes (*SDG*s). Thus, senescence is characterized by a decrease in chlorophyll, as well as in the content of total RNA and protein. This early phase of senescence is followed by an up-regulation of senescence-associated genes (*SAG*s) that aid in the remobilization of biomolecules. At the terminal phase of senescence, plants lose antioxidant capacity and the release of ROS increases (Leshem, 1988; Zimmermann and Zentgraf, 2005). At this final stage of senescence, cells undergo lipid peroxidation, DNA degradation and the nuclei, mitochondria, membranes, and vacuoles disintegrate.

Tremendous progress has been made in deciphering the complex developmental senescence process, particularly with the analysis of the senescent leaf transcriptome by sequencing of more than 10 000 cDNAs that were derived from mRNAs isolated from senescent *Arabidopsis* leaves (Guo *et al.*, 2004). This work revealed more than 130 transcriptional regulators and 182 genes whose products are components of signal transduction pathways in senescent leaves. One hundred and sixteen of these genes are predicted to be involved in protein turnover. In another study, analysis of changes in global gene expression patterns during developmental leaf senescence in *Arabidopsis* has identified more than 800 genes whose transcript abundance increases (Buchanan-Wollaston *et al.*, 2005). This study also revealed significant differences in hormone-regulated gene expression during natural senescence and dark-induced senescence. Furthermore, more than 100 *SAG*s have been isolated from a variety of plant species such as *Arabidopsis thaliana*, asparagus (*Asparagus officinalis* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), oilseed rape (*Brassica napus* L.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.) (Buchanan-Wollaston, 1994; Weaver *et al.*, 1997). Many of these *SAG*s, including proteases, protease regulators, 1-aminocyclopropane-1-carboxylate (ACC) oxidase, RNAases, glutamine synthetases, lipases, and metallothioneins, have senescent-related functions. However, the biochemical function of many of these genes remains to be identified. The gene *SAG12*, encoding a cysteine protease, is exclusively expressed during age-regulated senescence and is not induced by stress conditions. It is therefore believed to be a reliable marker for natural leaf senescence (Grbic, 2003; Lohman *et al.*, 1994; Weaver *et al.*, 1998; Noh and Amasino, 1999). Other *SAG* genes encode pathogenesis-related proteins (*LSC94*, *LSC222*), glutathione-S-transferase, and catalase and have defence-related functions (Buchanan-Wollaston, 1997; Weaver *et al.*, 1997). Furthermore, various transcription factors are differentially regulated during senescence (Chen *et al.*, 2002; Hinderhofer and Zentgraf, 2001; Robatzek and Somssich, 2001). The targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis* have been identified (Miao *et al.*, 2004).

Gene regulation during leaf senescence is very complex, because various environmental cues and endogenous

factors can induce leaf senescence. He and co-workers (He *et al.*, 2001) analysed the regulation of the leaf senescence-specific expression of a reporter construct in 125 *Arabidopsis* enhancer trap lines by six senescence-promoting factors, including ABA ethylene, jasmonic acid (JA), brassinosteroids, darkness, and dehydration. The authors found that one, two, or more of these factors regulate the expression of individual *SAG*s. Furthermore, there is increasing evidence that AA plays an important role in conjunction with various plant hormones in regulating gene expression during senescence.

Oxidative stress, i.e. increased levels of ROS, has been demonstrated to promote the expression of *SAG*s. Navabpour and co-authors (Navabpour *et al.*, 2003) investigated the expression of *LSC54*, encoding a metallothionein protein, and *LSC94*, encoding pathogenesis-related protein 1 (PR1), in response to the ROS generator silver nitrate (Morris *et al.*, 2000; Navabpour *et al.*, 2003) and the ROS quencher AA. Silver nitrate-treated leaves showed strongly elevated expression of *LSC54* and *LSC94* compared with the water control, whereas expression of the small subunit of Rubisco decreased in the treated leaves. However, treatment with AA in addition to silver nitrate abolished an increase in *LSC54* and *LSC94* expression. This suggests that AA is involved in the transcriptional regulation of some *SAG*s during oxidative stress-induced senescence. Consistent with its function during natural senescence, expression of *SAG12* was not elevated under the applied oxidative stress conditions.

More direct evidence for the role of AA in regulating the expression of *SAG*s is provided by a study that revealed an up-regulation of select *SAG* transcripts in the AA-deficient *Arabidopsis* mutant *vtc1* when grown under a long-day photoperiod (16 h; Barth *et al.*, 2004), suggesting that AA-deficiency induces a senescent phenotype. However, the developmental senescence marker *SAG12* was not induced (C Barth, PL Conklin, unpublished results), suggesting that other *SAG*s that play critical roles in cellular degradation and nutrient remobilization accumulate during senescence (e.g. *SAG13* and *SAG15*; Barth *et al.*, 2004). Similarly, it has been reported that *SAG12* is not induced in response to ozone, a known promoter of senescence, whereas other *SAG*s are up-regulated during ozone exposure (Miller *et al.*, 1999). The hypothesis that AA deficiency causes premature senescence is confirmed by the observation that dark-induced senescence occurs more rapidly in leaf discs of *vtc1* than of the wild type (results in faster chlorophyll loss; Barth *et al.*, 2004; Conklin and Barth, 2004). It should be noted that dark-induced senescence may be different from developmental senescence (Buchanan-Wollaston *et al.*, 2005). However, when the gene that is defective in *vtc1*, GDP-mannose pyrophosphorylase, is expressed in antisense orientation in transgenic potato, transgenic lines have a decrease in AA and undergo developmental senescence faster (Keller *et al.*, 1999). In a separate study, the expression of other, not typical *SAG*s, such as *PR-1*, *PR-2*, and *PR-5* (as well as the GSH:GSSG ratio) was also found to be elevated in the AA-deficient *vtc1* and *vtc2* mutants relative to that in the wild type (Pastori *et al.*, 2003; Pavet *et al.*, 2005). However, by contrast, under the conditions of these studies (short-day photoperiod of 10 h), the AA-deficient mutants exhibit delayed (not accelerated) senescence. Based on these results, Pavet and co-workers concluded that the abundance of AA modifies the threshold for activation of plant defence responses via redox mechanisms that are independent of the natural senescence programme. Alternatively, it is quite possible that the shorter photoperiod affects the onset of senescence differently in AA-deficient plants, leading to this difference in the senescence phenotype of *vtc1* under different light regimes. Indeed, in a study of senescence in different *Arabidopsis* ecotypes, senescence of the early flowering ecotypes was significantly accelerated by a long-day photoperiod (Levey and Wingler, 2005).

Ascorbic acid, phytohormones and the regulation of senescence

The plant hormones ABA, SA, jasmonic acid (JA), and ethylene are known to promote senescence, whereas GA is known to inhibit senescence and promote flowering. It is becoming increasingly clear that various hormone-mediated signalling pathways form an interactive network. Some evidence for a link between these plant hormones, senescence-associated processes, and AA is also mounting. However, a more simplified theory linking AA levels, ROS generation, and senescence will also be discussed.

Abscisic acid is commonly known to induce senescence. Evidence has accumulated that connects ABA accumulation to the endogenous AA content and its redox status with regard to the plant response to drought (Zhang *et al.*, 2001; Chen and Gallie, 2004; Hu *et al.*, 2005). As mentioned above, ABA levels are elevated in *vtc1* grown under short days (Pastori *et al.*, 2003). Therefore, it could be predicted that this elevation in ABA levels via alteration of AA would lead to an early induction of senescence. Indeed, the opposite occurs: the mutant has delayed senescence when grown under a short-day photoperiod (Pavet *et al.*, 2005). Clearly, there is a missing piece in our understanding of the role of ABA in senescence induction in a background of low AA. The level of ABA in long day-grown *vtc1* (which senesces prematurely) is not known. Therefore, it is not possible at the present time to connect ABA levels to the timing of senescence and AA levels using the *vtc1* mutant as a model.

The level of SA and the presence/absence of SA signalling components correlate in a positive manner with senescence and with the expression of *SAG*s (Quirino *et al.*, 1999; Morris *et al.*, 2000; Pegadaraju *et al.*, 2005). For example, mutant plants accumulating SA, such as *cpr5* and

ssi2, grown under long days, (Bowling *et al.*, 1997; Shah *et al.*, 2001), contain high basal levels of *SAG13*, *SAG21*, and *SAG27* transcripts (Pegadaraju *et al.*, 2005) and exhibit premature senescence. As mentioned above, SA levels are elevated in the AA-deficient mutant *vtc1* when grown under long-day conditions (Barth *et al.*, 2004). It is assumed that the low AA level promotes (part of) the senescence programme and that the elevated level of SA is thus an indirect effect. However, the fact that low AA directly induces SA synthesis cannot be excluded and remains to be investigated. Furthermore, and in contrast to the findings cited above and to the presented hypothesis (Fig. 1), SA has also been reported to inhibit senescence, presumably through the inhibition of ethylene biosynthesis (Huang *et al.*, 1993). Therefore, the precise role of SA in regulating flowering and senescence requires further investigation.

In addition to the stress hormones ABA and SA, JA has also been shown to have a role in controlling gene expression during senescence (He *et al.*, 2001; Navabpour *et al.*, 2003). Exogenous application of JA caused premature senescence in attached and detached *Arabidopsis* wild-type leaves. By contrast, JA treatment of the JA-insensitive mutant *coi1* did not result in such changes, suggesting that the JA-signalling pathway is required to promote leaf senescence. Interestingly, with the use of cDNA microarrays, Sasaki-Sekimoto *et al*. (2005) found that among the transcripts affected by jasmonates are genes involved in AA biosynthesis and recycling. In fact, the *VTC1* and the *VTC2* genes, both of which are involved in AA biosynthesis, and the AA recycling enzyme MDAR were significantly up-regulated in JA-treated samples, strongly supporting a role of JA in the metabolism of AA. In support of this idea, Wolucka *et al*. (2005) recently reported an increase in *de novo* synthesis of AA in methyl jasmonate-treated *Arabidopsis* and tobacco Bright Yellow-2 suspension cells. Whether or not the regulation of AA biosynthesis and metabolism by JA is related to the developmental senescence programme remains to be determined.

Finally, high levels of GA have been shown to inhibit senescence (Goldwaithe, 1972; Manos and Goldwaithe, 1975). However, the GA-deficient *Arabidopsis* mutants *ga1-3* and *ga1-6*, which never flower or exhibit delayed flowering, respectively, exhibit delayed senescence compared with the wild type in short days (Wilson *et al.*, 1992). In accordance with this observed relationship between low GA levels and delayed senescence in short day-grown *Arabidopsis*, the delayed senescence in *vtc1* grown under short days (Pavet *et al.*, 2005) could be explained, at least in part, by a GA deficiency in this AA-deficient mutant. By contrast, long-day conditions have been shown to result in a decline in the GA_1 level in vegetative tissue with increasing photoperiod in G2 dwarf pea plants, which therefore senesce faster (Zhu and Davies, 1997). However, whether or not this scenario occurs in *vtc1* under long-day conditions (in which this mutant has accelerated senescence) remains to be tested. Moreover, this speculation implies that high levels of GA would accelerate senescence, which is in contrast to reports in the literature (Goldwaithe, 1972; Manos and Goldwaithe, 1975). Finally, interactions of GA with the senescence inhibitor cytokinin and other regulatory genes in the GA response pathway have to be taken into account as well (Greenboim-Wainberg *et al.*, 2005).

Ascorbic acid deficiency, photoperiod, flowering, and senescence: a connection

It is well known that senescence correlates with loss of antioxidant capacity and consequently with an increase in ROS (Leshem, 1988; Zimmermann and Zentgraf, 2005). According to the 'free radical theory of ageing' proposed by Harman in 1956 (Harman, 1956), it would be predicted that low levels of AA result in a pronounced production of ROS compared with the wild type regardless of the growth conditions. As a result, damage of the photosynthetic apparatus would be increased, leading to a faster decline in photosynthetic activity in AA-deficient tissue, and thus accelerate senescence. Leaf senescence is triggered by a decline in photosynthetic processes (Hensel *et al.*, 1993). However, alterations in photosynthetic activity, antioxidant capacity and the amount of ROS produced have been reported neither under long (C Barth and PL Conklin, unpublished results) nor under short-day conditions (Veljovic-Jovanovic *et al.*, 2001; Pastori *et al.*, 2003). Hence, it appears that the flowering and senescence phenotypes linked to the endogenous AA content are not connected to changes in photosynthetic capacity. However, it cannot completely be ruled out that there are changes in ROS levels or photosynthesis in *vtc1*. It is possible that AA deficiency causes very subtle and gradual changes in these processes that may be difficult to detect. Moreover, photosynthetic activity has not yet been measured over the course of development in *vtc1*.

A possible role of ROS in the induction of senescence raises another important question that is still a matter of debate. Can senescence be considered a form of apoptosis or programmed cell death (Zimmermann and Zentgraf, 2005, and references therein)? If ROS are mainly involved in the induction of cell death, but not in developmental senescence, then that could possibly explain the reported phenotype by Pavet *et al*. (2005). However, these authors did not observe elevated levels of ROS in *vtc1*.

Based on the data discussed in this review, it is hypothesized that low levels of AA cause accelerated flowering and senescence under long-day conditions and delayed flowering and senescence under short-day conditions through alterations in phytohormone levels that are at least partially dependent on photoperiod (Fig. 1). It is hypothesized that the low levels of AA cause GA deficiency and that the GA flowering pathway is favoured under short-day conditions, leading to delayed flowering and presumably

also delayed senescence (see above). High levels of ABA may contribute to the delayed flowering of *vtc1* through the autonomous pathway (Fig. 1). Furthermore, under short days, senescence is delayed. By contrast, under long days, flowering and senescence are accelerated in *vtc1*. High SA content and decreased levels of GA may, in a photoperiod-dependent manner, result in early flowering and senescence under low AA conditions. It has been suggested that a low GA level, in conjunction with increased levels of auxin, is important for increased partioning of nutrients from the vegetative into reproductive organs (Zhu and Davies, 1997).

In conclusion, an attempt has been made to connect some very intriguing observations that have been reported for the AA-deficient mutant *vtc1* in terms of flowering time and the onset of senescence. In doing so, some light has been shed on the role of AA in controlling flowering and senescence. Due to its essential function as a co-factor for the biosynthesis of GA and ABA, AA appears to influence not only the endogenous level but also signalling of these plant hormones, and thus affect developmental flowering and senescence in a presumably photoperiod- and/or circadian rhythm-dependent manner. Other hormones such as SA (and ethylene) may also be affected by AA. In addition, the redox status of AA may play a role in signalling in this interconnected phytohormone network. However, there are obviously still large gaps to fill in order to elucidate the precise role of AA in regulating the final stages of plant development.

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