Effects of Cytokinin Production under Two *SAG* Promoters on Senescence and Development of Tomato Plants

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Received: February 7, 2006; Accepted: April 26, 2006

Abstract: Two promoters of senescence-associated *Arabidopsis* genes, *SAG12* and *SAG13,* were used in tomato plants to express *IPT* that catalyzes the rate-limiting step in cytokinin biosynthesis. Expression of these heterologous promoters in tomato plants was analyzed using the reporter gene β-glucuronidase. Both promoters are expressed in tomato leaves in a manner similar to their expression in *Arabidopsis* plants. The *SAG12* promoter is very specific to senescing leaves, whereas the *SAG13* promoter is expressed in mature leaves prior to the onset of visible senescence and its expression increases in senescing leaves. Expression of both promoters in tomato tissues other than leaves was very low. *IPT* expressed under the control of *SAG12* and *SAG13* promoters (P_{SAG12}*::IPT* and P_{SAG13}*::IPT,* respectively) resulted in suppression of leaf senescence and advanced flowering, as well as in a slight increase in fruit weight and fruit total soluble solids (TSS). However, expression of P_{SAG13}*::IPT* also led to stem thickening, short internodal distances and loss of apical dominance. In contrast to the autoregulation of P_{SAG12}*::IPT, *P_{SAG13}*::IPT* is expressed at higher levels in mature leaves. This difference is likely due to P_{SAG13}*::IPT* exhibiting two phases of expression – a senescence-independent expression prior to the onset of senescence that is not subjected to autoregulation by cytokinin, and enhanced expression throughout senescence which is autoregualted by cytokinin. This moderate different autoregulated behavior of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* markedly influenced plant development, emphasizing the biological effects of cytokinin in addition to senescence inhibition.

Key words: Cytokinin, IPT, promoter, SAG, senescence, tomato.

Abbreviations:
SAG: senescence-associated gene
TSS: total soluble solids
IPT: isopentyl transferase
GUS: β-glucuronidase

Plant Biol. 8 (2006): 579 – 586

DOI 10.1055/s-2006-924240 · Published online August 1, 2006
ISSN 1435-8603

Introduction

Leaf senescence is an active orderly process characterized by a reduced rate of photosynthesis, loss of chlorophyll, leaf yellowing, degradation of proteins, and recycling of nutrients to young tissues (Gan and Amasino, 1997; Smart, 1994). Leaf senescence is regulated by the combined action of external and internal factors such as light intensity, nutrient availability, leaf age, and reproductive stage (Gan and Amasino, 1997; Smart, 1994). Plant hormones such as auxins, gibberellins, ethylene, abscisic acid, and cytokinins are also involved in the regulation of senescence. Cytokinins, which are thought to be synthesized mainly in the roots and transported to the shoots via the xylem (Gan and Amasino, 1996), have been implicated in several aspects of plant development and are key components in the regulation of plant senescence (Buchanan-Wollaston, 1997; Gan and Amasino, 1997; Nam, 1997; Gan and Amasino, 1996; Santokh et al., 1992 a, b; Van-Staden et al., 1988). Endogenous cytokinin concentrations decrease in senescing plant tissues (Nooden et al., 1990; Van-Staden et al., 1988; Skene, 1975) and exogenous application of cytokinin retards senescence (Gan and Amasino, 1996).

Transgene-encoded cytokinin biosynthesis was initially studied in tobacco *(Nicotiana tabacum)* using constitutive or inducible over-expression of the *IPT* gene from *Agrobacterium tumefaciens,* which encodes isopentenyl transferase. This enzyme catalyzes the rate-limiting step for the addition of dimethylallyl to the N6 of 5-AMP to form isopentenyl AMP, which is the precursor of all other cytokinins (Chen, 1997; McGaw and Burch, 1995). Over-expression of the *IPT* gene in transgenic plants led to elevated foliar cytokinin concentrations and delayed leaf senescence. However, the high cytokinin levels were largely detrimental to growth and fertility (McKenzie et al., 1998; Wang et al., 1997; Hewelt et al., 1994; Van Loven et al., 1993; Li et al., 1992; Smart et al., 1991; Smigocki, 1991; Medford et al., 1989). Gan and Amasino (1995) devised autoregulated cytokinin production that delayed leaf senescence in transgenic tobacco plants without altering the plant phenotype. This strategy exploited a highly senescence-specific promoter *(P_{SAG12})* from an *Arabidopsis* gene *SAG12* encoding a cysteine protease (Lohman et al., 1994) fused to the *IPT* gene from the Ti plasmid of *A. tumefaciens*. The fused P_{SAG12}*::IPT* gene was activated only at the onset of senescence in the lower mature tobacco leaves (Gan and Amasino, 1995). This resulted in cytokinin biosynthesis in the leaves, which inhibited leaf senes-

cence and consequently repressed further expression of the promoter, thus preventing cytokinin over-production.

The effect of P_{SAG12}*::IPT* expression in transgenic plants has also been assessed in lettuce, petunia, *Nicotiana alata* (Jasmine tobacco), maize, and broccoli (Young et al., 2004; Chang et al., 2003; McCabe et al., 2001; Schroeder et al., 2001; Gan and Amasino, 1996). P_{SAG12}*::IPT* delayed leaf senescence in lettuce, Jasmine tobacco, and petunia, and attenuated petunia corolla senescence. However, it had no effect on leaf senescence in broccoli and maize, and only reversed maize pistil abortion during its flower development (Chen et al., 2001; Young et al., 2004). Nevertheless, the effect of autoregulated production of cytokinin on fruit development has not been investigated.

SAG13 is also a senescence-associated *Arabidopsis* gene, encoding a protein related to short-chain alcohol dehydrogenases, whose expression increases during senescence (Gan, 1995). However, while *SAG12* exhibited no detectable expression until after significant visible yellowing, *SAG13* exhibited clearly detectable expression shortly (2 days) before the onset of visible senescence (Weaver et al., 1998; Gan, 1995).

The aim of this study was to examine whether the *Arabidopsis* P_{SAG12} and P_{SAG13} promoters could be used as senescence-specific promoters in tomato plants and to study the effect of *IPT* expressed under these promoters on tomato leaf senescence, and plant and fruit development.

Materials and Methods

Tomato transformation

Transformation was performed with MP-1, a tomato *(Lycopersicon esculentum)* line known for its high transformation efficiency (Barg et al., 1997). The transformation procedure was essentially as described by McCormick (1991) with P_{SAG12}*::GUS* and P_{SAG12}*::IPT* (Gan and Amasino, 1995). Similar to P_{SAG12}*::GUS* and P_{SAG12}*::IPT*, P_{SAG13}*::GUS* and P_{SAG13}*::IPT* were constructed by replacing the P_{SAG12} with P_{SAG13} (Gan, 1995). T0 and T1 independent transgenic plants were analyzed using the polymerase chain reaction (PCR) for the presence of *IPT* and *GUS* genes. Homozygous plants were identified by segregation analysis on a medium with kanamycin sulfate (50 – 100 mg L^{-1}).

Analysis of GUS *(β-glucuronidase) activity*

GUS activity was determined fluorometrically as described in Jefferson et al. (1987).

RNA extraction and cDNA preparation

Samples from wild-type (MP-1), P_{SAG12}*::IPT* (529-9, 529-19) and P_{SAG13}*::IPT* (766-13, 766-20) independent tomato lines were collected from mature and old leaves of plants grown under regular fertilization conditions in a greenhouse. The samples were immediately frozen in liquid nitrogen and stored at – 80 °C. Total RNA was isolated from the leaves using the TRI-REAGENT (Molecular Research Center. Inc.) according to the manufacturer's protocol. To prepare cDNA for real-time PCR, the RNA was treated with DNase (RQI-RNase free DNase, Promega Corporation, USA) and tested as a template in a PCR amplification as a negative control. First strand cDNA synthesis was carried out for each tissue by reverse transcription using the Superscript II pre-amplification system (Gibco BRL Life Technologies, UK) according to the manufacturer's instructions.

Real-time PCR analysis

The primers used for the expression analysis of *IPT* (GenBank accession no. X14410) were: CAG GAA AGA CAT CGA CTG CGA and GCT TCC GGT TGA TAG TTG AGG AC (GenBank accession no. X14410). The primers were designed to yield fragments of approximately 100 bp. Quantitative RT-PCR reactions contained the first strand cDNA of each tissue as a template, specific primers, and SYBR Green Master Mix (*PE* BioSystems) in a final volume of 25 µl. Fluorescence increments of each reaction were simultaneously monitored using the GeneAmp 5700 Sequence Detection System (version 1.3, *PE* BioSystems, CA, USA). Amplifications were performed for 40 cycles, consisting of 30 s at 95 °C, 1 min at 60 °C, and 1 min at 72 °C, and an initial pre-heating at 95 °C for 15 min.

Total soluble solids (TSS)

Portions of fully ripened fruits were used for determination of TSS, using an N-1 hand-held refractometer (Atago, Tokyo, Japan).

Photosynthetic activity and chlorophyll and carotenoid contents

The CO_2 uptake rate was measured using a portable photosynthesis measuring system (model Cl-301; CID, Vancouver, WA) in mature leaves under greenhouse natural saturating light (600 to 800 mmol m^{-2} s^{-1} photosynthetically active photon flux density) in the morning (10:00 – 12:00 am).

Chlorophyll and carotenoid contents were determined by acetone extraction according to Zhang and Kirkhan (Zhang and Kirkham, 1996).

Results

Expression of Arabidopsis P_{SAG12} *and* P_{SAG13} *promoters in transgenic tomato plants*

Transgenic tomato plants were generated with the reporter gene β-glucuronidase *(GUS)* joined to P_{SAG12} and P_{SAG13} promoters *(*P_{SAG12}*::GUS* and P_{SAG13}*::GUS)* in order to analyze the expression pattern of the *Arabidopsis* P_{SAG12} and P_{SAG13} in tomato plants. Twenty-six plants with P_{SAG12}*::GUS* and 19 plants with P_{SAG13}*::GUS* were obtained. Expression of P_{SAG12}*::GUS* and P_{SAG13}*::GUS* was determined as a function of leaf age in F2 homozygous plants obtained from several independent transgenic lines for each promoter. P_{SAG12}*::GUS* expression was very specific to senescing leaves, whereas P_{SAG13}*::GUS* had a relatively low expression in non-senescing mature leaves and a higher expression in senescing leaves (Fig. **1**). Expression of P_{SAG12}*::GUS* and P_{SAG13}*::GUS* was also examined in roots, flowers, young and mature fruits, and in seeds of developing and mature fruits (data no shown). In most tissues the observed expression was four to five orders of magnitude lower than in senescing leaves. It was concluded that P_{SAG12} is mainly expressed in senescing leaves of tomato plants while P_{SAG13} is also expressed in mature leaves prior to the onset of senescence.

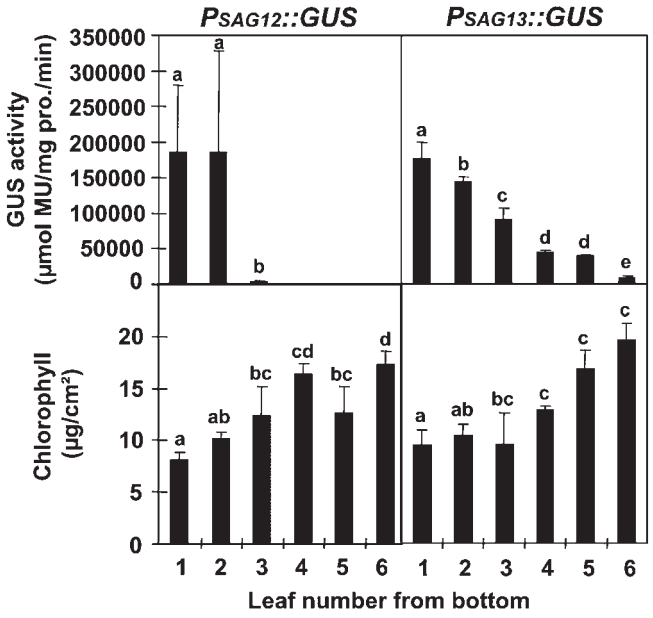

Fig. 1 Expression of P_{SAG12}*::GUS* and P_{SAG13}*::GUS* in transgenic tomato plants. Expression of *GUS* was determined by *GUS* activity in consecutive leaves, starting from senescing leaves at the bottom of the plants as determined by leaf yellowing (not shown) and chlorophyll content. No activity or endogenous fluorescence was detected in leaves of wild-type plants. The results are the mean of two samples ± SD. Similar results were obtained in several repeats from independent plants. Levels not connected by same letter are significantly different (*t*-test; $\alpha = 0.05$).

P_{SAG12}::IPT *and* P_{SAG13}::IPT *delay senescence of tomato leaves*

Transgenic tomato plants were generated with either P_{SAG12}*::IPT* or P_{SAG13}*::IPT*. Northern blot analysis demonstrated that most tested plants exhibited *IPT* expression in mature/senescing leaves (data not presented). However, due to the autoregulated inhibition of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* by the putatively produced cytokinin, mRNA expression could not be relied on as a means for choosing plants with the most effective level of expression of P_{SAG12}*::IPT* and P_{SAG13}*::IPT*. The phenotype of F2 plants homozygous for the transgene was therefore characterized. Independent P_{SAG12}*::IPT* and P_{SAG13}*::IPT* transgenic lines exhibited clear inhibition of cotyledon and leaf senescence throughout plant development (Fig. **2**). Higher photosynthesis rates as well as chlorophyll and carotenoid contents were observed in consecutive leaves of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* plants compared to wild-type plants; the largest difference was mainly manifest in older leaves (Fig. **3**). The autoregulatory nature of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* was observed by RT-PCR analysis, in which expression of both constructs was detected mainly in the lowest senescing leaves (Fig. **4**). However, basal expression of P_{SAG13}*::IPT* in mature non-senescing leaves persisted, as opposed to the complete autoregulated repression of P_{SAG12}*::IPT*.

The effect of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* on dark-induced senescence was also examined. Detached non-senescing leaves of the same age were darkened. After 10 days in the dark, leaves of wild-type plants yellowed and had a low chlorophyll content, whereas the leaves of P_{SAG13}*::IPT* plants were still green and exhibited a higher chlorophyll content (Fig. **5**).

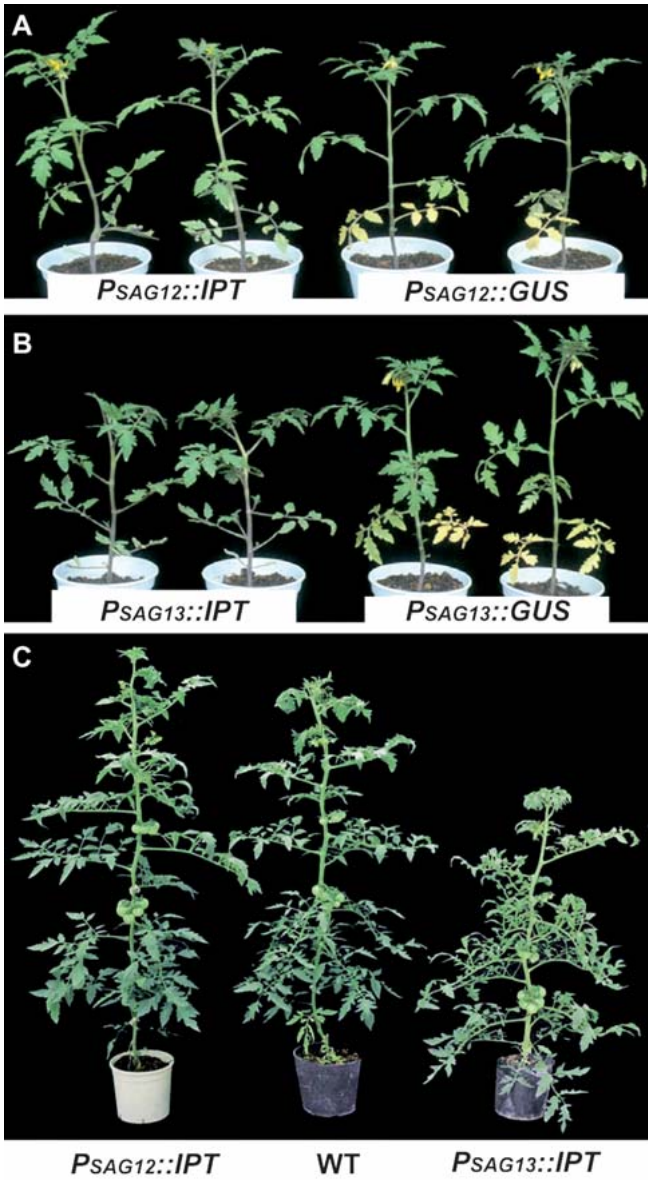

Fig. 2 P_{SAG12}*::IPT* or P_{SAG13}*::IPT* delay senescence of tomato leaves. (**A**) Independent lines of plants expressing P_{SAG12}*::IPT* (529-9 and 529-19). (**B**) Independent lines of plants expressing P_{SAG13}*::IPT* (766-13 and 766-20). Plants expressing P_{SAG12}*::GUS* and P_{SAG13}*::GUS* served as control. (**C**) P_{SAG12}*::IPT* (529-9), P_{SAG13}*::IPT* (766-20), and WT (control) plants after fruit setting. The advanced flowering (demonstrated by the first fruit cluster) and the proximity of fruit clusters (that appear after every three leaves) due to short internodes of P_{SAG13}*::IPT* are apparent.

Developmental effects of P_{SAG12}::IPT *and* P_{SAG13}::IPT

P_{SAG12}*::IPT* and P_{SAG13}*::IPT* plants flowered earlier, with the first inflorescence appearing after the 11th leaf in P_{SAG12}*::IPT* plants and after the 8 – 10th leaf in P_{SAG13}*::IPT* plants, compared to the 12th leaf in the control plants (Fig. **6A**). Otherwise, P_{SAG12}*::IPT* plants appeared normal, whereas P_{SAG13}*::IPT* plants were stunted with shortened internodes and thicker stems (Figs. **2C, 6B, C**), but no differences were observed in the rate of leaf appearance. In addition, P_{SAG13}*::IPT* plants had reduced apical dominance and accumulated anthocyanins in the stem (not shown). The effect of P_{SAG12}*::IPT* and P_{SAG13}*::IPT* on plant longev-

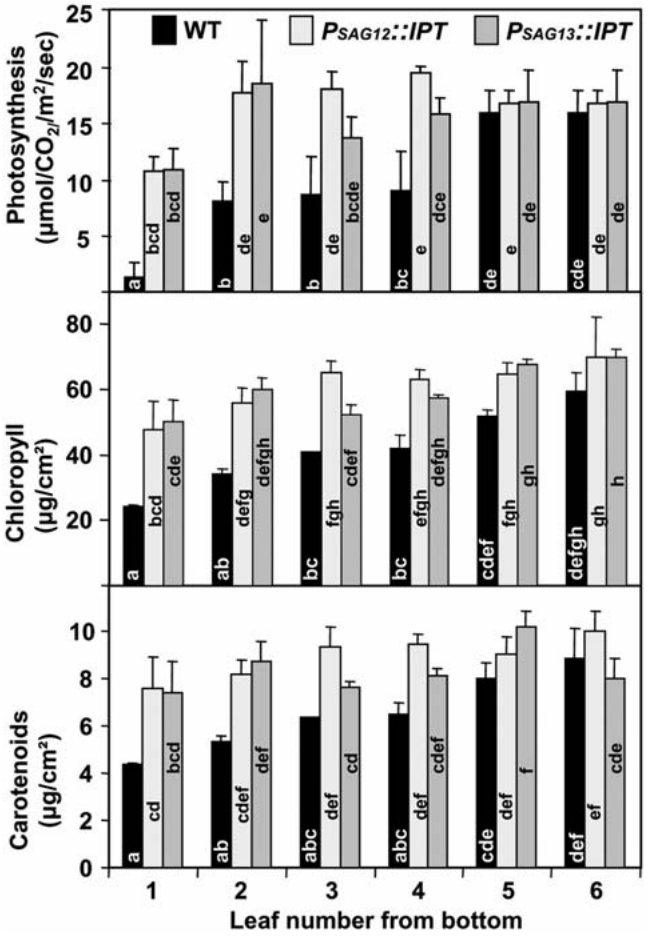

Fig. 3 Photosynthesis rates and chlorophyll and carotenoid contents in P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants. The values are the mean of the measurements of two leaves at each position of each line ± SD. Similar results were obtained with independent P_{SAG12}::*IPT* and P_{SAG13}::*IPT* lines. Levels not connected by same letter are significantly different (*t*-test; $\alpha = 0.01$).

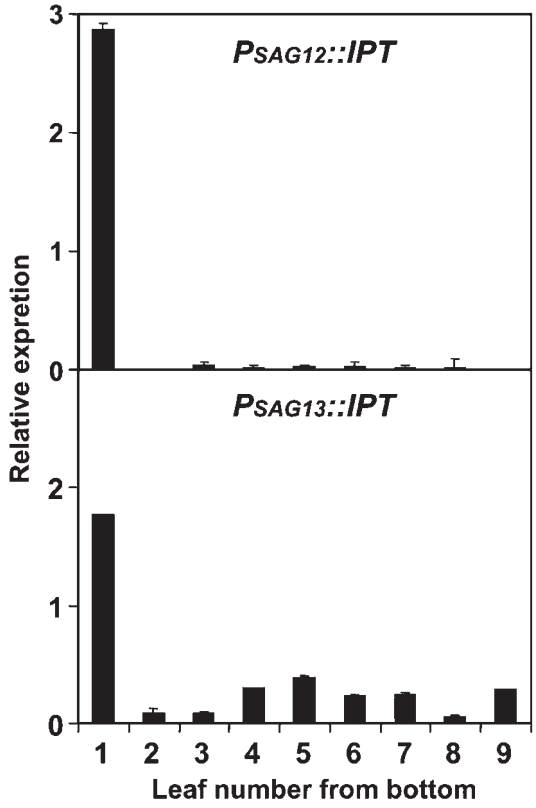

Fig. 4 Expression of P_{SAG12}::*IPT* and P_{SAG13}::*IPT*. Expression levels of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* were determined by real-time PCR in consecutive leaves of 529-9 and 766-20 lines, starting from the bottom of the plants. Data was normalized with that of the rRNA. The results are the mean of three repeats ± SE.

ity could not be examined as MP-1, the wild-type line used in this study, is an indeterminant line.

The effect of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* on fruit development was examined. Fruit number, fruit weight, and total soluble solids (TSS) were measured in five consecutive fruit stalks in transgenic and wild-type plants. No significant differences were observed among the stalks of each line. Total fruit number and fruit weight were not affected, except for a small increase in fruit weight in line 529 – 19 of the P_{SAG12}::*IPT* plants. However, an increase in TSS was observed in both P_{SAG12}::*IPT* lines examined and in one of the P_{SAG13}::*IPT* lines (Fig. **7**). A delay in chlorophyll degradation in the pulp surrounding seeds of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* was also observed (Fig. **8**).

Discussion

In this study, the use of two promoters of *Arabidopsis SAG* genes, *SAG12* and *SAG13,* was compared in tomato plants. The *SAG12* promoter is specifically expressed in senescing tomato leaves. This was also the case in tobacco *(N. tabacum* and *N. alata),* lettuce, petunia, and maize, although expression of IPT under SAG12 did not effect leaf senescence in maize (Young et al., 2004; McCabe et al., 2001; Schroeder et al., 2001; Gan and Amasino, 1997). The *SAG13* promoter is also expressed in tomato plants in a manner similar to that observed in *Arabidopsis,* i.e., its expression begins before the onset of visible leaf yellowing and increases in senescing leaves (Weaver et al., 1998; Gan, 1995). These results support the conservation of *SAG* expression throughout senescence in various plant species. *SAG12* and *SAG13* promoters are also expressed upon dark-induced senescence in detached tomato leaves and retard chlorophyll degradation (Fig. **5**). Expression of IPT under the AGPase S1 promoter in tomato plants also resulted in delayed senescence of detached leaves (Luo et al., 2005). Yet, Chen et al. (2001) found that in detached leaves and florets of broccoli only P_{SAG13}::*IPT* plants exhibited retardation of chlorophyll degradation.

SAG12 and *SAG13* promoters are also expressed to a small extent (four to five orders of magnitude lower than in leaves) in flowers, green fruits (mainly *SAG13*), and in seeds taken from green fruits. However, unlike *N. alata* and petunia (Chang et al., 2003; Schroeder et al., 2001), no effect on flower senescence was observed in tomato, perhaps due to the very low expression in flowers. However, the low expression of *SAG12* and *SAG13* in developing seeds may explain the delayed chlorophyll degradation in the columella around the seeds of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants (Fig. **8**).

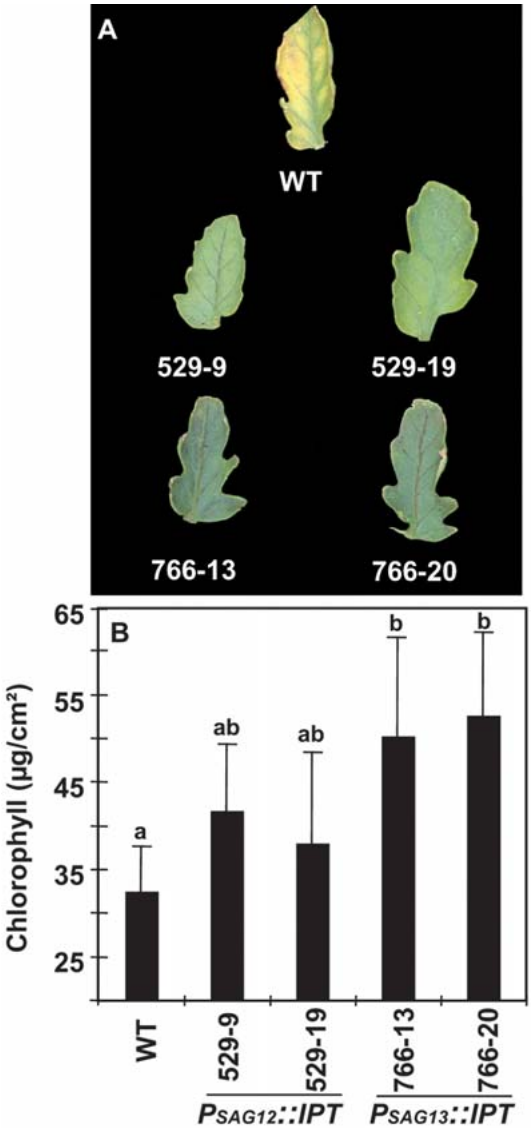

Fig. 5 Delayed senescence of detached leaves of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants. (**A**) Detached mature leaves of the same age were incubated 10 days in the dark. P_{SAG12}::*IPT* plants are represented by lines 529-9 and 529-19 and P_{SAG13}::*IPT* plants are represented by lines 766-13 and 766-20. WT are wild-type control plants. (**B**) Chlorophyll content in the detached leaves after 10 days incubation in the dark. The results are the mean of five leaves ± SD. Levels not connected by same letter are significantly different (*t*-test; $\alpha = 0.01$).

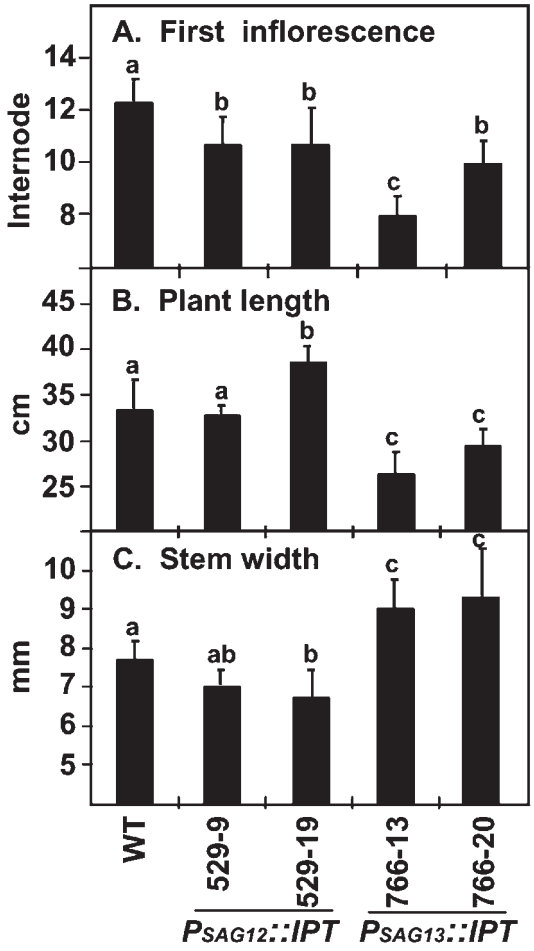

Fig. 6 Developmental effects of P_{SAG12}::*IPT* and P_{SAG13}::*IPT*. (**A**) Appearance of first inflorescence. (**B**) Plant length. (**C**) Stem width, measured between leaves 3 and 4. Stem width and plant length were measured in 2-month-old plants, and are the mean of eight plants of each line ± SD. Levels not connected by same letter are significantly different (*t*-test; $\alpha = 0.01$). WT are wild-type control plants.

The autoregulated behaviour of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* in tomato plants was observed by analysis of *IPT* expression in mature and senescing leaves of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants. High expression of *IPT* under both promoters was observed only in the very old yellow senescing leaves (Fig. **4**). It is assumed that expression of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* was feedback inhibited in upper leaves that exhibit delayed senescence (compared to the control plants). In contrast to P_{SAG12}::*IPT,* low expression of P_{SAG13}::*IPT* was observed in leaves from the upper layers of the canopy of P_{SAG13}::*IPT* plants, indicating that the basal expression of P_{SAG13}::*IPT* in mature non-senescing leaves was not suppressed by cytokinin (Fig. **4**). This could be related to the fact that expression of *SAG13* begins in mature leaves prior to the onset of visible senescence (Weaver et al., 1998; Gan, 1995). Perhaps P_{SAG13}::*IPT* might have two phases of expression, i.e., a senescence-independent expression prior to the onset of senescence that is not subjected to cytokinin autoregulation, and enhanced expression throughout senescence which is suppressed by cytokinin and could be detected only in the very old senescing leaves.

Inhibition of P_{SAG13}::*IPT* expression in senescing leaves contradicts results reported for detached *Arabidopsis* leaves in which cytokinin repressed the expression of *SAG12* but not that of *SAG13* (Noh and Amasino, 1999). The different behaviour of detached and attached leaves has also been shown in relation to the effect of glucose on *SAG12* expression. Glucose inhibited expression of *SAG12* in detached leaves (Noh and Amasino, 1999) but induced expression of *SAG12* in attached leaves (Wingler et al., 2005). These results are in agreement with a recent study presenting similarities but also differences between natural and dark-induced leaf senescence (Buchanan-Wollaston et al., 2005).

Analysis of mature leaves from the upper layers of the canopy of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants demonstrated slightly, but significantly, higher levels of photosynthesis rates as well as chlorophyll and carotenoid contents, compared to the wild-type plants. Naturally, mature non-senescing leaves exhibit developmental decline in their photosynthetic activity (Wingler et al., 1998). Since expression of P_{SAG12}::*IPT* is entirely autoregulated and is strictly confined to senescing leaves, the delayed decline in photosynthetic activity of P_{SAG12}::*IPT* plants suggests that cytokinins are transported to the younger leaves, as shown for tobacco plants (Jordi et al., 2000). Unlike P_{SAG12}::*IPT,* the delayed decline in photosynthetic activity of P_{SAG13}::*IPT* might be attributed to the basal expression of P_{SAG13}::*IPT* in the upper layers of the canopy.

The slightly different expression pattern of *SAG12* and *SAG13* promoters had a profound effect on plant development. P_{SAG12}::*IPT* plants exhibited overall normal growth and development, whereas P_{SAG13}::*IPT* plants were stunted due to short internodal distances and had thicker stems. Lettuce plants expressing P_{SAG12}::*IPT* also had thicker panicles (McCabe et al., 2001). The thicker stem observed in tomato plants could be the result of a mechanism similar to that suggested by McCabe et al. (2001) for lettuce, in which cytokinins increase the internal production of reducing sugars, perhaps by the induction of apoplastic invertases (Balibrea Lara et al., 2004; Roitsch and Ehness, 2000). Accumulation of reducing sugars may result in increased osmotic pressure, increased water uptake, and cell expansion (Bewli and Whitam, 1976), which may lead to stem thickening. Stem thickening due to both cell division and cell enlargement was also observed in tobacco plants expressing *IPT* under the non-specific chalcone synthase promoter (Wang et al., 1997). However, unlike lettuce panicles, stem thickening of P_{SAG13}::*IPT* tomato plants was accompanied by shortening of the internodes, thus implying a trade-off between horizontal and vertical expansion.

In contrast to P_{SAG13}::*IPT* tomato plants and P_{SAG12}::*IPT* lettuce plants (McCabe et al., 2001), P_{SAG12}::*IPT* tomato plants did not have thicker stems, nor has such an effect been reported for P_{SAG12}::*IPT* tobacco plants (Cowan et al., 2005; Jordi et al., 2000; Gan and Amasino, 1995). Furthermore, as opposed to P_{SAG12}::*IPT* lettuce plants (McCabe et al., 2001), no accelerated senescence of young leaves nor delayed flowering were observed in P_{SAG12}::*IPT* tomato and tobacco plants (Cowan et al., 2005; Jordi et al., 2000; Gan and Amasino, 1995). On the contrary, P_{SAG12}::*IPT* tobacco (Cowan et al., 2005) and tomato plants, and more so P_{SAG13}::*IPT* tomato plants, exhibited earlier flowering. The effect of autoregulated cytokinin production on the Solanaceae species (tomato and tobacco) thus appears to be similar.

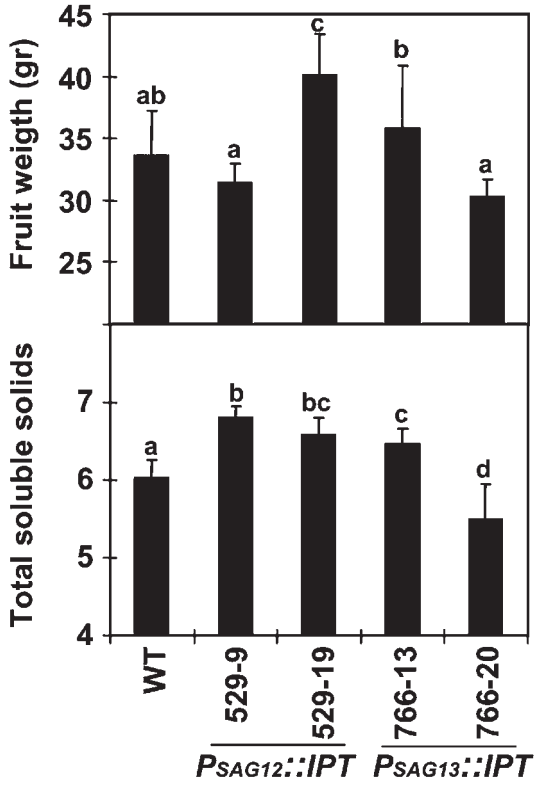

Fig. 7 Effects of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* on fruit development. Fruits from five consecutive stalks of eight plants from each line were examined. Stalks had up to seven fruits each. Hence, the results are the mean of more than 200 fruits from each line ± SD. Levels not connected by same letter are significantly different (*t*-test; $\alpha = 0.05$). No significant differences were observed among the stalks of each line. P_{SAG12}::*IPT* plants are represented by lines 529-9 and 529-19 and P_{SAG13}::*IPT* plants are represented by lines 766-13 and 766-20. WT are wild-type control plants.

Cowan et al. (2005) found that earlier flowering of P_{SAG12}::*IPT* tobacco plants coincided with slow expansion of young leaves. The slow expansion was interpreted to be the result of the observed reduced natural translocation of nitrogen from senescing leaves to young leaves (Cowan et al., 2005; Jordi et al.,

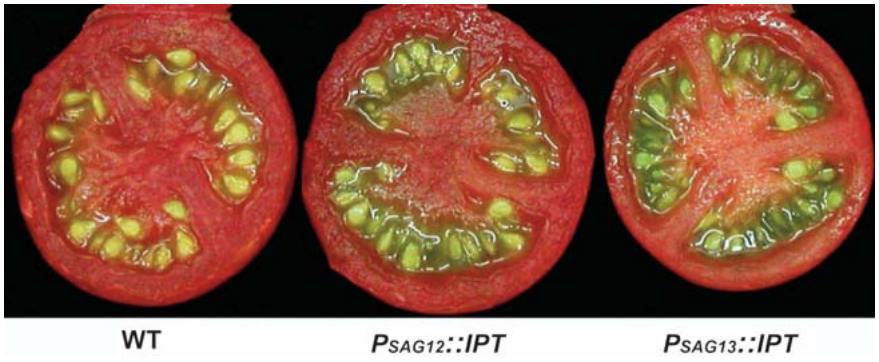

Fig. 8 Chlorophyll in the columella of P_{SAG12}::*IPT* and P_{SAG13}::*IPT* plants.

2000). It is well known that, under nitrogen deficiency, carbohydrates not used in nitrogen metabolism may be used in anthocyanin synthesis that may accumulate in the stems (Taiz and Zeiger, 1998), as was indeed observed in the P_{SAG13}*::IPT* plants. Since cytokinins enhance the sink strength (Cowan et al., 2005; Balibrea Lara et al., 2004; Guivarc'h et al., 2002; Roitsch and Ehness, 2000), it is possible that the basal expression of P_{SAG13}*::IPT* in non-senescing leaves modified the sink-source relations of these leaves, turning them into sinks that, in combination with anticipated reduced translocation of nitrogen, led to anthocyanin synthesis. The advanced flowering, reported for P_{SAG12}*::IPT* tobacco plants (Cowan et al., 2005) and manifested mainly in P_{SAG13}*::IPT* plants, might therefore be the effect of moderate stress conditions imposed by a modified sink-source relationship.

The comparison between P_{SAG12}*::IPT* and P_{SAG13}*::IPT* plants emphasizes the need for timely production of cytokinin in order to delay senescence without additional effects. Having that, a positive effect on fruit yield might also be obtained as a result of delayed leaf senescence, due to potential translocation of cytokinin to fruits, or due to local production of cytokinin in fruits.

Acknowledgements

This research was supported by The Israel Academy of Science Foundation grant No. 582/01, by Binational Agricultural Research and Development (BARD) grant No. IS-2894-97 and IS-3326-02C, and by Binational Science Foundation (BSF) grant No. 97-00250. This manuscript is a contribution from the Agriculture Research Organization, The Volcani Center, Bet Dagan, Israel, No. 135/2005 series.

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Editor: J. T. M. Elzenga