Response of *Nicotiana alata* to Insertion of an Autoregulated Senescence-inhibition Gene

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**Abstract**. *Nicotiana alata* Link and Otto (Jasmine tobacco) was transformed with an autoregulated senescence-inhibition gene construct P\textsubscript{SAG12}–IPT encoding isopentenyl transferase via *Agrobacterium*-mediated transformation. Transformation was confirmed by polymerase chain reaction. Transgenic plants exhibited up to 2- to 4-fold fewer senesced leaves, 29% longer in situ flower life, 26% more shoot dry weight, and a 32% to 50% reduction in flowers per branch. Additionally, transgenics were 28% shorter and had up to 174% more branches, indicative of cytokinin overproduction and a lack of tight autoregulation of P\textsubscript{SAG12}–IPT. Variation among independent transgenics suggests selection for enhanced P\textsubscript{SAG12}–IPT is feasible. Our observations of increased branching and in situ flower longevity, as well as reduced plant height and flowers per branch provide new information on P\textsubscript{SAG12}–IPT and its potential value for biological study and horticultural application.

Leaf senescence is a pressing problem for annual bedding plants like *Amaranthus* L., *Nicotiana* L., *Pelargonium* L. Her. Ex Ait., *Petunia* Juss., and *Zinnia* L. By middle to late summer, plant attractiveness is often compromised by mature senescing leaves, especially on stressed plants. Leaf senescence avoidance and chlorophyll retention is often associated with cytokinin level (Mok, 1994; Nooden, 1988a, 1988b; Van Staden et al., 1988). Recently, molecular methods have advanced our understanding of the role of cytokinins in plant development (Buchanan-Wollaston, 1997; Gan and Amasino, 1997; Klee, 1994; Weaver et al., 1997).

Lohman et al. (1994) identified senescence-associated genes (SAGs) from *Arabidopsis thaliana* (L.) Heynh., one of which has patterns of expression that are highly senescence specific. Messenger RNA from this SAG is detectable only during senescence. Its promoter (P\textsubscript{SAG12}), was fused to a *Agrobacterium tumefaciens* gene encoding isopentenyl transferase (IPT). This construct is referred to as P\textsubscript{SAG12}–IPT (Gan and Amasino, 1995). IPT catalyzes the first, rate-limiting step in cytokinin biosynthesis. This chimeric gene functions as an autoregulated senescence-inhibition system. P\textsubscript{SAG12}–IPT transgenic *Nicotiana tabacum* L. were developmentally and morphologically normal, except for exhibiting delayed leaf senescence and increased seed yield and plant biomass (Gan and Amasino, 1995, 1996). Our study evaluated the potential use of P\textsubscript{SAG12}–IPT to improve the ornamental qualities of *Nicotiana alata* (Jasmine tobacco).

**Materials and Methods**

**Senescence inhibition system**

Gan and Amasino (1995) describe the strategy used to create the autoregulatory senescence inhibition system.

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basal ends of the shoots were inserted 1 to 2 mm into TS medium without BA for rooting. After three weeks, eight rooted plants were transferred to a medium of 1 soil : 1 perlite : 1 sphagnum peat (by volume). At flowering, each of the eight putative transgenic plants and the five WT plants were crossed with N. alata ‘Domino Crimson’ (pollen donor). Seeds from these crosses were obtained from five putative transgenic plants (T1, T2, T3, T5, and T7) and the WT.

Selection for transgenics

Twenty-five milligrams of seed from WT, T1, T2, T3, T5, or T7 were disinfested by stirring for 12 min in a 30% Clorox (v/v, 1.58% NaOCl, Clorox Co.) solution containing one drop of Tween 20 per 100 mL then rinsing three times in sterile deionized water. Seeds were divided into three subsamples and each subsample was sown on the surface of 35 mL of SS medium in 100 × 20-mm petri dishes and the dish sealed with parafilm (American Natl. Can, Greenwich, Conn.). Cultures were maintained in a growth chamber in a completely random design under the conditions described previously. Green (kanamycin-resistant) and white (kanamycin-susceptible) seedlings were counted after 4 weeks in culture. Data were analyzed by chi-square goodness-of-fit tests.

Polymerase chain reaction (PCR) screen

Leaf tissue was harvested from N. alata and stored at –80 °C for 7 d. DNA was extracted from 100 mg of young leaves of WT, T2, T3, T5, T7, and T2xT5 using a plant miniprep kit (DNeasy; Qiagen, Mississauga, Ontario Canada). Tissue was ground with a 1.5-mL pellet pestle (Kontes Glass Co., Vineland, N.J.). No liquid nitrogen was used in extraction steps. Regions of P<sub>SAG12</sub>-IPT that were amplified included a SAG12-IPT gene fragment using a SAG12 forward primer (22-mer) and an IPT reverse primer (19-mer), and an nptII gene fragment using an NPT II gene primer pair (22- and 21-mer) (Table 1). The PCR reaction was prepared containing a final concentration of 1× PCR reaction buffer, 2.0 mm MgCl<sub>2</sub>, 0.2 mm dNTP mix, 0.5 mm of each primer, 100 ng template DNA, 1.5 units of Taq DNA polymerase (Promega, Madison, Wisc.), and distilled water to a total volume of 30 mL. The amplification was carried out in a thermocycler (model 9700; Perkin Elmer Applied Biosystems, Inc., Foster City, Calif.) using 94 °C for 1 min, then 30 cycles of 94 °C for 30 s, 30 s at 54 °C for IPT or 56 °C for SAG12-IPT or 62 °C for nptII, 72 °C for 1 min (nptII and IPT) or 2 min (SAG12-IPT); and finally 72 °C for 10 min. Ten microliters from each PCR reaction was loaded on either a 1% (SAG 12 analysis) or 2% (IPT and nptII analyses) agarose gel and run at 120 V for 1.5 h. Gels were stained with ethidium bromide and the sizes of the amplified fragments were determined by comparison to a 1-kb DNA ladder (New England Biolabs, Beverly, Mass.).

Leaf senescence and flower longevity

**Trial 1.** Twenty green seedlings from the above study for each of WT, T1, T2, T3, T5, or T7 were planted on 30 Aug. 1995 in the growing medium described above. These plants were acclimated and grown to flowering in a glasshouse at the University of Wisconsin, Madison (Ball, 1998). Plants were fertilized weekly with 200 mg L<sup>−1</sup> of a 20N–8.8P–16.6K water soluble fertilizer (Peter’s 20–20–20; Scott’s-Sierra Hort. Products Co., Marysville, Ohio). Thirteen weeks after planting, fertilizer application ceased for 10 plants per accession and continued weekly for the remaining 10 plants per accession. These are henceforth called truncated and continuous fertilizer regimes, respectively. At 16 weeks of age (21 Dec. 1995), when flowering began, in situ flower longevity was determined. Flower longevity was measured as the number of days from flower opening to flower senescence (flower wilting) on the first two open flowers on each plant. At 23 weeks of age (7 Feb. 1996), senesced leaves (leaf 75% or more necrotic) were counted and the plants discarded.

**Trial 2.** Evaluation of leaf senescence was repeated in a polyculture on a new population of plants for seeds sown 6 Feb. 1996. These plants were grown from the same seed lots as in Trial 1 and handled in the same manner. Physiological maturity of these plants (based on the date of first flower opening) occurred 5 weeks earlier than the plants in Trial 1 due to exposure to longer photoperiods, higher natural light levels, and higher temperatures in the glasshouse. Therefore, fertilizer application was stopped on one-half of the plants at 8 weeks of age, and leaf senescence data were collected at 18 weeks of age (5 July 1996).

**Trial 3.** Leaf senescence and in situ flower longevity were measured on a second population of plants grown in a polyculture. Seeds were sown 13 Sept. 1996 and handled as described for Trial 1. Accession T3 was excluded due to lack of seeds. In situ flower longevity was determined starting at 11 weeks of age (26 Dec. 1996) on continuously fertilized plants. At 13 weeks of age, fertilizer application ceased on one-half of the plants. Senesced leaves were counted on 20 Mar. 1997 when the plants were 23 weeks old.

Leaf chlorophyll analysis

Discs were removed from each of the four oldest leaves on 23-week-old plants from Trial 3 using a 1 cm cork borer. Eight plants of WT, T1, T2, T3, or T7 were sampled from the continuous and truncated fertilizer regimes. Discs from each plant were placed in a test tube with 2 mL 96% ethanol in darkness at 4 °C for 48 h. Ethanol was decanted and saved. This process was repeated two more times to obtain a total of 6 mL of ethanol extract. Extracts were analyzed with a spectrophotometer (model DU-50; Beckman Instruments, Inc., Schaumburg, Ill.) at absorbencies of 665 and 649 nm, for chlorophyll <i>a</i> and chlorophyll <i>b</i>, respectively. Chlorophyll content was calculated using the following equations (Wintermans and Demots 1965):

\[
	ext{Chlorophyll} \; \text{content} = \frac{(A_665 – A_649)}{2} 
\]

\[
	ext{Chlorophyll} \; \text{content} = \frac{(A_665 + A_649)}{3} 
\]

\[
	ext{Chlorophyll} \; \text{content} = \frac{(A_665 \times 0.061) + (A_649 \times 0.543)}{1.60} 
\]

Table 1. Segregation in vitro for green (kanamycin resistant) and white (kanamycin susceptible) Nicotiana alata seedlings produced by crossing the parental genotypes into N. alata ‘Domino Crimson’.

| Parental genotype<sup>a</sup> | Green (no.) | White (no.) | Expected ratio | χ² | P |
|-----------------------------|------------|------------|----------------|----|---|
| WT                          | 0          | 201        | 0:1            | 0  | 1 |
| T1                          | 64         | 49         | 1:1            | 1.99 | 0.16 |
| T2                          | 132        | 121        | 1:1            | 0.48 | 0.49 |
| T3                          | 25         | 24         | 1:1            | 0.02 | 0.89 |
| T5                          | 29         | 27         | 1:1            | 0.07 | 0.79 |
| T7                          | 90         | 97         | 1:1            | 0.26 | 0.61 |

<sup>a</sup>WT = wild type and T1, T2, T3, T5, and T7 = putative <i>P<sub>SAG12</sub></i>-IPT transgenic plants.
Chlorophyll \( a = (13.7A_{649nm} - 5.76A_{665nm}) \times 6 \) mL ethanol \( \times 4 \) cm\(^2\) leaf tissue.

Chlorophyll \( b = (25.8A_{649nm} - 7.6A_{665nm}) \times 6 \) mL ethanol \( \times 4 \) cm\(^2\) leaf tissue.

Total chlorophyll = chlorophyll \( a + \) chlorophyll \( b \).

**Growth analysis**

Data were collected on plant height (growing medium surface to top of plant), number of branches, and number of flowers on 23-week-old plants under the truncated fertilizer regime from Trial 3. Shoot dry weight (DW) was recorded on shoots from 23-week-old continuously fertilized plants from Trial 3. Shoots were dried by forced air at 60 °C for 4 d before weighing.

**Data analysis**

Analysis of variance (ANOVA) procedures were used for analysis of quantitative data; the statistical computing package used was SAS Systems for Mixed Models (Littell et al., 1996). For analyses using data from a single trial only, the treatments were fertilizer and genotype; both of these factors were considered as fixed. For analyses using data from two trials (no analyses used data from all three trials), a factor for trial was added to the model. This factor was considered as random. This trial factor was viewed as a block; however, since blocks are not necessarily additive, interactions between trials and fixed factors were assessed for significance. Unless otherwise stated, \( P = 0.05 \) for all tests.

If the fertilizer by genotype interaction was significant, pairwise comparisons between all pairs of genotypes were performed for each fertilizer regime. If the interaction was not significant but the genotype main effect was, pairwise comparisons were conducted for the genotype means (averaged across the fertilizers). Pairwise comparisons, rather than a single comparison of WT versus the mean of the transgensics, were used since independent transgensics performed quite differently.

It was noted early, that use of original data resulted in heteroscedastic variances, in violation of usual ANOVA assumptions. A \( \log_{10} \) transformation resulted in homogenous variances. Thus, our analyses were conducted on the \( \log_{10} \) scale; however, data in the tables and figures are presented as nontransformed data.

**Results and Discussion**

**Selection for transgenics.** Ratios for chi-square tests did not deviate significantly from expected 0:1 or 1:1 ratios (green : white seedlings) for the progeny of WT or the five putative transgenic genotypes, respectively, grown in vitro on selection medium (Table 2). Our predictions for segregation were based on results reported previously for \( P_{SAG12-IPT} \) acting as a monogenic dominant trait (Gan and Amasino, 1995).

**PCR screen.** Successful amplification occurred using primers that target the \( SAG12-IPT \) region in genotypes T5, T7, and \( T2 \times T5 \); the \( IPT \) region in \( T2, T5, T7, \) and \( T2 \times T5 \); and the nptII region in \( T2, T3, T5, T7, \) and \( T2 \times T5 \) (Fig. 1). No PCR-ampified products were detected for WT using any of the primer pairs, \( T3 \) using the \( IPT \) primer pair, or for \( T2 \) or \( T3 \) using the \( SAG12-IPT \) primer pair. Results for \( T5, T7, \) and \( T2 \times T5 \) suggest integration of the full construct occurred, whereas results for \( T2 \) suggest it was missing the \( SAG12 \) promoter terminus. \( T3 \) appeared to be missing the \( SAG12 \) and \( IPT \) segments. All transgensics were nptII positive. Even though \( T1 \) was not confirmed to be transgenic by PCR and \( T2 \) and \( T3 \) were missing segments of the construct, \( T1, T2, T3, T5 \) and \( T7 \) will henceforth be referred to as transgenic.

**Leaf senescence.** In Trial 1, the transgenic plants showed little or no leaf senescence at 23 weeks of age, whereas the WT plants had four to eight senesced leaves at the base of the stem (Figs. 2 and 3). The number of senesced leaves from Trial 1 (Feb. 1996) was affected significantly by a fertilizer \( \times \) genotype interaction (Table 3). WT and \( T3 \) had significantly more senesced leaves under the truncated fertilizer regime than plants that were continuously fertilized, whereas the response of \( T7 \) was reversed (Fig. 3A). Genotypes \( T1, T2, T3, T5 \) and \( T7 \) responded similarly to fertilizer regimes. Overall, the main effect of fertilizer was nonsignificant, whereas the main effect of genotype was significant (Table 3). WT plants had two to 14 times more senesced leaves than the transgenic plants (Fig. 3B). Significant variation existed among the transgenic genotypes for the number of senesced leaves, with mean values ranging from 0.4 to 2.6.
Table 3. Likelihood estimates for the number of senesced leaves on Nicotiana alata as influenced by fertilizer and genotype in Trial 1.

| Source of Numerator Denominator variation | df | df | F |
|------------------------------------------|----|----|---|
| Fertilizer (F)\(^x\) | 1  | 101 | 0.08\(^\text{NS}\) |
| Genotype (G)\(^x\) | 5  | 101 | 22.95\(^****\) |
| F × G | 5  | 101 | 4.34\(^**\) |
| Residual | 101 | | 0.05 |

\(^x\)Continuous or truncated.
\(^x\)Wild type and PSAG12-IPT transgenics T1, T2, T3, T5, and T7.
\(^\text{NS},**,**\)Nonsignificant or significant at \(P \leq 0.05\) or 0.01, respectively.

Leaf senescence in July 1996 (Trial 2) and March 1997 (Trial 3) was influenced significantly by fertilizer × genotype and trial × genotype interactions (Table 4), indicating genotypes responded differently to different fertilizer regimes and trials. The fertilizer × genotype interaction was due primarily to differences in magnitude of response to fertilizer regimes for WT and T7, compared to T1, T2, and T5 (Fig. 4A). Generally, there was a trend for plants under truncated fertilizer treatment to have more senesced leaves than those plants fertilized continuously. Transgenic plants given either fertilizer treatment had fewer senesced leaves than WT given continuous fertilizer. The main effects of fertilizer and genotype were significant (Table 4). Transgenic genotypes had from 2- to 5-

Table 4. Likelihood estimates for the number of senesced leaves on Nicotiana alata as influenced by fertilizer, genotype and trial in Trials 2 and 3.

| Source of Numerator Denominator variation | df | df | F |
|------------------------------------------|----|----|---|
| Fertilizer (F)\(^y\) | 1  | 159 | 8.07\(^*\) |
| Genotype (G)\(^y\) | 4  | 4.99 | 6.23\(^*\) |
| F × G | 4  | 159 | 3.41\(^*\) |

\(^y\)Analysis on log\(_{10}\) transformed data.
\(^*\)Continuous or truncated.
\(^x\)Wild type and putative PSAG12-IPT transgenics T1, T2, T5, and T7.
\(^\text{NS},**,**\)Nonsignificant or significant at \(P \leq 0.05\) or 0.01, respectively.
Table 5. Likelihood estimates for leaf chlorophyll content of Nicotiana alata leaves as influenced by fertilizer and genotype in Trial 3.

| Source of variation | Chlorophyll \( \frac{a}{b} \) (F values) | a | b | a : b | Total |
|---------------------|-----------------------------------------|---|---|------|-------|
| Fertilizer (F)\(^{y}\) | 1 | 108.86 | 103.66 | 47.46 | 119.18 |
| Genotype (G)\(^{y}\) | 4 | 34.10 | 22.27 | 35.84 | 31.15 |
| G × F | 4 | 2.44 | 1.14 | 10.76 | 2.01 |
| Residual | 63 | 3.98 | 1.03 | 0.02 | 8.66 |

\(^{x}\)Evaluation at 23 weeks of age.

\(^{y}\)Continuous or truncated.

Table 5. Likelihood estimates for leaf chlorophyll content of Nicotiana alata leaves as influenced by fertilizer and genotype in Trial 3.

fold fewer senesced leaves than WT plants (Fig. 4B). Leaf senescence of T2, an apparent promoterless transgenic (Fig. 1) was similar to WT. Fertilizer truncation caused a 44.5% increase in senesced leaves over plants given continuous fertilizer (Fig. 4C).

Similar trends in number of senesced leaves were reported with the same chimeric gene in N. tabacum ‘Wisconsin 38’ (Gan and Amasino, 1995); with the IPT gene in N. tabacum ‘Samsun’ using the Glycine max L. heat shock promoter HS6871 (Smart et al., 1991); in Solanum tuberosum L. using the IPT gene and the native IPT promoter (Ooms et al., 1991); and N. tabacum ‘Petit Havana’ using a promoterless IPT gene inserted randomly into the genome (Hewelt et al., 1994).

Number of senesced leaves varied among genotypes of N. alata when trials were done at different times of the year (Table 4). Gan and Amasino (1997) suggested regulation of SAG expression is multifactorial. Thus, growing conditions could cause variation in gene expression. Expression of IPT in G. max under control of an auxin-inducible promoter resulted in differences in leaf senescence between plants grown in a growth chamber and in vitro (Li et al. 1992). Likewise, Scorza et al. (1994) reported response differences due to the rolC gene (gene product: cytokinin-b-glucosidase) in N. tabacum ‘Wisconsin 38’ grown under different photoperiods and irradiance. Thus, further study is warranted to characterize the effects of P\(_{\text{SAG12}}\)-IPT under different environmental conditions.

**Leaf Chlorophyll.** The ratio of chlorophyll \( a \) to \( b \) was influenced significantly by a genotype \( \times \) fertilizer interaction (Table 5). A higher chlorophyll \( a : b \) ratio was observed for WT, T1, and T2 under continuous fertilizer application, whereas the ratio for T5 and T7 was similar under both regimes (Fig. 5). Genotype and fertilizer main effects influenced leaf chlorophyll \( a, b, a : b \) ratio, and total leaf chlorophyll (Table 5). Leaves of transgenic genotypes had higher chlorophyll \( a, b, a : b \) ratio, and total chlorophyll than WT (Fig. 6A–D). Significant variation was found among transgenic genotypes for chlorophyll measured. T7 had the greatest values followed by T5, T2, and T1, respectively.

Continuous fertilization produced leaf tissues with higher chlorophyll values than truncated treatments (Fig. 6E–H).
higher chlorophyll content of transgenics compared to WT suggests the transgene is functioning to delay chlorophyll degradation whether or not the promoter is fully inserted. This information supports our leaf senescence results reported above.

**Flower Longevity.** Comparison of flower longevity in situ of plants grown under continuous fertilizer in a glasshouse in December 1995 (Trial 1) and a polyhouse in December 1996 (Trial 3) resulted in significant main effects for genotype and location/year, and a nonsignificant interaction (Table 6). Flower longevity in situ varied between WT, and T1, T5, and T7, and among transgenics (Fig. 7A). WT and T2 flowers lasted ≈18 d with longevity increasing 2 to 4 d for T1, T7, and T5, respectively. Flowers lasted 4 d longer in the glasshouse in December 1995 than in the polyhouse in December 1996 (Fig. 7B). Our results of increased in situ flower longevity provide new information on P_{SAG12-IPT} affecting floral organs.

**Growth Analysis.** Transgenic plants appeared more compact and dense than WT plants (Fig. 8). Genotypes varied signifi- cantly for shoot dry weight (DW), plant height, number of branches, and flowers per branch, but the number of flowers per plant did not differ among the genotypes (Table 7). In addition, variation existed among WT and transgenics for shoot dry weight, plant height, and number of branches (Fig. 9A–C). Shoot DW ranged from 42 g for WT to 53.1 g for T7 (Fig. 9A). Increases in shoot DW were reported also with P_{SAG12-IPT} in *N. tabacum* (Gan and Amasino, 1995) and with the cytokinin biosynthesis gene T-cyt in *S. tuberosum* (Ooms et al., 1991). Plant height ranged from 46.8 cm for WT to 33.6 cm for T7 (Fig. 9B). Transgenic plants branched more (175.6 to 246.6 branches) than WT (89.6 branches) (Fig. 9C). Branching was more profuse in upper portions of transgenic plants than in WT plants (Fig. 10). Our results with P_{SAG12-IPT} in *N. alata* showed concurrent phenotypes of reduced plant height and increased branching. However, plant height and branching are developmental processes completed usually before the onset of tissue senescence. Our results thereby suggest P_{SAG12-IPT} in *N. alata* was not regulated tightly (i.e. expressed only during tissue senescence) but expressed sufficiently during plant development to cause a reduction of plant height and an increase in branching: responses typically associated with cytokinin over-production (Klee, 1994).

Although the number of branches was greatest in transgenics, the number of flowers did not differ significantly from those observed on WT plants (Table 7 and Fig. 9D). More flowers existed per branch on WT plants (2.2), than on transgenic plants (1.1 to 1.5) (Fig. 9E). Despite these results, the increased number of branches observed on transgenic *N. alata* offers a potential for increasing numbers of flowers per plant. Transgenic *N. tabacum* expressing rolC produced more flowers per plant than wild type controls (Scorza et al., 1994). In our study, transgenics appeared to produce more flowers per plant than WT plants (Fig. 9D). Although means of wild type and transgenic plants were not significantly different, this effect might have been masked by the truncated fertilizer treatment, leading to inadequate nutrients, and less metabolites for allocation to flowering. Stress can promote young flower bud abortion and/or abscission while other organs are only slightly affected (Halevy, 1987). Therefore, further investigation of the influence of P_{SAG12-IPT} on flowering under normal fertilizer application is warranted.
Table 7. F values for shoot dry weight (DW), plant height, number of branches, number of flowers, and flowers/branch on Nicotiana alata as influenced by genotype and fertilizer regime in Trial 3.

| Source of variation | Shoot DW* (g) | Plant ht* (cm) | Branches/† (no.) | Flowers/† (no.) | Flowers/branch† |
|---------------------|---------------|----------------|------------------|----------------|----------------|
| Genotype            |               |                |                  |                |                |
| Genotype            | 4             | 5.41**         | 3.94*            | 6.70**         | 1.48**         |
| Residual            | 36*, 31†, 29* | 37.41          | 34.85            | 3729.67        | 4975.02        | 0.29          |

**Data collected from plants grown under continuous or truncated fertilizer, respectively.
*Wild type and putative \( P_{SAG12-IPT} \) transgenic plants T1, T2, T5, and T7.
†Error degrees of freedom for shoot DW; plant height; and number of branches, flowers, and flowers/branch, respectively.
**Nonsignificant or significant at \( P \leq 0.01 \) or 0.001, respectively.

Fig. 9. (A) Shoot dry weight (DW), (B) plant height, (C) number of branches, (D) number of flowers, and (E) number of flowers per branch on Nicotiana alata as influenced by genotype under a truncated fertilization regime in Trial 3. WT = wild type and T1, T2, T5, and T7 = putative \( P_{SAG12-IPT} \) transgenic plants. Mean separation by pairwise \( t \) tests.

**Phenotype expression.** Variation in phenotype existed among the five transgenic \( P_{SAG12-IPT} \) N. alata genotypes for all traits evaluated in this study, except for the number of flowers per plant. Similarly, phenotype of transgenic clones varied on promoterless IPT and rolC N. tabacum (Hewelt, 1994; Scarza et al., 1994), and T-cyt S. tuberosum (Ooms et al., 1991). The large amount of variation detected among the few transgenic genotypes evaluated in our study indicates potential for varying levels of \( P_{SAG12-IPT} \) expression.

In conclusion, our observations of increased branching, increased in situ flower longevity, reduced plant height, and fewer flowers per branch provide new information on the characteristics of \( P_{SAG12-IPT} \) transformants and their potential value for biological study and agricultural application. Suppression of leaf senescence and increased in situ flower longevity through expression of \( P_{SAG12-IPT} \) in annual bedding plants and perennials would help maintain more aesthetically pleasing plants throughout the growing season. However, studies are needed to determine whether winter hardiness would be compromised in perennials by the transgene altering redistribution of nutrients during senescence and cold acclimation. Potentially, this chimeric gene could reduce leaf loss on indoor tropical foliage plants during acclimation from high light crop production to low light interiorscapes as well. In addition, the shorter, more compact phenotype exhibited by the plants transformed with \( P_{SAG12-IPT} \) opens the possibility for its use in regulating plant growth.

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