Special Research Report #301: Plant Breeding & Genetic Engineering

Genetic Transformation of Petunia for Delayed Leaf and Flower Senescence

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BACKGROUND

The postproduction quality of many ornamentals is affected by leaf and flower senescence. While ethylene causes premature senescence, cytokinins are known to delay senescence. The ability to create transgenic plants with altered cytokinin synthesis has overcome problems associated with the uptake, translocation, and metabolism of exogenously applied cytokinins. Increases in endogenous cytokinin levels can be obtained by transforming plants with isopentenyltransferase (ipt), the gene encoding the rate-limiting step in cytokinin biosynthesis. The ipt gene can be targeted toward senescing tissues using the senescence specific promoter SAG12.

METHODS & RESULTS

Transgenic Plants

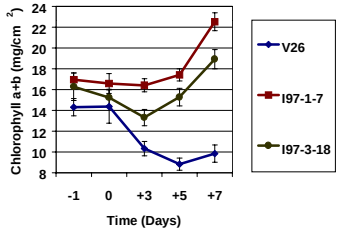
Petunia x hybrida 'V26' was transformed with the P_{SAG12}-IPT gene construct created by Dr. Richard Amasino (University of Wisconsin). Independently transformed lines were identified which exhibited a delayed leaf and flower senescence phenotype. All lines were confirmed to contain the ipt gene and the leaves and flowers had detectable levels of ipt transcript. Enhanced cytokinin levels were detected in two independently transformed lines. This confirmed that the ipt gene was producing a functional enzyme, resulting in increased cytokinin production.

Drought-induced leaf senescence

Petunia plants were grown until flower buds were just showing color. Subsequently, watering was discontinued. After a 24 h period visibly wilted plants were rewatered. Transgenic IPT plants showed little or no visible signs of leaf chlorosis compared to wild type.

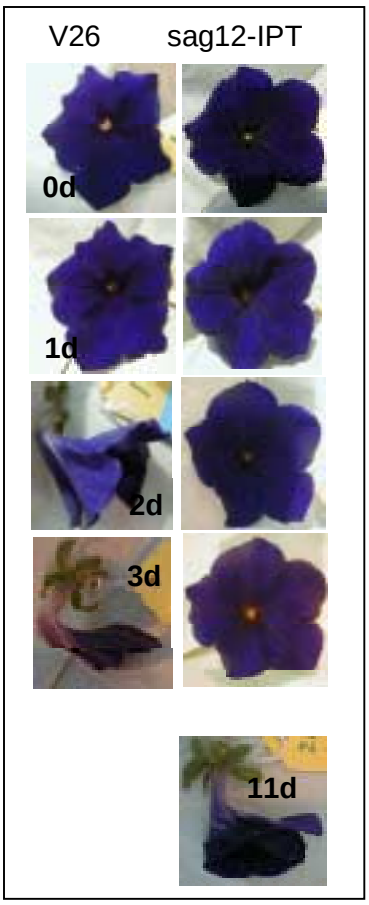
Leaf senescence following drought stress

Chlorophyll was extracted from leaves in order to quantify leaf yellowing and senescence. Transgenic leaves had an increase in chlorophyll while wild type leaves exhibited a steady decline following drought stress.



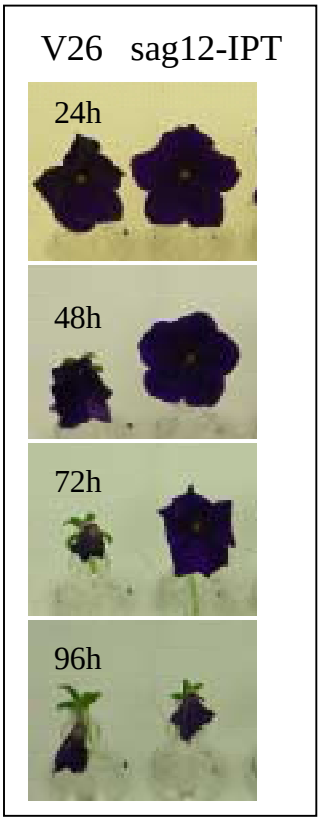
Leaf chlorophyll content following drought stress

Flower longevity
Flowers of sag12-IPT Petunia plants had delayed flower senescence after pollination and during natural aging. Floral senescence was delayed 6 to 9 days relative to wild type flowers.



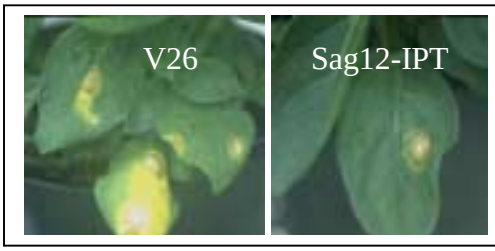
Flowers after pollination (days)

Ethylene sensitivity
Wild type and transgenic Petunia flowers were treated with 2 µl L^{-1} ethylene for 12 h in airtight chambers and then placed in air. Wild type flowers wilted within 24 h, while sag12-IPT flowers did not wilt until 72 h after removal from ethylene. Also, longer exposures to exogenous ethylene were required to induce endogenous ethylene production from transgenic flowers (data not shown).



Flowers at various times after treatment with 2 µl L^{-1} ethylene

Enhanced disease resistance
Wild type V26 and IPT Petunia plants were inoculated with *Cercospora* sp. The development of lesions and leaf chlorosis was more severe on wild type than sag12-IPT plants.



Cercospora infection

CONCLUSIONS

1. Transformation of petunias with sag12-IPT produced plants with enhanced cytokinin production.
2. IPT petunias had delayed leaf and flower senescence.
3. IPT petunias were more resistant to drought stress.
4. IPT petunias had enhanced resistance to Cercospora infection.
5. IPT petunia flowers were less sensitive to ethylene

IMPACT TO THE INDUSTRY

Transgenic Petunia plants have improved post-production longevity and quality. They will be more attractive in the landscape with enhanced bloom display, decreased leaf yellowing following water stress, and increased resistance to disease. Plants will also be able to withstand retail environments where they could be exposed to water stress and/or ethylene.

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