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Genetic Approaches to Improve Cold Tolerance of Petunia

Ryan M. Warner
Department of Horticulture, Michigan State University, East Lansing, MI 48824

American Floral Endowment
Funding Industry Solutions Through Research and Education
Phone: 703-838-5211
Fax: 703-838-5212
E-mail: afe@endowment.org
Website:www.endowment.org

BACKGROUND

Developing frost-tolerant bedding plants will help to expand the early spring and fall bedding plant markets. Unfortunately, few bedding plant crops are strongly frost-tolerant, limiting the crops available for early spring and fall sales.

Freezing tolerance of many species increases following exposure to low, non-freezing temperatures, through a process termed “cold acclimation”. A group of related genes (called “CBF” genes) have been identified as important regulators of this process.

The objectives of this research were : (1) to evaluate freezing tolerance and horticultural performance of transgenic petunia plants expressing the CBF3 gene from *Arabidopsis* and (2) to evaluate freezing tolerance of wild relative *Petunia* species as potential genetic sources of improved cold tolerance.

MATERIALS AND METHODS

Influence of altered CBF expression on stress tolerance.
We developed transgenic petunia lines constitutively expressing the CBF3 gene from Arabidopsis.
Freezing tolerance was evaluated using an electrolyte leakage assay, which estimates cellular damage due to freezing temperature exposure, either without cold acclimation (plants grown at 68 ºF), or after cold acclimation (plants grown at 68 ºF, then exposed to 59 ºF for one week, 50 ºF for one week, and then 37 ºF for three days).

Evaluation of wild Petunia species as genetic sources of stress tolerance.
Freezing tolerance of *Petunia axillaris* (two accessions), *P. exserta* and *P. integrifolia* was evaluated with or without cold acclimation as described above.

RESULTS

Freezing tolerance of plants expressing the AtCBF3 gene.
Plants that constitutively expressed the *AtCBF3* gene were more freezing tolerant than wild type *Petunia* ‘Mitchell’, both before and after cold acclimation (Figure 1). In the absence of cold acclimation, wild type plants had an EL_{50} temperature (the temperature resulting in severe damage to cellular membranes) of -1.4 ºC (29.5 ºF). In contrast, the transgenic lines expressing *AtCBF3* had non-acclimated EL_{50} ranging from -5.5 to -6.5 ºC (22 to 20 ºF). Following cold acclimation, plants had a further increase in freezing tolerance. Following cold acclimation, freezing tolerance of wild type plants increased to -6.5 ºC (20 ºF), while freezing tolerance of

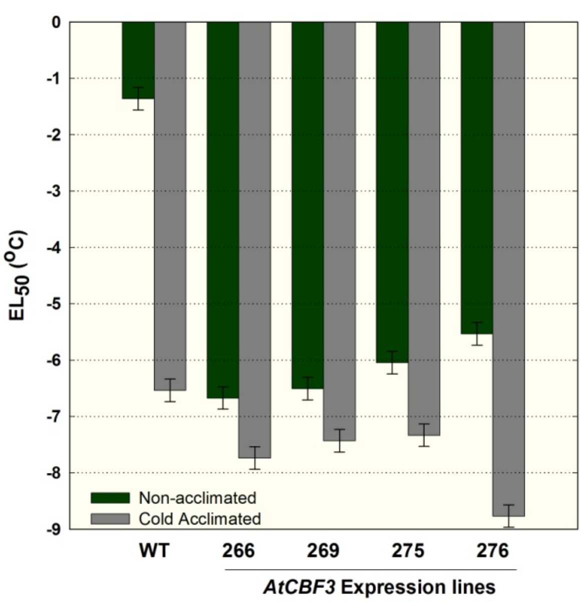

Figure 1. Plants expressing the AtCBF3 gene exhibit increased freezing tolerance compared to wild type (WT) Petunia ‘Mitchell’ with or without cold acclimation. Lower EL_{50} temperature indicates greater freezing tolerance.

plants expressing *AtCBF3* had an additional increase, ranging from -7.3 ºC to -8.7 ºC (18.9 to 16.3 ºF).

In addition to improving freezing tolerance, constitutive expression of *AtCBF3*, also resulted in dwarfism and delayed flowering (Figure 2). The dwarfism may be desirable and reduce the need for PGRs, though clearly the delay in flowering in undesirable.

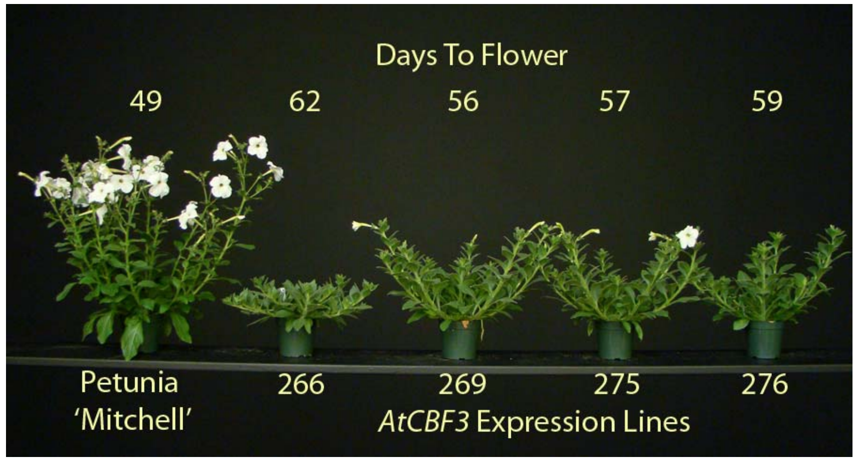

Figure 2. Flowering of plants expressing AtCBF3 was delayed compared to wild type Petunia, but plants were much more compact.

The CBF genes have also been shown to improve drought tolerance in several crops. However, preliminary experiments did not reveal any improvement in drought tolerance of petunia (data not shown).

Variation in cold acclimation ability of wild Petunia species. Wild species varied in freezing tolerance following, but not before, cold acclimation. Without cold acclimation, all species had EL_{50} values around -2 ºC (28.4 ºF). However, following cold acclimation, freezing tolerance varied considerably, with *Petunia exserta* the least freezing tolerant, and *P. axillaris* accession 28548 exhibiting the greatest freezing tolerance (Figure 3), which is greater than *Petunia hybrid*a.

IMPACT TO THE INDUSTRY

This research has identified potential genetic sources for the improvement of frost tolerance in petunia, either through traditional breeding approaches utilizing *P. axillaris* germplasm with superior cold acclimation ability, or through the transgenic approach of manipulating expression of "CBF" genes.

Identifying genetic sources for improving stress tolerance of petunia can benefit not only floriculture breeding companies but also consumers by developing stress-tolerant crops. Development of more cold-tolerant bedding plant species will increase the diversity of crops available for early spring or fall sales.

Email address:
warnerry@msu.edu

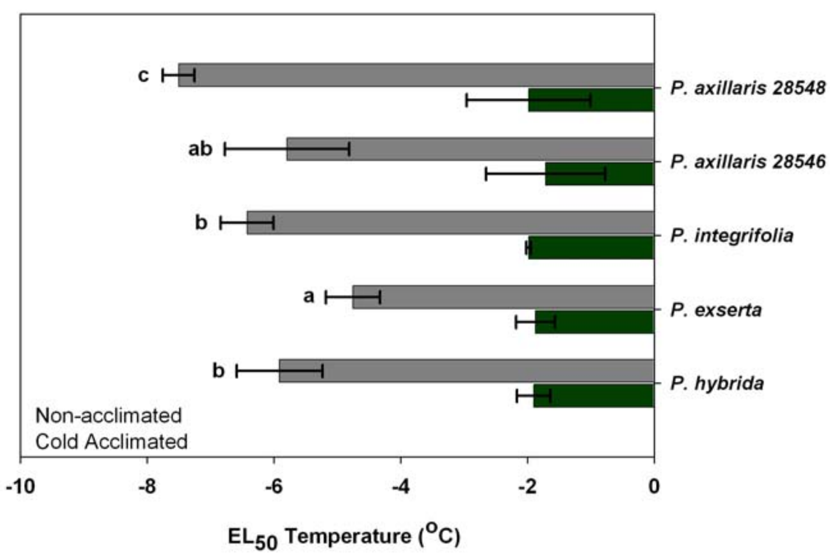

Figure 3. Wild relative Petunia species vary in freezing tolerance following cold acclimation.