

Floriculture Germplasm Enhancement Using Genetic Engineering to Improve Postproduction Quality



Michelle L. Jones¹, Shuangyi Bai¹, Laura J. Chapin¹, and Charles Krause²
¹Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691
² US Department of Agriculture, Agriculture Research Service, Wooster, OH 44691



PROJECT OBJECTIVES

1. Identify genes and proteins involved in senescence
2. Characterize expression of senescence-related genes during development and following drought and nutrient stress
3. Investigate the regulation of senescence-related genes by the plant hormone ethylene
4. Identify the best gene targets to enhance postproduction quality and performance using genetic engineering.

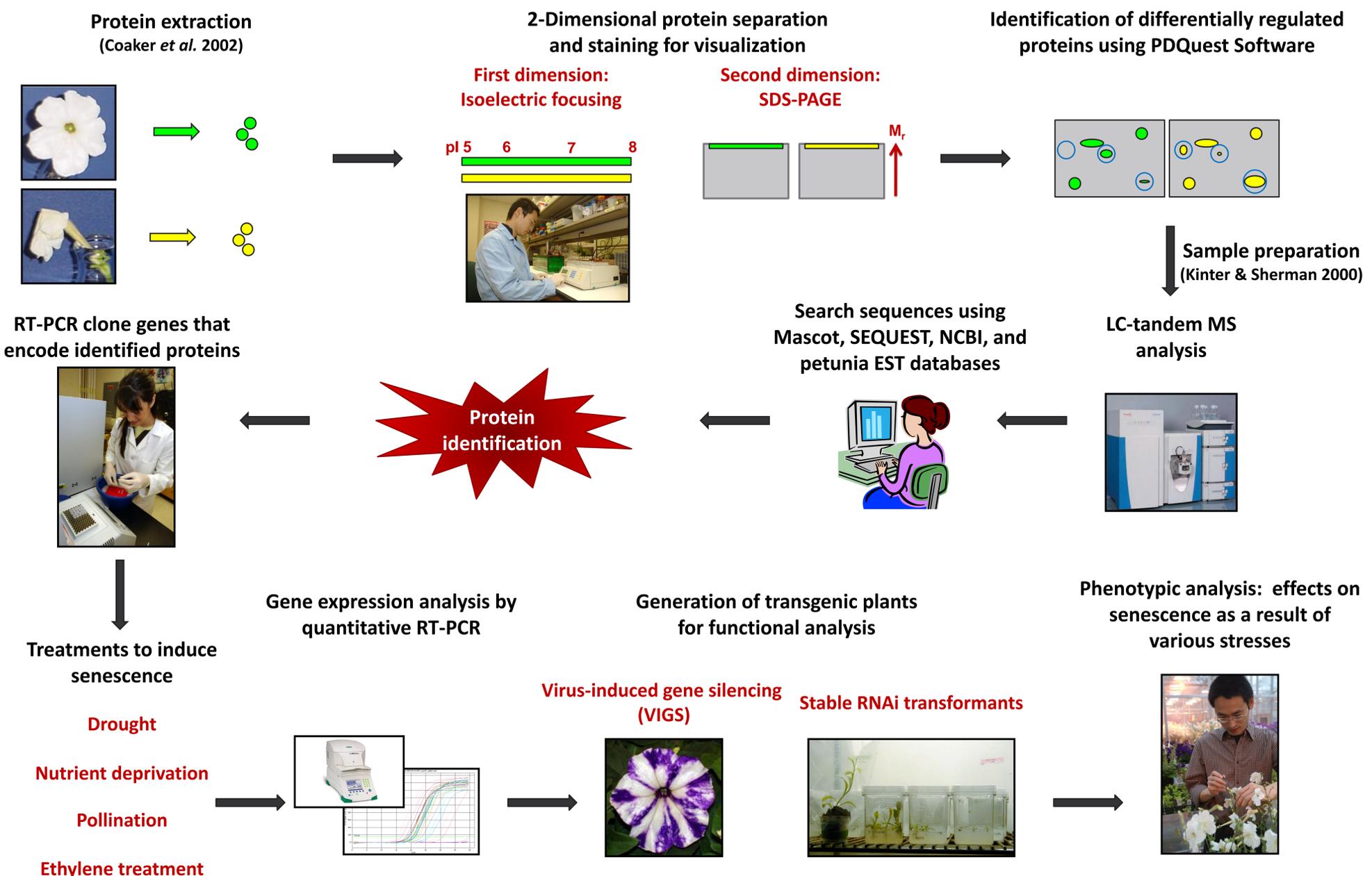
INTRODUCTION

Senescence represents the last phase of plant development. This is a genetically controlled process that allows the plant to systematically dismantle the cells of senescing leaves and petals so that essential nutrients can be remobilized to developing tissues before the dying organs are shed. The developmental senescence (i.e. age-related) of many plants is controlled by the plant hormone **ethylene**. Exposure to ethylene gas during greenhouse production or during shipping or retailing can accelerate the senescence of floriculture crops and these crops may become unsalable. Postproduction exposure to environmental stresses, including drought, can also reduce plant quality and shelf life. The premature senescence of flower, leaves and whole plants during any part of the supply chain leads to decreased sales and decreased profitability. This has recently been referred to as **shrink**. Losses from postproduction shrink may be from 5 to 15% (Healy, 2009). While shrink can be reduced by proper postproduction care and handling, plants with delayed senescence and enhanced stress tolerance (the result of traditional breeding or genetic engineering) will have the longest shelf life. A detailed understanding of the genetic control of senescence is required to efficiently modify both developmental and stress-induced senescence. While plant development is controlled by changes in the expression of genes, many processes like senescence are also controlled directly by changes in the proteins that are encoded by these genes (i.e. post-transcriptional regulation). This project uses **proteomics** to identify the biochemical pathways controlling senescence. Proteomics is the large-scale study of proteins.

RELEVANCE TO FLORICULTURE INDUSTRY

This research provides valuable knowledge about the execution of senescence in plants and how senescence is influenced by ethylene (Chapin & Jones, 2009) and environmental stresses. This information will be used to increase the shelf life of flowering plants, which will reduce postproduction shrink and increase the profitability of floriculture producers.

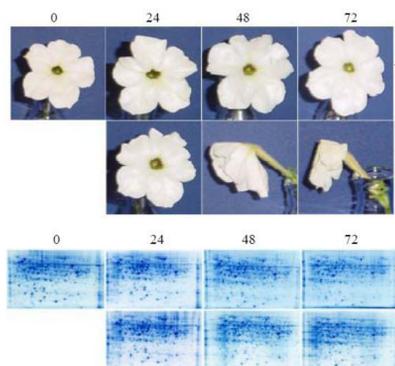
METHODS



RESULTS

Proteomic Analysis

Figure 1: Proteomics was used to identify protein changes during the pollination-induced senescence of *Petunia x hybrida* 'Mitchell Diploid' corollas.



- ❖ Total soluble proteins were extracted from pollinated (P) and unpollinated (U) corollas (3 replicate samples containing 8 corollas) at 0, 24, 48 and 72 h after flower opening.
- ❖ Separation by 2-dimensional gel electrophoresis (2-DE) and staining with GelCode Blue resulted in the detection of approximately 600 protein spots in each gel.
- ❖ Protein differences between the pollinated and unpollinated corollas at each time point were identified using PDQuest image analysis software and the experimentally determined cut-off of 2.1 fold for $P < 0.05$.

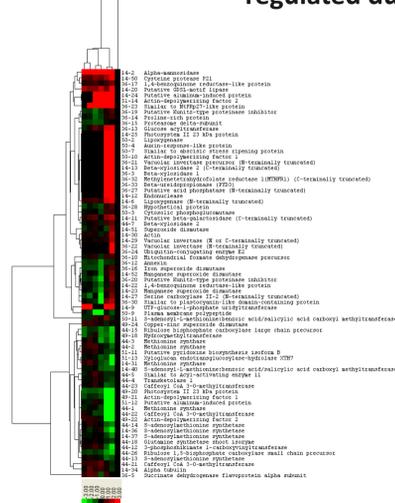
Table 1: Proteomic analysis identified proteins that were up regulated and down regulated during petal senescence

Differentially expressed proteins	Time point and treatment					
	24P vs. 24U		48P vs. 48U		72P vs. 72U	
	Number	% (total)	Number	% (total)	Number	% (total)
Up regulated z	0	0	73	13	112	20
Down regulated y	0	0	41	7	57	10
Total	0	0	114	20	169	30

z , up regulated proteins were those that increased in abundance or were newly detected in pollinated corollas

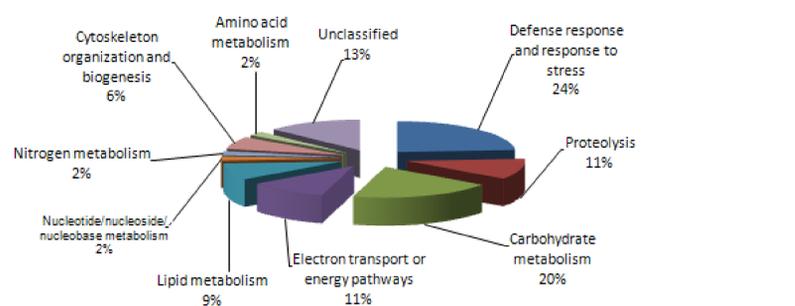
y , down regulated proteins were those that decreased in abundance after pollination or were detected in unpollinated but not pollinated corollas

Figure 2: Liquid chromatography tandem mass spectroscopy and database searches led to the identification of 73 proteins that were differentially regulated during corolla senescence.



- ❖ The **red** color indicates proteins that increased in abundance during senescence and which may have a functional role in senescence.
- ❖ Some proteins were detected only in senescing (48P & 72P) corollas. These may be the result of new protein synthesis or the post-translational modification of existing proteins.
- ❖ The **green** color indicates proteins that decreased in abundance during senescence.
- ❖ **Black** indicates no change in abundance compared to corollas on the day of flower opening (0h).

Figure 3: Functional classification of the senescence up regulated proteins indicates which biochemical pathways are involved in corolla senescence.

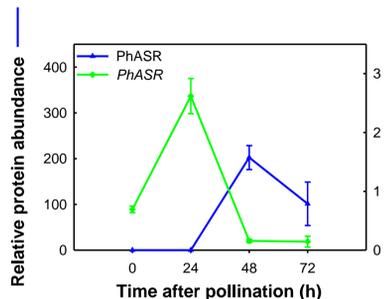


- ❖ Defense and stress response proteins may protect the corolla from pathogen and stress damage during senescence.

- ❖ The majority of the proteins involved in macromolecule metabolism encoded enzymes involved in dismantling cell walls, proteins, lipids and nucleic acids.

Gene Expression Analysis

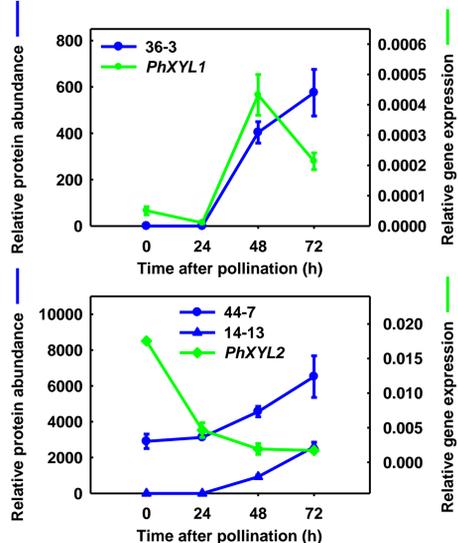
Figure 4: One protein that was detected only in senescing corollas was identified as an abscisic stress ripening protein (ASR).



- ❖ Expression of the **PhASR gene** was highest at 24 h after pollination and it may function in the initiation of the senescence program.

- ❖ The ASR gene family in tomato is induced by dehydration, abscisic acid (ABA) and cold stress.
- ❖ ASR may encode a transcription factor.
- ❖ The petunia **ASR protein (PhASR)** was detected only at 48 and 72 h after pollination.
- ❖ ASR genes have not been shown previously to be involved in petal senescence.
- ❖ The petunia gene encoding ASR was cloned by RT-PCR and gene expression was determined by qRT-PCR.

Figure 5: Multiple differentially regulated spots were identified as Beta-xylosidases.



- ❖ Beta-xylosidases are involved in cell wall polysaccharide disassembly or modification and may function in cell wall degradation.
- ❖ **PhXYL1** mRNA abundance also increased during pollination-induced petal senescence.
- ❖ Two spots contained the PhXYL2 protein and both increased in abundance at 48 and 72 h after pollination.
- ❖ mRNA abundance of **PhXYL2** decreased after pollination.

DISCUSSION

- ❖ Novel proteins not previously reported to be involved in senescence were identified using proteomics.
- ❖ Protein identifications in petunia were possible due to ESTs generated by David Clark (UF).
- ❖ mRNA abundance did not always correlate with protein abundance.
- ❖ PhASR may function as a transcription factor that controls the initiation of senescence in flowers and leaves.
- ❖ Research is ongoing to evaluate these proteins in transgenic plants.

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COLLABORATORS

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Jonathan Frantz (USDA/ARS, Toledo, OH)
Michael Kinter and Belinda Willard (Cleveland Clinic Proteomics Lab)
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