



Funding Generations of Progress
Through Research and Scholarships

Final Report on Gus Poesch Grant
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Research project description

Autophagy is a biological process by which the cell encapsulates and transports certain cytosolic content (organelles, proteins, pathogens) to the vacuole to degraded it into primary building compounds (amino acids, lipids, carbohydrates) which are reused in other cellular processes. The structure in charge of capturing, transporting and delivering the cellular contents is called an "autophagosome". At the molecular level, autophagy is regulated by the autophagy-related (Atg) genes, which were first identified in yeast (Liu and Bassham 2012). I am interested in the role of autophagy in flower senescence because this is also a process by which the plant degrades the components of the petals to allow for recycling of important nutrients and compounds to the developing seed (Shibuya 2012). An understanding of this process of petal degradation and how it is regulated by internal signals (i.e. plant hormones like ethylene) and environmental stresses is the key to regulating the longevity of flowers by delaying petal senescence.

Functional characterization studies of the Atg genes have revealed the role of autophagy in plant development, and abiotic and biotic stress responses. Autophagy deficient plants are characterized by an accelerated senescence and hypersensitivity to nutrient, salt and drought stress (Liu and Bassham 2012). An RNA-sequencing project in the Jones lab has identified a number of Atg genes that increase in abundance during petunia petal senescence suggesting a role for autophagy in flower development and senescence (Broderick *et al.* 2014). Although the study of autophagy has progressed considerably, most of the studies have been performed in *Arabidopsis* or agronomic crops. However, a deeper understanding of autophagy in floricultural crops is highly needed due to its role in senescence and stress responses, both important aspects of the production and quality of floricultural commodities.

The goal of my Master's research project is to understand the role of autophagy during flower senescence and abiotic stress responses in petunia. The research project has three objectives: (1) characterize the expression patterns of the Atg genes during the senescence of flowers and the response to abiotic stresses, (2) characterize the function of Atg genes in the response to salinity and nutrient deficiency, and (3) monitor autophagosome formation in senescing petals via transient transformation of the GFP-ATG8 fusion protein.

Impact of the project

During the life cycle of floriculture crops, they can encounter a variety of stresses either during

the production or retailing phase. Production scheduling and post-harvest quality of ornamental crops can be detrimentally affected by abiotic stresses such as: temperature, salinity, drought and nutrient deficiency. Therefore, for the floriculture industry it is very important to understand how ornamental crops respond to their environment. A better understanding of the physiological responses of plants to their environment has vital importance not only to ameliorate current quality issues due to abiotic stresses but also to face the future more extreme stress conditions predicted to be caused by global warming (Walthall *et al.* 2013).

Flowering plants are commercialized due to the beauty of their blooms. Therefore, flower longevity is an important characteristic for floriculture crops as it determines shelf life. Flower longevity is determined genetically but can be accelerated by abiotic stresses. Blooms should not only last long enough for the plant to be sold, but also to satisfy the customer with a sufficient display life in the home or garden. In addition, flower longevity is even a more determining factor for cut flower industry due to the more stressing process that it takes to transport the flowers from the fields to the customer. Therefore, a greater understanding of the biological processes underlying the senescence of flowers is of high interest for floriculture industry. A better understanding of the physiology of floricultural crops will allow to generate research based technologies and practices to improve the production of high quality plants with long lasting flowers. This would directly benefit producers since their product will be better looking and have a greater shelf life.

Importance of attending the ASHS (American Society For Horticultural Science) conference

My interest in plant sciences and floriculture crops drove me to pursue a Master's degree in Horticulture at The Ohio State University. I started in the DC Kiplinger Floriculture Crop Improvement laboratory of Dr. Michelle Jones in Fall 2015. This program has given me a unique opportunity to get more involved in floriculture and science. Currently, I am thinking ahead about opportunities that will allow me to further enhance my professional development and obtain a career in the Floriculture industry. That is the reason why I want to attend to the ASHS conference in 2017. I look forward to contributing to the improvement of floricultural production through the research I am doing, and I think that the ASHS conference is a wonderful opportunity to share my research with other scientists, and to obtain feedback and comments that can help me to improve my thesis. This opportunity not only will benefit my research but also my personal and professional development as it is a unique opportunity to develop relationships with people around the US that share my interest in floriculture. Furthermore, I will be able to see the research that other scientists are doing and what problems they try to address. By the end of the conference I will have a broader vision of opportunities and problems of horticulture as well as a bigger network connection of people with whom I could potentially work in the future.

FINAL REPORT: Presenting at ASHS (American Society for Horticultural Science) conference

The 2017 ASHS conference took place at Waikoloa, Hawaii from Sep 19th to the 22nd, 2017. I had the chance to present part of the research I did during my Master's degree at OSU. This experience started with an amazing visit around the Big Island of Hawaii. I could learn how volcanos have shaped the ecosystems there. We observed the diversity of native and introduced

plants species in Hawaii, specially the very diverse orchid collections. Before going to graduate school, I worked with tropical cut flowers. Thus, visiting the anthurium breeding program was an amazing opportunity to get ideas and useful information about this crop. Although my main interest is floriculture, it was great to visit the research station ran by the University of Hawaii, where research on tropical fruits is performed. In addition to the research part of the conference, I had the chance to visit and learn from local growers. All together this experience provided me with a good vision of the horticulture system in the Big Island. The conference was also a unique opportunity to obtain useful information from the different sessions and posters, and to learn what other types of research is being conducted in the field of Horticulture. I got the opportunity to present an oral seminar in one of the floriculture sessions. This was a fantastic opportunity to practice my research communications skills. I obtained excellent feedback about the project and met other graduate students and faculty conducting floriculture research. The social events were also very useful for me as I was able to establish a good network of people involved in horticulture. I was very happy to find one of my college professors, and an undergraduate student presenting research done in my home country, Ecuador. I am finishing my MS in December 2017, and the career development sessions were very useful for me as I search for job opportunities. Overall, attending to the ASHS conference let me get more involved in horticulture due to the up-to-date information shared and the connections I was able to make.

References

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ASHS abstract:

The role of autophagy in the response of *Petunia* × *hybrida* ‘Mitchell Diploid’ to low fertility and salinity stress.

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Abiotic stresses reduce the visual quality of ornamental plants and consequently cause economic losses for growers. Plant responses to stress involve several physiological, morphological, and molecular changes. Autophagy, a process of cellular degradation, is important for normal plant development and stress responses. During autophagy, double membrane structures (autophagosomes) capture the cytosolic contents and deliver them to the vacuole. Autophagosome formation is regulated by the autophagy-related (ATG) genes. Here, we describe how the ATG genes of petunia (*PhAtg4*, *PhAtg5*, *PhAtg6*, *PhAtg7*, *PhAtg8a*, and *PhAtg13*) are regulated in response to low fertility and salinity stress. During low fertility stress, upregulation of the ATG genes was accompanied by a decrease in leaf chlorophyll content. In contrast, under salinity stress the ATG genes were either downregulated or not affected even when significant changes were observed in stomatal conductance, leaf temperature, photosystem II efficiency, and electrolyte leakage. To further investigate autophagy, wild type petunia and transgenic RNAi lines targeting endogenous *PhAtg6* were grown for six weeks using three irrigation solutions: control (100 ppm N from 15-5-15), low fertility (10 ppm N from 15-5-15), and salinity (100 ppm N from 15-5-15, 80mM NaCl). Growth index (GI), dry weight (DW), days to flower anthesis (DF), number of leaves per plant (LP), leaf area (LA), and chlorophyll content (SPAD) were measured to evaluate the stress responses in wild type and transgenic petunias. Under control conditions, transgenic lines showed lower SPAD values, delayed flowering, a reduction in number of leaves per plant, and an increase in leaf area. Low fertility stress did not affect GI, LP, and LA; however, DW and leaf chlorophyll content were higher in transgenic plants. Salinity treatment reduced growth and leaf area, but not the number of leaves in transgenic lines. Although the autophagy molecular machinery was activated in response to the low fertility treatment, salinity stress did not have the same effect. Disruption of *PhAtg6* expression affected normal development of petunia and its ability to tolerate low fertility and salinity stress. By identifying the function of the autophagy related genes, we aim to increase our understanding of plant development and abiotic stress tolerance.