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Project title: Improving *Petunia* production efficiency through cultivar selection and development of molecular tools.

Project Summary

Rising energy costs are forcing greenhouse growers to become more efficient in the way they produce bedding plants. In fact, in a recent survey, greenhouse producers identified energy and labor costs as the two biggest threats to profitability for the industry. Both of these costs are a direct function of how long a crop is in the greenhouse. Reducing crop production time is one critical component of increasing production efficiency. For this project, two approaches were taken to reduce crop production time. The first involved evaluating crop production time of numerous cultivars *Petunia* cultivars to identify early flowering varieties. Selecting early flowering varieties among currently available cultivars is a *short-term* approach to improving production efficiency. The *long-term goal* of this project is to develop *Petunia* cultivars exhibiting earlier flowering under the non-optimal production conditions of Michigan (low light, cool temperatures, short days during the production season) as a means to improve production efficiency. For this second approach to improving production efficiency, we developed resources that will be useful for understanding the genetic control of crop timing and allow the breeding of earlier flowering cultivars.

Project Approach

The specific goals for this project were to:

1. Screen currently available *Petunia* germplasm to identify early flowering cultivars for Michigan conditions and make recommendations for Michigan growers.
2. Evaluate interspecific hybrid *Petunia* populations for early flowering under non-optimal conditions to assess genetic diversity.
3. Develop genetic linkage maps for segregating populations of *P. axillaris* × *P. exserta* and *P. integrifolia* × *P. axillaris* to aid our long-term goal of identifying molecular markers for early flowering.

Project Director Dr. Ryan Warner and graduate student Joseph Tychonievic were responsible for phenotyping the panel of petunia cultivars and mapping populations for early flowering, and extracting DNA from the populations for Simple Sequence Repeat (SSR) marker genotyping for the development of genetic linkage maps. Co-PD Dr. Cornelius Barry oversaw the bioinformatics analysis of the sequencing data for SSR identification and evaluation.

Goals and Outcomes Achieved

Goal 1. We evaluated flowering time in response to temperature for 17 petunia cultivars, comprising both trailing and upright varieties. Plants were grown at 14, 17, 20, 23 and 26 °C. While decreasing temperature increased time to flower of all varieties, there was considerable variability in the increase in flowering time as temperature decreased (Table 1). For example, decreasing temperature from 20 to 17 °C increased time to flower by 11 days for petunia 'Dreams Rose Morn' and 'Prism Sunshine' while increasing time to flower by 17 days for 'Double Cascade Blue'. Additionally, flowering time of several wild *Petunia* species that have potential as sources of genetic diversity for breeding programs were evaluated. The increase in flowering time as temperature decreased from 20 to 17 °C was only 7 days for *Petunia exserta* and *Petunia axillaris*, less than any of the commercial cultivars evaluated. These data indicate

that there is sufficient variation in crop timing among current commercial cultivars for growers to select cultivars exhibiting minimal delay in flowering with decreasing temperature and that wild relatives of the cultivated petunia may be useful genetic sources for breeding programs to develop faster flowering cultivars. We have also determined the impact of photoperiod on flowering time for the wild species. While all species were classified as quantitative long-day plants, there was considerable variability in the strength of the photoperiodic response which may be exploited to breed cultivars with reduced photoperiod sensitivity.

Table 1. Increase in days to flower resulting from the indicated drop in temperature for several petunia cultivars and species.

	Temperature drop from:	
Petunia genotypes	20 to 17 °C	20 to 14 °C
Bravo Pink	13 days	34 days
Daddy Sugar	15	33
Double Cascade Blue	17	31
Double Pirouette	13	32
Double Sonata	14	35
Dreams Rose Morn	11	26
Frost Blue	14	35
Prism Sunshine	11	28
Ultra Burgundy	15	37
Petunia axillaris	7	31
Petunia exserta	7	26
Petunia ×*hybrida* 'Mitchell'	13	35
Petunia integrifolia	14	27

Goal 2. We have evaluated flowering time and crop quality traits for two interspecific hybrid *Petunia* F_2 populations. The populations are hybrids of *P. axillaris* × *P. exserta* and *P. integrifolia* × *P. axillaris*. The common garden petunia (*Petunia hybrida*) is a hybrid between *Petunia axillaris* and *Petunia integrifolia*. Therefore, we are essentially recreating the germplasm base for *P. hybrida*. As *P. hybrida* has been inbred for commercially desirable traits over the last 100 years, much genetic diversity has been lost. Recreating the original cross allows us to recapture much of that diversity and introgress novel traits back into the commercial gene pool.

Variability in development rate across Petunia *genotypes* The rate of node appearance (i.e. leaf unfolding rate) is one factor determining crop timing. Many greenhouse crop growers in northern climates have reduced greenhouse temperature in an attempt to reduce heating costs. Leaf unfolding rate is a temperature dependent process; therefore lowering greenhouse temperature decreases the rate of development, leading to increased production time. Developing cultivars with faster development rates at lower temperatures is one long-term strategy for improving the energy efficiency of greenhouse crop production. Initial experiments indicated that the wild relative species of the cultivated petunia, *P. axillaris* and *P. integrifolia* have higher leaf unfolding rates than any of nine elite *Petunia hybrida* cultivars (Fig. 1A), and that the interspecific hybrid F_2 populations *P. axillaris* × *P. exserta* and *P. integrifolia* × *P.*

axillaris exhibit transgressive segregation for leaf unfolding rate and node number below the first flower (Fig. 1B and C).

To further analyze variability in crop timing and quality traits among the two interspecific mapping populations, approximately 200 F_2 individuals of each mapping population were grown under a cool temperature regime (17 °C). Plants were evaluated for crop timing traits, including the leaf unfolding rate and the number of nodes forming below the first flower, as well as several crop quality parameters, including flower size (width and length), flower bud number, branch number and plant height.

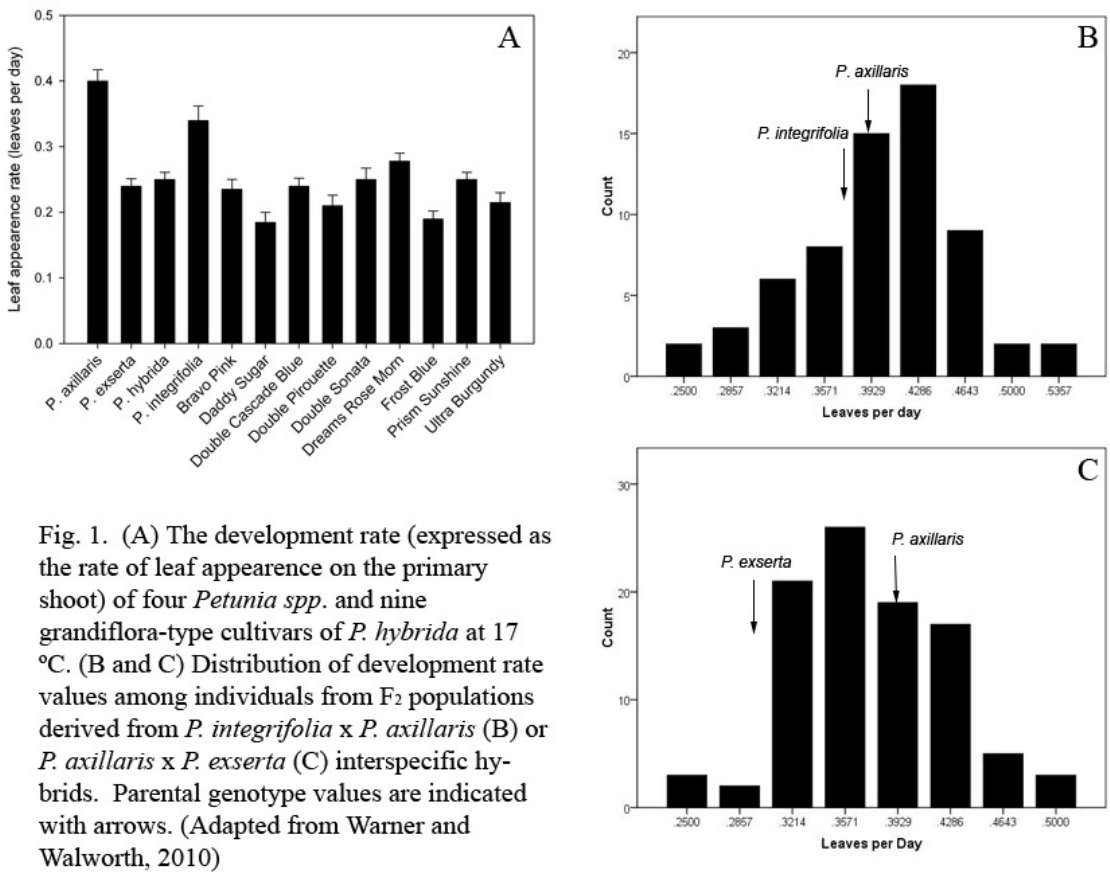

Fig. 1. (A) The development rate (expressed as the rate of leaf appearence on the primary shoot) of four *Petunia spp*. and nine grandiflora-type cultivars of *P. hybrida* at 17 ºC. (B and C) Distribution of development rate values among individuals from F_2 populations derived from *P. integrifolia* x *P. axillaris* (B) or *P. axillaris* x *P. exserta* (C) interspecific hybrids. Parental genotype values are indicated with arrows. (Adapted from Warner and Walworth, 2010)

Both populations exhibited transgressive segregation for all traits evaluated, including development rate (Fig. 2A and B), flower width (Fig. 2C and D) and the number of flower buds at first flowering (Fig. 2E and F). Correlation analysis was conducted to evaluate potential genetic linkage between traits. Interestingly, fast development rates were positively correlated high flower bud number (Table 2; i.e. faster developing plants also produced more flower buds at first flowering). Also, fast development rate was negatively correlated with days to flower (Table 2), meaning faster developing plants flowered earlier in time. These data suggest that selecting for fast development rate is a viable strategy for reducing crop production time without negatively impacting crop quality parameters.

Fig. 2. Population distributions for development rate (A and B), flower width (C and D) and flower bud number (E and F) among the interspecific hybrid F_2 populations *P. axillaris* × *P. exserta* and *P. integrifolia* × *P. axillaris*.

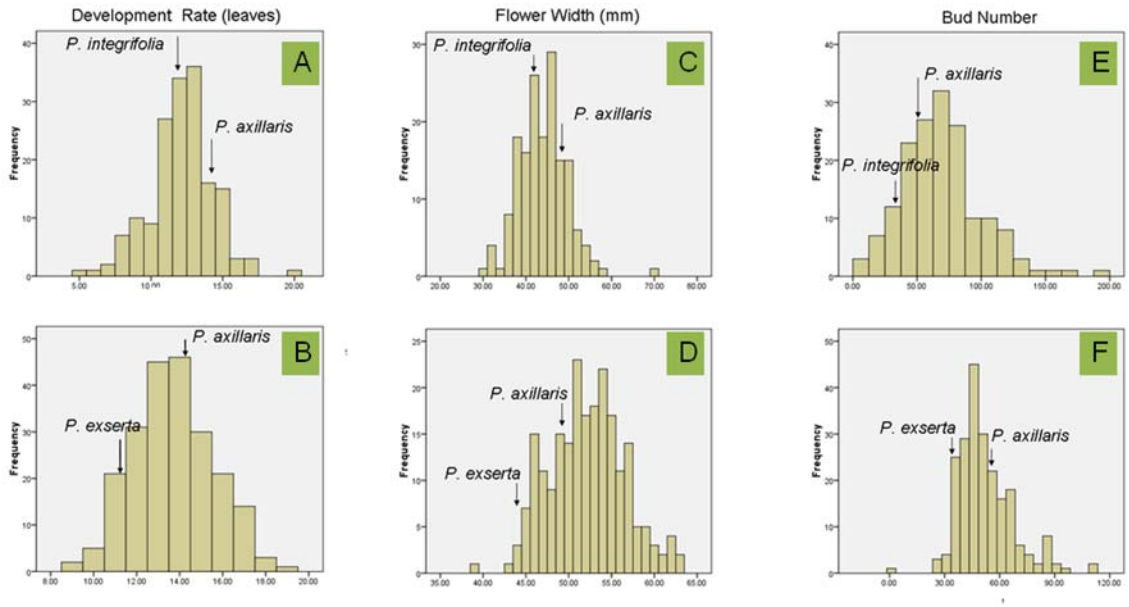

Table 2. Pearson correlation coefficients for crop timing and quality traits in two interspecific hybrid *Petunia* F_2 populations. Values followed by *, **, or *** represent significant correlations at $P<0.05$, 0.01 and 0.001, respectively.

Trait	Days	Flower Width	Flower Length	Plant Height	Flower Buds	Leaves
			P. integrifolia × *P. axillaris*			
Width	0.134					
Length	0.011	0.060				
Height	0.112	0.332***	0.441***			
Buds	0.164*	-0.099	0.183*	0.058		
Leaves	0.474***	-0.165*	0.097	-0.036	0.418***	
Rate	-0.508***	-0.256***	0.128	-0.093	0.19*	0.255***
			P. axillaris × *P. exserta*			
Width	0.083					
Length	-0.072	0.066				
Height	0.173*	-0.079	0.084			
Buds	0.188**	0.265***	0.033	0.040		
Leaves	0.374***	-0.033	-0.103	0.186**	0.394***	
Rate	-0.213**	0.032	0.003	0.120	0.280***	0.559***

These phenotypic data will be utilized to identify genetic loci controlling these traits following generation of the genetic linkage map (Objective 3). These data indicate that both populations exhibit considerable diversity for all of the evaluated traits, which will greatly facilitate identifying molecular markers associated with these traits that can be utilized in petunia breeding programs.

Goal 3. *SSR marker and genetic linkage map development*

To develop genetic linkage maps, we are utilizing molecular markers termed simple sequence repeats (SSRs), or "microsatellites", which are based on DNA sequences consisting of short repeated strings of nucleotides (e.g. TGTGTGTGTG or GACGACGACGAC). We have mined the currently available DNA sequences for *Petunia axillaris* to identify SSRs that are being used to screen for DNA polymorphisms across the parents of our mapping populations. Within the approximately 20,000 available sequences, we have identified over 500 SSR sequences fitting the selection criteria of a minimum of seven repeats for di-nucleotide motifs, five for tri-nucleotide motifs, four for tetra-nucleotide motifs, and three for penta-nucleotide motifs. We have screened these markers on our populations to identify polymorphic markers. Additionally, we screened over 100 SSR markers developed for *Petunia hybrida* and identified numerous markers that will be useful for developing genetic linkage maps on our populations.

We identified 94 polymorphic SSRs between *P. axillaris* and *P. integrifolia* and 67 polymorphic SSRs between *P. axillaris* and *P. exserta*. We have genotyped 164 individuals from the *P. integrifolia* × *P. axillaris* population with 51 markers to generate a preliminary genetic linkage map (Fig. 2). Additionally, 10 dCAPS markers developed in Dr. Cris Kuhlemeier's lab at University of Bern, Switzerland have been screened on this population and integrated into the linkage map.

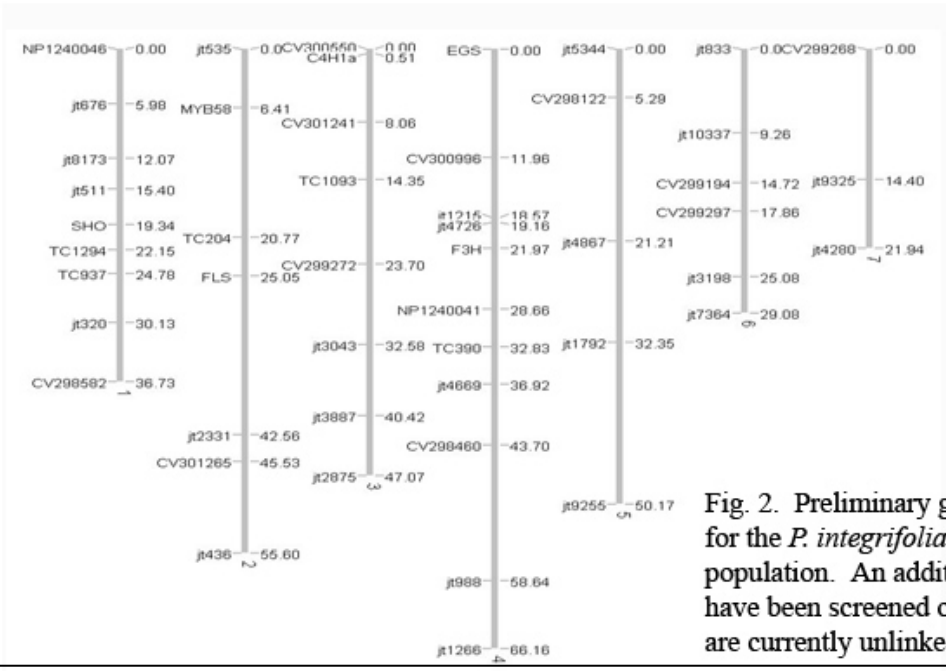

Fig. 2. Preliminary genetic linkage map for the *P. integrifolia* x *P. axillaris* population. An additional 12 markers have been screened on the population but are currently unlinked.

Beneficiaries
This work will benefit multiple sectors of the floriculture industry as well as the broader research community. The cultivar selection work will directly benefit greenhouse growers, particularly those in northern locations such as Michigan, by identifying cultivars that require less energy to produce. Our work has show that proper cultivar selection can reduce crop production time by 15-20%, thereby also reducing fuel usage for heating. The result for the genetic mapping of crop timing traits will benefit floriculture crop breeders by providing tools to breed faster developing cultivars. The genetic resources developed through this project (the mapping populations, molecular markers and genetic linkage maps) will benefit commercial floriculture breeders as well as the broader petunia research community. While we are focused on crop timing traits, these populations also exhibit considerable variation for a number of crop quality traits, including flower size and color, branching habit and plant height, and will therefore be useful for breeding and understanding the genetic control of these traits as well.

Lessons Learned
The results of this project indicate that while current petunia cultivars do vary in development rate, the range of variation is limited, especially to the range exhibited by the wild progenitor species of *Petunia hybrida*. This suggests that the wild species will be very useful sources of genetic diversity to introgress into the commercial breeding germplasm in order to breed faster developing and earlier flowering varieties. Our results also indicate that crop timing traits such as leaf unfolding rate and leaf number below the first flower are quantitative traits (i.e. controlled by multiple genes). Therefore, developing molecular markers for these traits should facilitate rapid introgression of superior alleles for crop timing traits while minimizing negative phenotypes (so called "linkage drag").

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