

BACKGROUND AND RESEARCH PROBLEM

Plants can respond to environmental change by changing their “appearance” or phenotype (phenotypic plasticity). For example, plants in high-density environments (competition) flower earlier than in low-density environments (no competition), i.e. reproducing before all of the resources needed are exhausted (Dorn et al., 2000). The ability to be plastic varies among different genetic strains, suggesting genes determine the level of plasticity. However, there is little consensus on the genetic mechanisms of plasticity (Schlichting and Pigliucci, 1993). In this study I propose examining the genetic mechanisms of plasticity in response to density (i.e. competition) when the seasonal environmental varies.

Seasonal changes in temperature act to regulate the timing of a plant’s life transitions. For example, in the annual plant *Arabidopsis thaliana*, when the seed germinates will affect when the plant flowers and thus its size and fitness, as large plants make more seeds. Seeds that germinate in fall and survive winter are bigger when they flower in early spring. Seeds that overwinter, i.e. delaying germination until spring, survive winter well but have very little time to grow and thus flower later in the spring at a smaller size. These two germination cohorts may represent a bet-hedging strategy that helps maintain genetic variation in the population for plasticity of flowering time (Donohue et al., 2005).

Changing the timing of flowering is also a strategy for dealing with competition. *A. thaliana* produces a large number of offspring with no mechanism to distribute seeds away from the maternal plant. Therefore, young seedlings often grow in very dense groups of plants, although different strains vary in their response to competition (Dorn et al., 2000). In a previous study, Lor et al. (in prep) asked if cold treating seeds (i.e. emulating spring germination) made a plant a better competitor than plants from seeds not cold treated (emulating fall germination); hypothesizing that early flowering induced by cold would automatically make a better competitor. They found that strains normally flowering later with cold were induced to flower early in competitive environment. Since the cold treatments were imposed first, these results suggest competition altered gene expression

patterns already induced by the cold. The differences among the strains in their responses to the cold provide a way to ask how competition can affect already cold-induced gene expression patterns.

OBJECTIVES:

The main objective of this study is to examine the gene expression differences among genetic strains grown in different combinations of interacting environments (cold and different types of competitors) to gain a better understanding of how genes respond within (on or off) interacting environments. Specifically, I will ask 1) if the gene expression changes triggered by the cold treatment affected by competition and 2) if the number of fruits (fitness) is affected.

METHODS:

Genetic Strains: I will use 6 genetically different strains of *Arabidopsis thaliana* called Recombinant Inbred Lines (RILs) developed by the faculty advisor. The RILs are made by crossing two wild strains (the parental strains) followed by inbreeding for many generations, which rearranges the two parental sets of genes into many unique multi-genic combinations. Because of inbreeding, individuals within an RIL strain are genetically identical to each other, but gene shuffling or recombination in each generation causes each RIL to be genetically different from every other RIL. The RILs differ in their plasticity, presumably because of variation in gene combinations, suggesting genes must work together or regulate each other to confer plasticity to the environment. I will measure gene expression and flowering time on 3 “focal” RILs with different types of plasticity to cold: an “insensitive” (no plasticity), a “sensitive negative” (flowers earlier with cold than no-cold), and a “sensitive positive” (flowers later with cold than no-cold). Additional RILs of the three plasticity types will act as competitors for the focal plant.

Environmental Treatments: Seeds will be placed on top of Sunshine SB300 universal soilless mix in 24cm x 48cm trays. Each tray is divided into 50 “pots” that are 4.5 cm x 4.5 cm. In each pot,

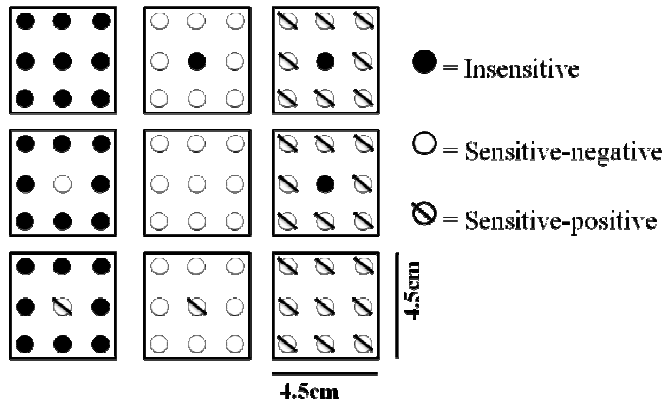


Figure 1: Competitive planting design with all possible combination between insensitive, sensitive-negative, and sensitive-positive plants. Each pot is a 3X3 grid with each dot spaced 1.5cm apart.

seeds will be placed 1.5cm apart in a 3x3 grid (Figure 1). To address the affects of competition against different strains, each pot will be randomly assigned a competitive treatment in which a focal seed is placed in the center of the pot while the competitors fill the rest of the grid. Each focal plant will be planted with competitors that are

sensitive negative, sensitive positive or insensitive RILs in all possible combinations. Thus, there are 3 RILs and 3 competitors for 9 competitive combinations.

In my previous experiment, I did not address the potential for interactions between a plant's roots, leaves or both. In this experiment, I will plant 10 replicates of each of the 9 competitive combinations outlined above (Figure 1) in four different types of high density treatments (Figure 2).

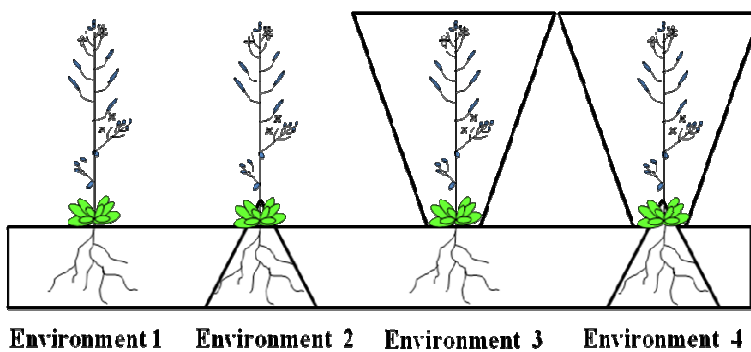


Figure 2: Environmental design for root shoot interactions.

In *environment 1*, roots and shoots interact. In *environment 2*, the roots of focal plants will be separated by a translucent cone but the shoots are allowed to interact. In *environments 3*, the shoots of focal plants will

be separated from competitors by a translucent cone but roots interact. In *environment 4*, both roots and shoots of the focal plant will be separated from competitors with translucent cones. Thus, there are a total of 360 pots or focal plants.

To address the effects of competition with and without cold treatment, 360 pots will be placed at 4°C for two weeks prior to placement in the greenhouse. At the end of the cold treatment,

the no-cold treatment will be planted and immediately be placed in the greenhouses on the 5th floor of Halsey Science Center for a total of 720 plants. The focal plants will be monitored daily until their death for the following traits: days to germination, days to flowering, size at flowering, fruit number, and life span.

Gene Expression Studies: To ask about the effects of competition on gene expression in response to cold, we will examine differences between genetic strains by measuring the amount of the molecule called RNA that is made by the plants in the different environmental combinations. A gene that is highly expressed makes more RNA than one less expressed. Microarrays measure how much RNA is made for each gene in the genome.

RNA will be extracted from leaves samples of each focal plant after the leaves of the focal plant interact (i.e. touch) with its nearest competitor but before the plant flowers. Thus, 2 leaves will be collected from the each focal plant for a total of 288 samples (12 competitions x 4 environments x 2 cold treatments x 4 replicates = 288 samples). RNA will be extracted using Qiagen® Plant RNeasy Extraction kit. Microarrays will use only the samples from plants that were cold treated and planted in environment 1 (roots and shoots interact). Genes discovered in this environment can then be assessed individually in the other environments at a later date.

Student Involvement and Time Commitment:

The student will be responsible for all plant care and phenotypic data collection. Sample collection and RNA extraction for microarrays will be conducted by the student. The faculty advisor will advise on proper data collection, data analysis, and laboratory techniques.

The plants will be planted in mid March 2010 and monitored until their natural deaths when the fruits will be counted. Care and data collection will take ~2-3 hours a day for ~120 days. RNA will be extracted in 2 batches of 24 samples, requiring three hours of work per batch totaling 6 hours.

The setup and run time for microarrays in sets of 3 is ~ 2-3 weeks. Several weeks will be needed to analyze data and write a manuscript. Thus, ~160 days is required to acquire data for the manuscript.

Students Educational Experience:

The student will master proper plant husbandry, statistical analysis, and advance laboratory techniques. This project will also prepare the student for the professional level in methods of scientific query. The proposal experience will help the student professionally and be a valuable asset to the student's career.

Project Outcome:

The data collected will provide insight into the genetic mechanisms behind plant response to competition and root/shoot interaction with respect to the cold. Results from this experiment should result in a publication in a peer-reviewed journal, a presentation at the Celebration of Scholarship, and contribute to a thesis by the student. Moreover, the faculty advisor has already identified some cold-sensitive genes in the cotyledon stage (embryonic leaves formed in the seed), so the results from this experiment will be used to compare to these data on early gene expression.

Budget Justification:

The faculty advisor already has sufficient number of microarrays, equipment, and supplies for the research project with the exception of the following items:

Materials and Supplies:

Qiagen® Plant RNeasy Extraction kit, Qiagen Corp.	\$250.00
<i>Arabidopsis</i> microarrays reagents	\$250.00
Total	\$500.00

LITERATURE CITED:

- Donohue, K., Dorn, L. A., Griffith, C., Kim, E., Aguilera, A., Polisetty, C. R., and Schmitt, J. (2005). Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution*, 59(4), 740-757.
- Dorn, L. A., Pyle, E. H., and Schmitt, J. (2000). Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution*. 54(6): 1982-1994.
- Lor, V., Rubin, M., Yakub, M., and Dorn, L. A. (in prep).
- Munir, J., Dorn, L. A., Donohue, K., and Schmitt, J. (2001). The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (*Brassicaceae*). *The American Journal of Botany*, 88(7), 1240-1249.
- Schlichting, C. D. and Pigliucci, M. (1993). Control of phenotypic plasticity via regulatory genes. *The American Naturalist*, 142(2), 366-370.

To Whom It May Concern:

8 February 2010

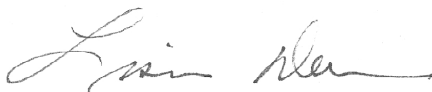
I am writing this letter in support of Vai Lor's proposal for a Graduate Student/Faculty Collaborative Grant. Vai has been either a graduate student or undergraduate researcher in my lab since spring 2006 so I am very familiar with his abilities. Vai is more than capable of conducting the research he has proposed here in both intellectual ability and work ethic. Vai has always been a hard worker in everything he has done and more importantly, he knows and accepts the sacrifices he must make to see a project done well. For example, he would never hesitate to spend a weekend or an evening in the lab if the situation warranted it. Moreover, he rapidly grasped the idea that the lab has common needs and always stepped right in to do basic lab tasks. Now that he is the senior student he has aptly assumed the job of making sure those support tasks get done. I always find myself checking on some basic support task, such as watering the stock plants in the greenhouse, only to find that Vai has already thought of it and has been making sure someone did it.

In support of his own development, Vai has never been daunted or distracted from the challenge of learning a new technique and perfecting it. This is an extraordinary gift as some students expect to be told what to do so they can do it. Vai needed only to be given a protocol with which he methodically worked out every detail so it gave him optimal results. Even some of my brightest students never quite grasped this essential ability.

Vai has also been rapidly developing his interests in scientific questions. Not only was this proposal written entirely by Vai with a few suggestions by me, the idea for this project is also entirely Vai's. This project was inspired by both the work he did as an undergraduate and the work of other students before him. Vai designed all the experiments with some minor changes suggested by me. Of all the students I have mentored in Oshkosh he is the first to present me with a proposal many days before the deadline that required little more than advice on focusing the questions and suggestions for re-designing figures for clarity.

I have no doubts that Vai has the work ethic and the ability to complete this task.

Sincerely,



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