Cotyledon stomatal density differentiation and quantitative genetic analysis of seedling traits in Impatiens capensis ecotypes

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Introduction

Impatiens capensis is a weedy annual plant that grows contiguously from the Eastern U.S. to the Rocky Mountains through a range of soil conditions, from the moist habitats of Rhode Island and Pennsylvania to very dry habitats in Colorado. This is possible through physiological and morphological changes in stomatal density, conductance, ABA production, and leaf growth (Biber et al. 2011; Manyam et al. 2016). These changes indicate regionally specific ecotypes of Impatiens capensis in areas such as Pennsylvania, Rhode Island, and Colorado.

Previous work has examined maternal effects on physiology in the Pennsylvania ecotype, however studies have not yet examined seedling traits across multiple Impatiens ecotypes. Using Scanning Electron Microscope (SEM) and physiological data, Colorado and Pennsylvania Impatiens capensis seedlings were analyzed for functional traits as well as heritability of photosystem efficiency and stomatal conductance.

In addition to simply measuring and comparing traits, heritability calculations can be used to provide insight into the evolutionary potential of a population. With the changing climate, germination and seedling success could be affected by unpredictable spring weather patterns. Knowing the evolutionary potential of these ecotypes of Impatiens capensis seedlings could bolster our understanding of how this species will respond to climate change in regionally specific ways.

Methods

Scanning Electron Microscopy (SEM) Imaging

Pennsylvania and Colorado seeds were germinated on 1% agar plates in a growth chamber at 24°C. Differences in photosystem efficiency were marginally significant, (Fig. 3) and stomatal densities for CO and PA seedlings. Means ± SE bars shown. [F=7.3, df=1, P<0.0002]

Greenhouse since early December of 2018. Maternal Control (Transpiration Rates)

In late February and early March of 2019, transpiration rates were measured on the 49 maternal Impatiens plants, 18 from Pennsylvania and 31 from Colorado. All plants had been growing in stable conditions at the Colorado College Greenhouse since early December of 2018. Stomatal conductance rates were measured on the newest leaf of each plant with a steady state leaf porometer (Decagon Devices).

Quantitative Genetic Analysis

Shoots from 44 lines (22 CO, 22 PA) were grown in the Colorado College greenhouse under stable conditions. After 13 days of growth, photosystem efficiency (PE) measurements were taken on the youngest cotyledon of each seedling with an Edwards 720L fluorometer (PE); using a black background paper to shade sunlight, after about 16 days of growth, stomatal height was measured using a camera to the smallest margin of the cell walls and the smallest stomatal aperture was measured using a camera to the smallest margin of the cell walls and the smallest stomatal aperture was measured using a camera. Each cotyledon was examined in four different frames on the SEM. Each frame the stomata were counted and converted to mean density by dividing each plant’s average number of stomata per frame by the average area of the frame (1.2mm2).

Results

1. Do Impatiens capensis seedlings from Pennsylvania and Colorado populations show differences in physiological and morphological traits such as stomatal density, stomatal conductance, and photosystem efficiency?

2. Does evolutionary potential differ between Pennsylvania and Colorado varieties of Impatiens capensis in seedlings linked to drought and photosynthesis?

Discussion

Functional Traits

- Region was a significant predictor of stomatal density in seedlings, but not of cuticular wax. Higher stomatal densities in the Colorado ecotype seedlings (Fig. 2) indicate that strategies related to water use efficiency are likely highly plastic throughout the transition to adulthood. In adult plants from CO, stomatal density is often lower than in other regions (Biber et al. 2014). The lack of cuticular wax in seedlings (Fig. 4) both populations contradicts what was found by Biber et al. (2011) and indicates that wax changes with developmental age.

- Differences in photosystem efficiency were marginally significant, showing PA seedlings to have slightly higher PE (Fig. 3). Higher stomatal densities in CO populations may be a compensatory mechanism for this lower PE; i.e. CO cotyledons may have greater gas exchange capacity to compensate for less efficient photosystems.

- Day of seedling emergence was not significantly different between populations (Fig. 5), CO lines were significantly taller than PA lines. The combination of increased stomatal density and marginally different PE values made the CO lines capable of faster growth even though plants emerged at the same time; this may translate into drought avoidance strategies in CO populations (Biber et al. 2011; Manyam et al. 2016).

Control for Maternal Environment

- Stomatal conductance of Colorado and Pennsylvania adult plants showed no significant differences (maternal plant genotypes: CO: 106.8±6.6 & PA: 96.7±7.9 nml H20/sec cm-2). The adult plants were under no stress in the stable greenhouse environment. If differing transpiration rates in maternal plants were shown, this could have influenced the stomatal density of their offspring. Again, this indicates that genetic differences between regions may have a greater effect on observed seedling trait values than any maternal effects. This control for maternal environment allows us to examine genetic variances.

Quantitative Genetic Analysis

Genetic variance was significantly different between populations. Colorado populations had higher Vg for stomatal conductance (Fig. 5), but lower Vg for photosystem efficiency (Fig. 6). These data align with the variation in physiological traits of each region. Colorado environments have variable water availability, but relatively stable light conditions, whereas Pennsylvania environments are opposite. This illustrates that variation within an environment maintains genetic variation in traits linked to those environmental factors.

Conclusions

Differences in physiological and morphological traits in Impatiens capensis seedlings from CO and PA are likely due to allelic variation within these populations caused by environmental selection pressures, such as water and light availability. Differences in evolutionary potential between these two regional ecotypes may have implications for the future of these populations as climate change becomes an even more predominant issue in these natural environments.

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