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Intraspecific interactions in *Arabidopsis thaliana* and the stomatal mutants *tmm1-1* and *sdd1-2*

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Abstract

Competition is a major density-dependent factor structuring plant populations and communities in both natural and agricultural systems. Seedlings of the model plant species *Arabidopsis thaliana* cv. Columbia, and the Columbia-derived stomatal mutants *sdd1* and *tmm1*, were grown under controlled conditions at increasing densities of 1, 10, 20, and 50 plants per pot. We demonstrate significant effects of time (days after planting), density, genotype, density and genotype, and the three-way interaction with time upon several fitness components (plant height, silique number, leaf biomass and flowering stalk biomass) in Columbia and these mutants.

Additional key words: biomass, competition, density, plant height.

Introduction

Intraspecific competition is a major density-dependent factor structuring plant populations in both native and agricultural systems (Grace and Tilman 1990, Keddy 2001, Tow and Lazenby 2001). The outcome of intraspecific competition allows vigorous individuals to dominate a population at the expense of less vigorous individuals (Harper 1977). Factors involved in determining the outcome include the density, spatial arrangement, and environmental conditions (Purves and Law 2002). Less, however, is known about the importance of fine-scale genetic differences among individuals concerned in this process. It is clear that different genotypes and cultivars can differ in intraspecific competitive ability (Aarssen and Turkington 1985, Cayenberghs *et al.* 2001, Pfeiffer *et al.* 2001, Damgaard and Jensen 2002, Gustafson *et al.* 2002, 2004). However, the development of mutants of model organisms, such as *Arabidopsis thaliana*, allows for investigation at an even finer genetic scale (Griffing 1989, Andalo *et al.* 2001, Ballare and Scopel 1997, Krannitz *et al.* 1991, Pigliucci and Hayden 2001, Damgaard and Jensen 2002, Fitter *et al.* 2002).

Analyzing genetic mutants of *A. thaliana* will be instrumental in understanding the effects of competition upon plants (Cahill *et al.* 2005). We have selected two EMS-derived genetic mutants, *sdd1-2* (stomatal density and distribution) and *tmm1-1* (too many mouths), with altered stomatal positioning and density to evaluate the effects of constrained space and nutrients upon plant growth and development (Hetherington and Woodward 2003). The *sdd1-2* mutant was first observed by Yang and Sack (1995) and subsequently characterized by map-based cloning (Berger and Altmann 2000). The corresponding gene product *SDD1* is a putative subtilisin-like serine protease believed to be directed at a signaling receptor (Berger and Altmann 2000). The *sdd1-2* mutation exhibits a 2 to 4 fold increase in stomata throughout the plant as well as stomata forming in clusters (von Groll *et al.* 2002, Berger and Altmann 2000). The role of *SDD* in altering the epidermal architecture of the plant is understood, but the exact molecular control within a cell is unclear. The *tmm1-1* mutant has a striking phenotype where stomata form next to each other or in giant clusters (Yang and Sack 1995, Larkin *et. al.* 1997). The mutation causes more stomata in the cotyledons, rosette leaves, and sepals while other parts of the plant may lack stomata entirely (which have stomata in the wildtype) (Larkin *et al.* 1997). The corresponding gene product *TMM* can function as both a negative or positive regulator of cells that enter the stomate-forming pathway (Nadeau and Sack 2002b). The gene was characterized by map-based cloning (Nadeau and Sack 2002a) and *tmm1-1* was found to encode a receptor-like protein (Serna and Fenoll 2002, Nadeau and Sack 2002b).

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Abbreviations: DAP - days after planting; *sdd* - stomatal density and distribution; *tmm* - too many mouths.

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In this study we demonstrate the suitability of mutants of *Arabidopsis* as a model system for investigating intraspecific competition among genetically similar members of the same species. The known and few genetic differences among the *Arabidopsis* mutants we choose provides for a more precise quantification of the differences among intraspecific competitors than among sibs or cultivars which differ genetically, but in an unknown or complex manner. Specifically, we evaluate here the role of *TMM1* and *SDD1* in understanding this process. We also use *Arabidopsis* mutants to seek a physiological understanding of intraspecific competition. While earlier studies demonstrate the outcome of competition in *Arabidopsis* in terms of plant performance and growth, the detailed physiological basis behind the observed patterns have not been fully characterized (Bazzaz and Stinson 1999). We hypothesized that while differing in only a few genes, the two *Arabidopsis* mutants *sddl1-2* and *tmm1-1* will show a different response to intraspecific competition in response to density and nutrient level.

Materials and methods

Plants: *Arabidopsis thaliana* ecotype Columbia *(Lehle Seeds,* Round Rock, TX, USA) and the Columbia-derived mutant *sddl-2* and *tmm-1* (kindly provided by F. Sack, The Ohio State University, Columbus, Ohio, USA) were used in this series of experiments. *Arabidopsis thaliana* seeds were surface sterilized, suspended in a solution of 0.15 % (m/v) agar and 10 µg cm^{-3} ampicillin, cold stratified (4 ºC, 48 h) and sown to 10 cm pots containing an autoclaved soil mix of *Perlite*, *Vermiculite*, and *Sphagnum* (1:1:1). Seeds were sown at a depth of < 1 cm in a circle (r = 5 mm) at the center of each pot. Plants were grown in a growth chamber (*AR-32L*, *Percival Scientific*, Boone, IA, USA) under controlled conditions (23 °C, 24-h continuous light, 200 µmol m-^{2}s^{-1} and 50 % relative humidity) and watered 3 times per week with a modified Hoagland's solution (Wood and Goldsbrough 1997).

Competition treatments, data collection and data analysis: For the initial analysis of intraspecific competition Columbia ecotype seeds were sown at nine densities (1, 5, 10, 15, 20, 30, 40, 50, and 100 plants per pot) with four replicates of each density. For subsequent experiments with Columbia, *tmm1-1* and *sdd1-2*, seeds of an individual genotype were sown at four densities (1, 10, 20, and 50) with four replicates of each density. Flowering stalk heights (soil level to tip) were measured 14 d after planting (DAP), 28 DAP, 42 DAP, and 56 DAP. At 49 DAP flowering stalk biomass and root biomass of each density for all four replicates were determined. Leaf mass was determined by randomly selecting 10 leaves from the rosette. Silique number was determined by counting each plant. Repeated measures *ANOVA* (Howell 1997) was used for analysis of all the measurements of height taken every two weeks for both the first and second experiment. Single factor *ANOVA* (Howell 1997) was performed for each dependent variable for the first experiment and two factor *ANOVA* (Howell 1997) was used to analyze the second experiment. The *ANOVAs* were analyzed using the *SAS System for Windows V8*. Normality for the *ANOVAs* was analyzed and checked using the *SAS System for Windows*.

Results

Intraspecific competition in *A. thaliana* Columbia*:* Seedlings of the Columbia ecotype were grown separately under controlled conditions at increasing densities of 1, 5, 10, 15, 20, 30, 40, 50 and 100 plants per pot. Root biomass, leaf biomass, flowering stalk biomass, silique number and height all differed significantly ($P < 0.0001$) according to increasing density (Table 1). Plant height (42 DAP) and silique number (56 DAP) decreased in a linear manner (Fig. 1) and similar results were seen with root biomass, leaf biomass, and flowering stalk biomass (data not shown). Both height and silique number significantly decreased relative to the control at a density of 10 plants per pot with a 50 % reduction seen at a density of 15 and 30 plants per pot, respectively. Based upon these data, we selected densities of 10, 20, and 50 plants per pot for subsequent experiments using the stomatal mutants *sdd1-2* and *tmm1-1* (see below).

Table 1. Single factor analysis of variance for flowering stalk height at 42 DAP, and root biomass, leaf biomass, flowering stalk biomass, silique number, and height at 56 DAP in *A. thaliana* Columbia. Degrees of freedom and F-values are given. *** - $P < 0.0001$.

	d.f.	MS	42 DAP	56 DAP
Root biomass	8	0.00304		10.87***
Error	27	2.80 E-04		
Leaf biomass	8	1.114		164.65***
Error	27	6.76 E-03		
Stalk Biomass	8	0.4535		133.02***
Error	27	0.0034		
Silique number	8	1124.57		184.54***
Error	27	6.094		
Height	8	602.17	323.63***	730.96***
Error 42 DAP	27	1.861		
Error 56 DAP	27	0.6652		

Intraspecific competition in *A. thaliana* Columbia and the mutants *sdd1-2* and *tmm1-1*: Seedlings of either Columbia, *sdd1-2*, or *tmm1-1* were grown under controlled conditions at increasing densities of 1, 10, 20, and 50 plants per pot, and the effect of intraspecific competition upon silique number, plant height, root biomass and flowering stalk biomass was evaluated (Fig. 2). For silique number (Fig. 2*A*) and height (Fig. 2*B*) Columbia declined in a linear fashion (see also Fig. 1), while *sdd1-2* and *tmm1-1* had maximal reduction at a density of 10 which is essentially unchanged at 20 or 50 plants per pot. The *sdd1-2* mutant differed significantly from both Columbia and *tmm1-1* for silique number at a density of 10 (Fig. 2*A*), and for height at a density of 50 (Fig. 2*B*). For root biomass, Columbia, *sdd1-2*, and *tmm1-1* had maximal reduction at a density of 10 (Fig. 2C), and *sdd1-2* and *tmm1-1* differed significantly from Columbia at a density of 50 plants per pot. For flowering stalk biomass, Columbia was reduced slightly at a density of 10, while *sdd1-2* and *tmm1-1* differed significantly from Columbia at a density of 10 and 50 plants per pot (Fig. 2*D*). Repeated measures *ANOVA* for plant height demonstrated a significant interaction between time (days after planting) ($P < 0.0001$), density ($P < 0.0001$), genotype ($P < 0.0001$), density and genotype ($P < 0.01$, Fig. 2*B*), and the three-way interaction with time ($P < 0.05$) (Table 2). This three-way interaction reflected increased growth of both mutants compared with Columbia at the lowest density between days 42 and 49 (data not shown).

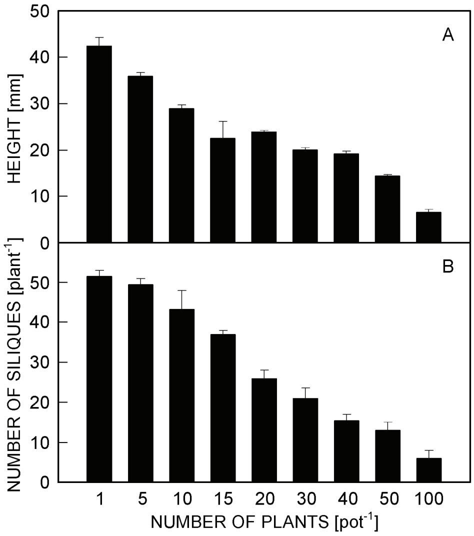

Fig. 1. Effect of intraspecific competition upon plant height (*A*) and silique number (*B*) in *A. thaliana* Columbia. Data presented represents 4 replicates, and *error bars* indicate standard deviation.

Table 2. Repeated measures *ANOVA* for flowering stalk height at 42 DAP and 49 DAP Columbia, *tmm1-1* and *sdd1-2*. The interactions between density, ecotype, and time are listed. Degrees of freedom, F-values, and Mean Squares (MS) are given. *** - $P \le 0.0001$, ** - $P \le 0.01$, * - $P \le 0.05$, NS - not significant.

	d.f.	MS	42DAP/49DAP
Time	1	56.84	279.08***
Density	3	2053.22	77.25***
Genotype	2	444.0	16.70***
Density × genotype	6	105.79	3.98**
Time × density	3	0.129	0.63 NS
Time × genotype	2	0.5578	2.74 NS
Time × density × genotype	6	0.501	2.46*
Error D × G	35	26.58	
Error T × D × G	35	0.204	

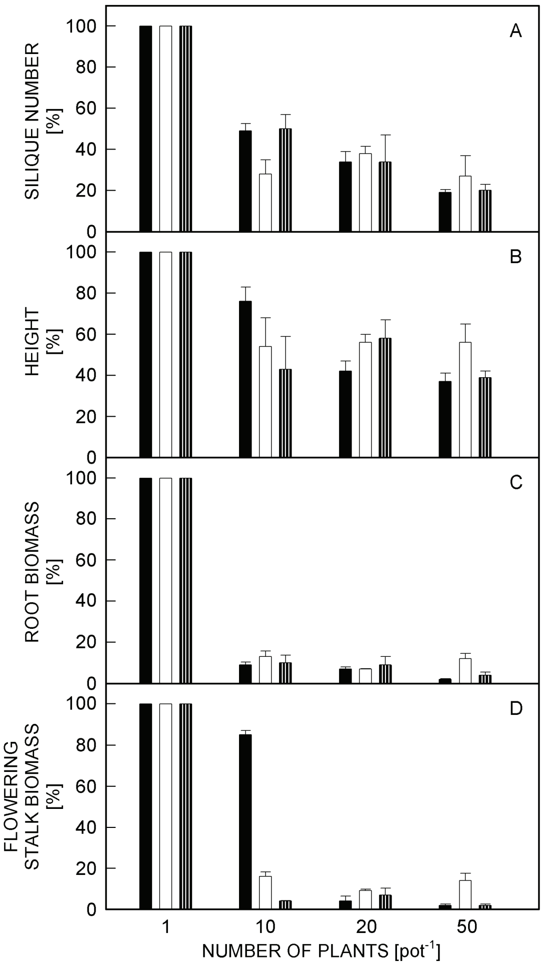

Fig. 2. Effect of intraspecific competition upon different characteristics in *A. thaliana* Columbia, *sdd1-2* and *tmm1-1* (*closed*, *empty* and *stripped columns*, respectively). Percent reduction for the second experiment was calculated using the averages from 4 replicates with the Columbia values normalized to 100 %. *A* - silique number, *B* - height, *C* - root biomass, *D* - flowering stalk biomass.

Discussion

Results from this study demonstrate that intraspecific competition in *A. thaliana* Columbia and the Columbia-derived mutants *sdd1-2* and *tmm1-1* were negatively impacted for several components of fitness: silique number, plant height, root biomass and flowering stalk biomass. In Columbia, silique number and plant height decreased in an essentially linear manner in response to increasing plant density (Figs. 1, 2*A*,*B*), while root and flowering stalk biomass decreased abruptly and did not decrease further in response to increasing density (Fig. 2*C*,*D*). Of greater interest is the fact that the mutants *sdd1-2* and *tmm1-1*, in sharp contrast to Columbia, exhibited a much lower threshold in their response to intraspecific competition. All fitness components significantly decreased at a density of 10 plants per pot, and did not decrease further in response to increasing competition (Fig. 2).

Aarssen and Clauss (1992) have analyzed the relationship of plant size and fecundity in *A. thaliana* and demonstrated that: *1*) larger plants had relatively low fecundities; *2*) seed viability did not depend on the amount of seeds produced per plant; *3*) and that fitness was not dependent on fecundity. However, biotic factors such as herbivory decrease fecundity of *A. thaliana*, and these decreases could be attributed to the allocation of resources to a variety of defense mechanisms (Mauricio 2001). Previous competition studies using *A. thaliana* have demonstrated that competition does not increase the cost to fitness of induced physiological responses such as systemic acquired resistance (SAR) (Cipollini 2002). Andalo *et al.* (2001) analyzed intergenotypic competition in *A. thaliana* in response to elevated CO_2 concentrations. Surprisingly, *Arabidopsis* performed better as a pure stand at current CO_2 levels and performed better as a mixture at elevated CO_2. In the short-term, elevated CO_2 increased the genetic diversity of the *Arabidopsis* stand.

Recently, Schluter *et al.* (2003) studied the response the *sdd1-2* mutant to altered irradiances and demonstrated that light-dependent alterations to stomatal density act independent of *SDD*. When exposed to high irradiances, low irradiance (200 - 250 μmol m^{-2} s^{-1}) adapted *sdd1-2* plants had identical net photosynthetic rates and stomatal conductance as compared to wild type. However, when exposed to elevated irradiances (400 to 1000 μmol m^{-2} s^{-1}) low irradiance adapted *sdd1-2* plants maintained higher net photosynthetic rates and accumulated higher amounts of both sucrose and hexoses. Our results demonstrate that at the highest density analyzed in this study (*i.e.* 50 plants per pot) (Fig. 2), *sdd1-2* is less sensitive to competition than both Columbia and *tmm1-1* for the fitness components of height (Fig. 2*B*), root biomass (Fig. 2*C*), and flowering stalk biomass (Fig. 2*D*). The enhanced photosynthetic rate of low irradiance adapted *sdd1-2*, coupled with the ability to accumulate greater amounts of sucrose and hexoses, may provide a mechanistic explanation for this observation. To our knowledge *tmm1-1* has not been analyzed per Schluter *et al.* (2003), however based upon the results of this study we would predict that the photosynthetic rate and sugar accumulation of low irradiance adapted *tmm1-1* exposed to elevated irradiances would be more similar to wild type than *sdd1-2*.

TMM1 and *SDD1* genes share a number of functions in mediating stomatal formation during leaf development that strongly suggest they can function as part of the same signal transduction pathway (von Groll and Altmann 2001, Serna and Fenoll 2002). However, the phenotypes of *tmm1-1* and *sdd1-2* are not completely co-incident and differ in several ways. Our results provide additional evidence that *TMM1* and *SDD1* function in the same pathway (Fig. 2). Both *tmm1-1* and *sdd1-2* have a lower threshold for responding to competition relative to Columbia, and do not respond to greater competition stress. However our results also provide evidence that *TMM1* and *SDD1* can act independently and differentially impact some components of fitness (Fig. 2*B*,*C*,*D*). Clearly *TMM1* and *SDD1* function in the same pathway (Von Groll and Altmann 2001, Serna and Fenoll 2002). However, *TMM1* may be responding to signals that do not originate with *SDD1*, or *SDD1* may be stimulating a signal transduction pathway independent of *TMM1*.

The stomatal mutants *sddl1-2* and *tmm1-1* show a different response to intraspecific competition in response to density. This contrast in intraspecific behaviour of genetic mutants provides a first demonstration that competitive interactions can be affected by just a few genes and supports the view that fine-scale genetic differences among individuals can have population-level implications (Ballare and Scopel 1997). Future experiments in our laboratories, utilizing DNA macro- and micro-arrays, will delineate the suite of genes differentially regulated by competition within these mutants, and evaluate the role of other genes predicted to be major determinants of interspecific competition.

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