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Genetic basis in plants for interactions with disease-suppressive bacteria

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ABSTRACT Plant health depends, in part, on associations with disease-suppressive microflora, but little is known about the role of plant genes in establishing such associations. Identifying such genes will contribute to understanding the basis for plant health in natural communities and to new strategies to reduce dependence on pesticides in agriculture. To assess the role of the plant host in disease suppression, we used a genetic mapping population of tomato to evaluate the efficacy of the biocontrol agent *Bacillus cereus* against the seed pathogen *Pythium torulosum*. We detected significant phenotypic variation among recombinant inbred lines that comprise the mapping population for resistance to *P. torulosum*, disease suppression by *B. cereus*, and growth of *B. cereus* on the seed. Genetic analysis revealed that three quantitative trait loci (QTL) associated with disease suppression by *B. cereus* explained 38% of the phenotypic variation among the recombinant inbred lines. In two cases, QTL for disease suppression by *B. cereus* map to the same locations as QTL for other traits, suggesting that the host effect on biocontrol is mediated by different mechanisms. The discovery of a genetic basis in the host for interactions with a biocontrol agent suggests new opportunities to exploit natural genetic variation in host species to enhance our understanding of beneficial plant–microbe interactions and develop ecologically sound strategies for disease control in agriculture.

Plants live in association with a rich diversity of microorganisms from the moment they are planted into soil as seeds. The most obvious and well studied plant–microbe interactions are those with pathogens that result in disease. For example, in tomato alone there are more than 30 named plant diseases caused by more than 40 different microbial species (1). However, plants also interact with beneficial microorganisms that suppress disease, enhance growth, fix atmospheric nitrogen, and solubilize and assimilate phosphorus and other nutrients (2). Much less is known about these beneficial plant–microbe interactions, particularly the role that plant genes may play in supporting or enhancing them. This situation represents a significant gap in our understanding of biology because most interactions of plants with microbes do not lead to disease, and, thus, we know the least about the most common plant–microbe interactions.

A promising application of beneficial plant–microbe interactions is microbial biocontrol, the use of beneficial microorganisms to suppress diseases caused by plant pathogens (3, 4). Though the subject of much research, the utility of microbial biocontrol in agriculture remains elusive because we lack understanding of the mechanisms of disease suppression in the face of complex and poorly understood ecological interactions (5).

The success of microbial biocontrol depends on the outcome of complex interactions among the plant host, beneficial

microflora, pathogens, and environment. Recent advances in genetics and molecular biology have provided tools to illuminate the mechanisms that underpin these interactions (6). Mechanisms contributing to disease suppression by microbial biocontrol agents include antibiosis, resource competition, parasitism, and induced resistance in the host (3, 4).

Efforts to improve the effectiveness of microbial biocontrol generally have concentrated on identifying or engineering new strains with enhanced attributes expected to increase disease suppression (7–10). Our goal was to provide a rigorous test of the role of plant genotype in a disease-suppressive microbial biocontrol interaction. Results of such a test are essential for exploiting genetic manipulation of crop plants to enhance the success of microbial biocontrol as proposed by Bliss and others (11, 12). Several previous reports have described variation among cultivars for disease suppression (12–16), colonization of the host (17), induction of resistance (13, 18), and induction of plant growth responses (19–21) by microbial biocontrol agents. Such phenotypic variation among cultivars may be, in part, the result of genetic variation and suggests genetic improvement of the host as an approach to development of superior biocontrol strategies.

We report results revealing that host genotype plays a significant role in the disease-suppressive interaction of plants with a microbial biocontrol agent. Our study made use of a previously established tomato mapping population (22) and an experimental system that we designed (15) by using the biocontrol agent *Bacillus cereus* UW85 (23) and the pathogen *Pythium torulosum* Coker and Paterson.

MATERIALS AND METHODS

Recombinant Inbred Line (RIL) Population. The mapping population used was derived from an interspecific cross of cultivated tomato and a related wild species, *Lycopersicon cheesmanii* (24). This population consists of 87 RIL (22), 61 of which produced sufficient seed for our study. We grew plants in the field from seed provided by D. Zamir (Hebrew University of Jerusalem), harvested fruits from single plants of each line, and extracted seeds as described previously (15). We were unable to produce a sufficient amount of seed from the *L. cheesmanii* parent; therefore, we omitted the parents from the phenotypic evaluations. The genetic map and marker data used to conduct the quantitative trait loci (QTL) analysis consists of 132 restriction fragment length polymorphisms distributed across the entire genome (22).

Disease Resistance. We evaluated host responses to inoculation with *B. cereus* and *P. torulosum* in two independent growth chamber experiments conducted as described previously (15). We used pathogen inoculum levels of 0, 5, 10, 20,

Abbreviations: QTL, quantitative trait loci; RIL, recombinant inbred line; BCI, biocontrol index; lod, logarithm of odds; PSS, proportion seedling survival.

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and 50 zoospores per seed in the first experiment and 0, 2, 5, 10, and 20 zoospores per seed in the second experiment. Ten seeds were planted for each of the three replicates of each of the five inoculum levels. We counted the number of healthy seedlings that emerged 16 days after planting and quantified resistance as the proportion seedling survival (PSS) in the pathogen treatment relative to the control.

Microbial Biocontrol. We evaluated four inoculum doses of the biocontrol agent obtained from 4-day cultures of *B. cereus* UW85, grown in half-strength tryptic soy broth, that were used undiluted or diluted (vol/vol) 1:1, 1:9, and 1:19 with sterile water. *B. cereus*-treated seeds (15) were evaluated at the *P. torulosum* dose of 10 zoospores per seed in both experiments. Biocontrol was quantified by the biocontrol index (BCI); $BCI = PSS$ in treatment with pathogen and biocontrol agent $- PSS$ in treatment with only the pathogen. To avoid possible problems associated with quantifying either resistance or biocontrol at a single inoculum dose (15), we calculated the mean PSS and BCI over the range of inoculum doses evaluated.

Bacterial Growth on the Seed. Growth was quantified as the change in population size of *B. cereus* UW85 on the seed from 0 to 48 h. *B. cereus*-treated seeds (15) were planted in autoclaved vermiculite in plastic pipette tips, watered with 1 ml sterile, distilled water, and placed in a growth chamber at 24°C. We measured the population size of *B. cereus* on the seed by placing the tip containing seed and vermiculite in a test tube containing sterile water, sonicating, and dilution-plating as described previously (15) to determine colony-forming units (cfu) per seed.

Rate of Seedling Emergence. We estimated days to 50% emergence of untreated seeds by fitting daily seedling emergence counts to a reparameterized version of the logistic equation used by Gan *et al.* (25), $Y = F/[1 + \exp(4S(T_{50} - t)/F)]$, where Y is the number of seedlings emerged, t is time in days, and T_{50} , S , and F are parameters to be estimated. This model describes an S-shaped curve relating time (x axis) to number of seedlings emerged (y axis). The parameters T_{50} , S , and F relate to the days to 50% emergence, variation for T_{50} among seedlings evaluated (slope of curve at T_{50}), and the maximum number of seedlings emerged (asymptote), respectively. We estimated values for these parameters by using nonlinear regression performed with PROC NLIN (SAS Institute, Cary, NC) using the Gauss-Newton method of iteration.

Data Analysis. To determine whether there was significant phenotypic variation among the RIL for the traits studied, we conducted ANOVA on the phenotypic data by using PROC GLM (SAS Institute). To examine the genetic basis for phenotypic variation, we conducted a QTL analysis by composite interval mapping by using the software package PLABOTL (26) with the following options: additive genetic model, cov select, F -to-enter = 3.5, RAIC = 3, and scanning interval of 2 cM (RAIC is the penalty value used when calculating the Akaike information criterion). The location of a QTL is defined as the position at which the logarithm of odds (lod) score reaches its maximum over the region being studied. We report QTL detected with a lod score >3.36 , corresponding to $P < 0.05$ and $P < 0.0004$ experimentwise and comparisonwise error rates, respectively. For each QTL, α (effect of wild-parent allele) is calculated as the regression coefficient for the corresponding QTL genotype in a multilocus regression model by using selected markers assuming no dominance (26). For example, an α value of 0.10 for a biocontrol QTL means that the estimated effect of the *L. cheesmanii* allele raises the BCI 0.10 unit. This result would mean that an individual that was homozygous for the *L. cheesmanii* allele at that locus would be 0.20 BCI unit higher than an individual that was homozygous for the *L. esculentum* allele at that locus, given that the two individuals otherwise were identical.

RESULTS

Phenotypic Variation for Host Effects. We observed significant phenotypic variation among the RIL for the host effect on disease suppression by the biocontrol agent *B. cereus* UW85 (Fig. 1*a*). This variation in the host is illustrated by a comparison of tomato lines supportive and nonsupportive of biocontrol. Inoculation of seeds not treated with the biocontrol agent, but treated with the pathogen (10 zoospores per seed), resulted in 2 and 17% seedling emergence for the RIL 14 and 37, respectively. The same level of pathogen inoculum applied to *B. cereus*-treated seeds resulted in 77% and 0% seedling survival, respectively.

We also observed significant phenotypic variation among the RIL for resistance to *P. torulosum* (Fig. 1*b*). This result was unexpected; despite the ubiquitous nature of *Pythium* species in soils and their wide host range (27, 28), there is little evidence for host resistance to seedling disease caused by these pathogens (15, 29–33).

Because colonization of the host by biocontrol agents is believed to be important for successful disease suppression in some systems (34–36), we measured growth of *B. cereus* on the seed during the first 48 h after planting. The change in population size on the seed from 0 to 48 h after planting ranged

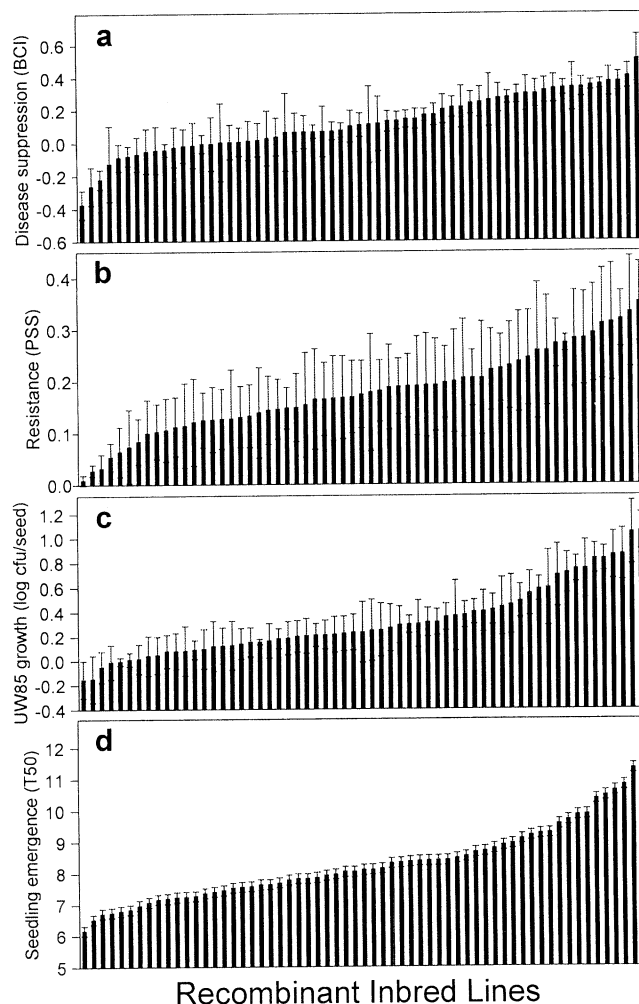


FIG. 1. Phenotypic variation among tomato 61 RIL for the traits: support of biocontrol (*a*), disease resistance (*b*), growth of UW85 on the seed (*c*), and rate of seedling emergence (T_{50}) (*d*). The RIL are arranged in ascending order for each trait independently. Bars represent the SEM for *a-c* and the asymptotic SE of the parameter estimate T_{50} for *d*. The phenotypic variation among the RIL for all four traits is significant ($P < 0.01$).

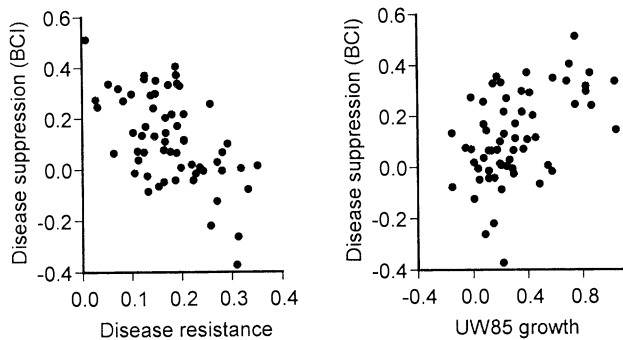


FIG. 2. Correlations among traits assessed for the RIL. (Right) Disease suppression and growth of *B. cereus* UW85 on the seed ($r = 0.50$, $P < 0.01$). (Left) Disease suppression and resistance to seedling damping-off ($r = -0.52$, $P < 0.01$).

from -0.15 to 1.05 log cfu per seed among the RIL (Fig. 1c). Subsequent experiments, using selected lines that differ dramatically in their support of growth of *B. cereus*, showed that the differences among lines continued to 96 h after planting, with nonsupportive lines continuing to support very little or no growth of UW85 (data not shown).

We also detected significant differences among the RIL for rate of seedling emergence in the absence of the pathogen (Fig. 1d). Because *P. torulosum* causes disease on germinating seeds or young seedlings, we reasoned that the rate of seed germination and emergence might affect resistance or disease suppression by UW85. The differences among the RIL for days to 50% emergence ranged from about 6 to 12 days after planting.

In addition to phenotypic variation among the RIL for the traits studied, we observed correlations among some of the traits. There was a negative correlation between resistance to *P. torulosum* and disease suppression (Fig. 2 Left). Growth of

UW85 on the seed was positively correlated to disease suppression (Fig. 2 Right).

QTL Associated with Host Effects. To determine the degree to which the phenotypic variation we observed was due to genetic variation, we used composite interval mapping to test the association of genetic markers with phenotypic variation. This approach provides an estimate of the number, position, and effect of genetic loci for a given trait. Our study utilized a relatively small population size (61 RIL) to estimate these parameters and, therefore, limited our ability to detect and accurately measure the effects of all QTL affecting these traits. We were, however, able to identify several QTL for each of the traits studied.

We detected three QTL, each located on a different chromosome, contributing to disease suppression by UW85 (Table 1). The allele contributed by the wild parent enhances disease suppression by UW85 at one of these loci (indicated by a positive α value in Table 1). The QTL *mbc* 5.1 had a relatively strong effect, individually accounting for more than 25% of the phenotypic variance. The multilocus model including all three QTL explained 38% of the phenotypic variance.

We also detected three QTL associated with resistance to *P. torulosum*. Two of these QTL had very small effects and are located on chromosome 4, and the other is located on chromosome 11. The multilocus model was not significant, suggesting that only the QTL detected on chromosome 11 had a significant effect. The allele contributed by the wild parent enhances resistance at this locus.

Three QTL explain 17% of the phenotypic variation for the host effect on growth of *B. cereus* on the seed. As in the case of biocontrol, these QTL are distributed on different chromosomes. The wild-parent allele has a negative effect on growth for two of the three QTL.

The rate of seedling emergence was the trait with the most phenotypic variation among the RIL and the most QTL detected. Six QTL on five chromosomes together explained about 30% of the phenotypic variation. At two of the QTL the

Table 1. Positions and effects of tomato QTL conditioning traits involved in the interactions with a microbial biocontrol agent (*B. cereus*) and a plant pathogen (*P. torulosum*)

Trait	QTL name	Chromosome*	Estimated QTL position, cM	lod score [†]	Partial r^2 [‡]	Substitution effect (α) [§]
Microbial biocontrol	<i>mbc</i> 5.1	5b	66	4.59	26.6	-0.142
	<i>mbc</i> 7.1	7	48	6.20	13.3	0.074
	<i>mbc</i> 10.1	10	56	3.85	15.5	-0.085
	Total			6.13	38.1	
Resistance to <i>P. torulosum</i>	<i>ptr</i> 4.1	4	0	3.74	1.2	0.011
	<i>ptr</i> 4.1	4	146	3.81	1.4	-0.011
	<i>ptr</i> 11.1	11	88	4.61	8.9	0.028
	Total			1.27	9.4	
Growth of UW85 on seed	<i>sgr</i> 4.1	4	122	5.27	8.1	-0.102
	<i>sgr</i> 10.1	10	10	5.34	6.7	-0.091
	<i>sgr</i> 11.1	11	42	3.49	3.5	0.069
	Total			2.19	17.3	
Seedling emergence (T_{50})	<i>sem</i> 1.1	1b	54	4.80	0.7	0.148
	<i>sem</i> 3.1	3a	42	4.66	12.7	0.487
	<i>sem</i> 3.2	3b	12	4.93	6.7	0.352
	<i>sem</i> 7.1	7	40	7.04	14.3	-0.663
	<i>sem</i> 9.1	9	50	5.05	5.4	0.307
	<i>sem</i> 10.1	10	0	4.40	6.6	-0.336
	Total			4.36	29.7	

*Chromosome designation on the published map (22), constructed from the interspecific cross between the domesticated *Lycopersicon esculentum* (cv. UC204 C) and a related wild species, *L. cheesmanii* (LA 483) (24), and consisting of 132 restriction fragment length polymorphism markers distributed throughout the genome.

[†]lod score, log of the likelihood odds ratio, is calculated as described previously (45). Threshold for detecting a QTR was 3.36.

[‡]The partial r^2 is an estimate of the percentage of the phenotypic variation explained by a QTL and is calculated as the square of the correlation coefficient from the regression model. The total phenotypic variation for a trait explained by the model is the square of the correlation coefficient obtained from the multiple-regression fit of the model that includes all putative QTL detected for the trait.

[§] α is the effect of substituting an allele from the domestic parent with the allele from the wild parent.

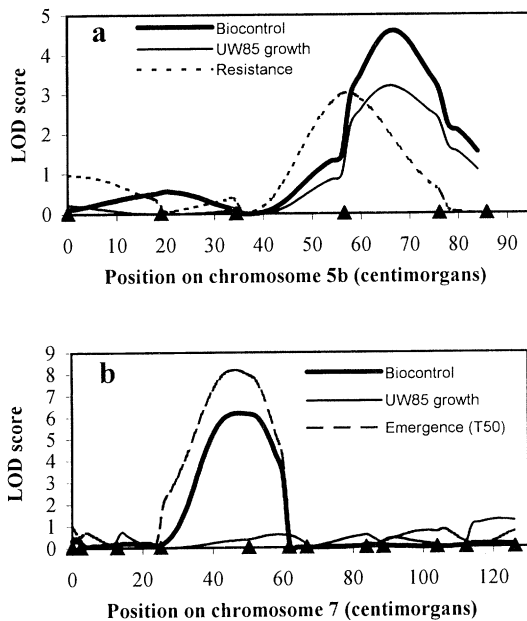


FIG. 3. Tomato QTL affecting interactions with a pathogen and biocontrol agent on chromosome 5 (a) and chromosome 7 (b). Solid triangles indicate the position of markers on the published map (22). On chromosome 5 the markers shown are CT93, TG503, TG619, TG23, TG69, and TG185 from left to right. On chromosome 7 the markers shown are TG499, CD65, TG438, TG20, TG572, CT84, TG170, CAB4, TG252, TG156, and CD54 from left to right.

wild parent had a negative effect on the rate of seedling emergence, whereas at the other four the wild parent had a positive effect.

Regions of the Genome Associated with Multiple Traits. In two regions of the genome, QTL for biocontrol mapped very near QTL for other traits. The biocontrol QTL *mbc* 5.1 maps to the same region of chromosome 5 as a QTL, detected just below the 3.36 lod threshold, associated with growth of *B. cereus* on the seed (Fig. 3a). The effect of the allele contributed by the wild parent is negative for both phenotypes. In another region, on chromosome 7, the biocontrol QTL *mbc* 7.1 is closely linked to *sem* 7.1, which is associated with the rate of seedling emergence (Fig. 3b). The effect of the wild-parent alleles for *sem* 7.1 and *mbc* 7.1 increases the rate of seedling emergence and enhance biocontrol, respectively. This region is not associated with growth of *B. cereus* on the seed.

DISCUSSION

This report documents a genetic analysis in plants of supportiveness of disease suppression by a microbial biocontrol agent. Several previous studies document phenotypic variation in plant health and nutritional status resulting from mycorrhizal (37) and *Rhizobium* (38) plant-microbe interactions. There is some evidence that modern breeding efforts in crop plants inadvertently have selected against hosting such beneficial microflora (39). Taken together, these results and ours suggest significant untapped potential to exploit genetic variation in the host through breeding to enhance beneficial interactions with microorganisms, just as plant breeding has harnessed tremendous genetic variation in plant germplasm to increase crop productivity and enhance the plant's ability to endure pathogens, pests, and harsh physical environments.

Our results, in terms of the number of QTL detected for disease suppression, are consistent with a recent evaluation of the power and precision of statistical approaches to QTL analysis (40). It is likely, given the relatively low percentage of

phenotypic variation explained by multilocus models, that our results represent an underestimate of the total number of QTL controlling these traits and that much larger populations would be necessary for a complete census of such QTL. When we analyzed these data with a lower lod threshold we identified several additional QTL and constructed multilocus models that explained more of the phenotypic variation (not shown).

The host effect on disease suppression is linked genetically to at least two other traits. There are two possible explanations for QTL associated with different traits mapping to the same locus. One possibility is that QTL for the traits are closely linked genetically but unrelated phenotypically. A second possibility is that multiple traits are controlled by a single locus (i.e., pleiotropy). In the latter case, a gene may have two functions, or the expression of one trait may be, in part, causal to the expression of another trait.

The coincidence of QTL for disease suppression and growth of UW85 on chromosome 5 suggests that the effect on microbial biocontrol is mediated, in part, by growth of the biocontrol agent on the host. The importance of growth of biocontrol agents on the host for successful disease suppression is widely accepted in the microbial biocontrol literature (3, 41, 42), but has been documented by results of only a few studies (34–36). The ability of a biocontrol agent to colonize the host is probably, in part, a result of its ability to grow, but other factors currently not well understood, such as persistence and competition for space, are likely to contribute as well.

The QTL for disease suppression on chromosome 7 was not associated with growth of UW85 but, rather, with rate of seedling emergence. Others (3) have speculated that metabolic activity, and not growth *per se*, of the microbial biocontrol agent is necessary for disease suppression. It is possible in our system that *sem* 7.1 is involved with some biological activity early in seedling development that affects metabolic activity or the expression of disease-suppressive factors by UW85 without influencing its growth. Experiments are underway in our laboratories to develop tools to monitor the host effect on the expression of one of the antibiotics produced by UW85 (43) known to be important for disease suppression, as well as identifying genes differentially regulated by different host genotypes that may be involved in disease suppression (A. Dunn, personal communication).

The phenotypic variation we observed for host effect on disease suppression was generated by crossing tomato to a related wild species. Conventional approaches for exploiting exotic sources of genetic variation involve phenotypic identification of an individual with a desirable trait and crossing it to an elite cultivar to introduce the new genes. An emerging new paradigm advocates a genetic- rather than phenotypic-based approach to identifying new genes by using molecular-linkage maps and marker-assisted selection (44). Tanksley and McCouch (44) have drawn attention to the emerging realization that often very desirable genes can be found in individuals with agriculturally undesirable phenotypes. Although insufficient seed supply prevented us from assessing the phenotypes of the parents used to generate the RIL used in this study, we predicted, based on the effects of the biocontrol QTL we identified, that the wild parent would be less supportive than the domestic parent. It nevertheless contributed an allele that is supportive of biocontrol at one of the three loci.

Permanent mapping populations in the form of RIL exist for several important crops; these are an exceptional resource that should be used to study the genetics of plant associations with beneficial microbes. Advances in the understanding of how plant genes affect beneficial plant-associated microbes will lead to sound, ecologically based strategies for future disease-control practices.

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