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Development of *fad7-1* single mutant *Arabidopsis thaliana* plants that are resistant to aphids

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ABSTRACT

Aphids are a group of sap-feeding insects that attack most of the world's crops. The loss of function of fatty acid desaturase7 (FAD7) in *Solanum lycopersicum* (tomato plant) induces aphid resistance that is dependent upon the accumulation of plant defense hormones such as salicylic acid (SA). Tomato lacks most of the genetic resources found in the model plant Arabidopsis (*Arabidopsis thaliana*). There is an analogous *fad7-1* line of Arabidopsis; however, the line has a background mutation, the *glabra-1 (gl1)*, that causes the absence of trichomes (small hairs), which are essential to plant defense. In order to study aphid resistance, a single mutant line of *fad7-1* mutants were developed using cross breeding between the *fad7-1/gl1* mutant and wild-type plants. Homozygous *fad7-1* mutants were then identified using the Kasajima DNA extraction method, followed by the use of single nucleotide polymorphism-polymerase chain reaction (SNP-PCR) primers using allele-specific PCR. A phenotypic screening was then performed to screen out the plants with the *glabra-1* mutation using the presence or absence of trichomes. Two single Arabidopsis *fad7-1* mutant lines were identified, and subsequently verified using a bioassay to be aphid resistant relative to other genotypes as seen in tomato.

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MEET THE STUDENT-AUTHOR

Kaleb Vaughn

I was born and raised in Fayetteville and graduated from Har-Ber High School in 2010. I graduated from Harding University with a degree in Molecular and Cellular Biology in spring of 2014 and will be attending medical school in the fall. I plan to continue research as a medical student and physician using the skills I learned in Fiona Goggin's lab.

I could not have completed this research without the members of the Goggin lab, including Dr. Fiona Goggin and Dr. Carlos Avila, who were patient and devoted in teaching me the necessary skills of a researcher. Due to their guidance my poster presentation won best undergraduate presentation at the 98th annual Arkansas Academy of Sciences Conference. I was also able to present this research as my senior seminar at Harding. I also am indebted to Dr. Steven Moore along with other Harding science faculty who saw my potential in research and encouraged me to pursue it.

INTRODUCTION

Organisms must have mechanisms by which they defend against predators. Plants have developed structural, chemical, and protein-based defense mechanisms designed to detect and defend against invading organisms. Aphids (plant lice) are a group of sap-feeding insects that attack most of the world's crops (Goggin, 2007). They reduce yield by stealing nutrients from the plant or by transmitting plant pathogenic viruses. In response to insect attack, plants release hormones that activate plant defensive signaling. A specific hormone is salicylic acid (SA), a signaling hormone that stimulates systemic acquired resistance throughout the plant via the increased expression of defense proteins. It has been shown that the loss of fatty acid desaturase7 (FAD7) in *Solanum lycopersicum* (tomato plant), due to a mutant allele (*fad7-1*), leads to an increased accumulation and signaling of SA along with an increased level of aphid resistance (Avila et al., 2012). However, the molecular mechanisms by which SA accumulation and signaling are enhanced in the *fad7-1* mutant in response to aphids are unknown.

Tomato is an excellent model to study plant-aphid interactions; however it lacks most of the genetic resources found in model plant Arabidopsis (*Arabidopsis thaliana*). There is an analogous *fad7-1* line of Arabidopsis; however, the line has a background mutation, the *glabra-1 (gl1)*, that causes the absence of trichomes (small hairs), which are essential to plant defense (Xia et al., 2010). Therefore, to identify the molecular mechanisms by which SA levels are increased due to the FAD7 mutation using the genetic resources found in Arabidopsis, a single mutant line of *fad7-1* plants must be developed, and aphid resistance must be confirmed in this line.

MATERIAL AND METHODS

Plant Material. We used the F2 segregating population of a previously made cross between Arabidopsis *fad7-1/gl1* with *sid2-2* salicylic acid mutant plant to screen for single mutant lines. The salicylic acid mutant was used for practical reasons that included its availability in the Goggin lab. The *sid2-2* mutation was screened out later in a progeny test.

DNA Extraction. The DNA was extracted from 3-5 mg of fresh tissue using the Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, Mo.) or the method reported by (Kasajima et al., 2004) consisting of a buffer solution of 200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, and 0.5% SDS. Then the buffer was diluted tenfold with TE buffer 910 mM Tris-HCl pH 8 and 1 mM EDTA. In order to speed-up tissue grinding, tissue was homogenized using 3 grinding glass beads in 1.5- or 2-ml tubes in a GenoGrinder (Spex Sample Prep, Metuchen, N.J.) tissue homogenizer at 1750 RPM for 30 s.

fad7-1 screening. The *fad7-1* mutation in Arabidopsis was identified to be a C to T transition in the *FAD7* DNA coding sequence, converting the amino acid at position 253 from proline to leucine (Xia et al., 2010). Single nucleotide polymorphism primers (SNP-primers) were designed by placing the wild-type or mutated base at the 3' end on the forward oligonucleotide primer. Both primer sets share the same reverse primer. Touchdown polymerase chain reaction (TD-PCR; Korbie and Mattick, 2008) was performed to increase amplification sensitivity and specificity using the following conditions for mutated allele: initial denaturation = 95 °C for 5 min; phase I = 95 °C for 45 s, 65/67 °C to 56/58 °C for 45 s (reducing 1 °C per cycle), and 72 °C for 45 s; phase II = 95 °C for 45 s, 55/57 °C for 45 s, and 72 °C for 45 s (20 cycles); and final extension at 72 °C for 5 min.

Gel Electrophoresis. The PCR amplification was run in 1% agarose gel at 250 V for 16 min (Fig. 1).

gl1 Screening. Presence or absence of trichomes was visually assessed with the help of a magnifying glass.

sid2-2 Screening. A progeny test was performed on 12 homozygous *fad7-1* F2-3 plants using a primer set which only amplifies DNA from plants having wild-type *SID2* gene (SID2(F) = 5'-TTCTCAATTGGCAGGGAGAC-3' and SID2(R) = 5'-AAGCCTTGCTTCTTCTGCTG-3') using the following PCR conditions: = 95 °C for 5 min initial denaturation; 95 °C for 45 s, 55 °C to 56 °C for 45 s, and 72 °C for 45 s (30 cycles); and final extension at 72 °C for 5 min.

Screening for fad7-1 Homozygous Mutant. The (*fad7-1/gl1 x sid2-2*) F2 population was first visually selected by the presence of trichomes and then screened by PCR for the presence of the *fad7-1* mutation (*fad7-1/gl1 x sid2-2*) F3 progeny test. Selected homozygous *fad7-1* mutant F2 plants were self-pollinated to obtain the F3 lines.

Aphid Reproduction Bioassay. Thirty-two plants of four genotypes: *fad7-1*, *fad7-1/gl1*, *gl1*, and *wild-type* were grown to maturity and then inoculated with three aphid adults per plant. Aphids were allowed to feed and reproduce for 72 hours before aphid numbers were assayed. Statistical analysis was completed using a Kruskal-Wallis with pairwise comparison post-hoc analysis.

RESULTS AND DISCUSSION

As expected, the wild-type *FAD7* primer set amplified a band from plants carrying the wild-type allele (the *sid2* parental line and one of the F2 plants shown), but not

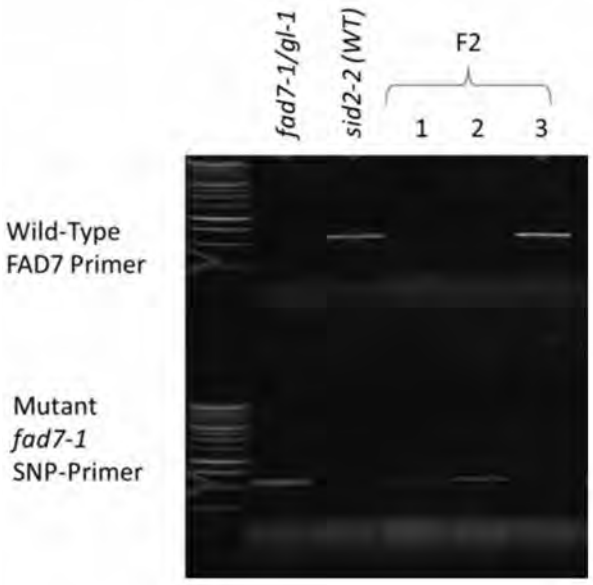

Fig. 1. Single Nucleotide Polymorphism-based polymerase chain reaction (PCR) screening was used to detect the presence of the wild-type and/or mutant alleles of the *FAD7* gene in a segregating population. From the left: lane 1 contains molecular weight markers, lane 2 contains PCR products amplified from a plant that is known to be homozygous for the mutant allele, lane 3 contains PCR products from a plant homozygous for the wild-type allele, and lanes 4 through 6 (labeled F2 1-3) are from 3 separate F2 plants from the *fad7-1/gl1 x sid2-2* cross. In two separate PCR reactions, the samples were amplified with primers for the wild-type allele (top), or the mutant allele (bottom).

from the *fad7-1* mutant plants. Conversely, the primer set designed to be specific to the *fad7-1* mutation amplified a band in the *fad7-1* mutant and two of the segregating F2 plants, but not the *sid2* parental line (Fig. 1). Heterozygous F2 plants show amplification with both primer sets (not shown). Twenty-four F3 plants for each of the 17 homozygous *fad7-1* mutant F2 selected plants were tested for *sid2-2* segregation using wild-type SID2 allele specific primers. We did not obtain a segregation ratio of 1 (*FAD7/FAD7*):2 (*FAD7/fad7-1*):1 (*fad7-1/fad7-1*), instead we observed a 1.1:1.5:1 ratio, respectively (19 *FAD7/FAD7*; 25 heterozygotes; 17 *fad7-1/fad7-1*). Alteration of expected segregation ratio may be due to low F2 sampling number and/or distortion by selecting *GL1* plants only.

Before the *sid2-2* progeny test, which would require a large volume of screening, DNA extraction methods were compared to discern which was the most efficient. The PCR amplification using DNA extracted using the method of Kasajima et al. (2004) yielded brighter bands as compared to the Extract-N-Amp Plant kit from Sigma (Fig. 2). Band intensity varied between different tissue homogenization treatments using Kasajima method, however extracting DNA in 1.5-ml tubes yielded brighter bands than using 2-ml tubes. Although, using rods to crush the DNA gives good DNA yield, using the tissue homogenizer reduced the time of sample preparation.

Two independent F2:3 lines have shown no segregation for the presence of the *SID2* allele (Fig. 3). Therefore, these two lines have a single mutation at the *FAD7* locus and are wild type for *SID2* and *GL1*. These mutant lines of Arabidopsis were aphid resistant relative to other genotypes after conduction an aphid reproduction assay ($P < 0.001$; Fig. 4).

Fig. 2. Comparison of DNA extraction methods. Polymerase chain reaction (PCR) was performed with primers for the wild-type SID2 allelle on DNA samples from 2 wild type plants that were collected by 4 different DNA extraction methods: 1) a commercial DNA extraction and amplification kit, the Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, Mo.); 2) a simple, one-step method reported by Kasajima and coworkers (Kasajima et al., 2004) using a GenoGrinder (Spex Sample Prep, Metuchen, N.J.) tissue homogenizer in 1.5-ml tubes; 3) the Kasajima method using tissue homogenizer in 2-ml tubes; and 4) the Kasajima method using a plastic pestle to crush the tissue in 1.5-ml tubes. Samples that gave brighter bands were assumed to contain higher-quality DNA with fewer PCR inhibitors.

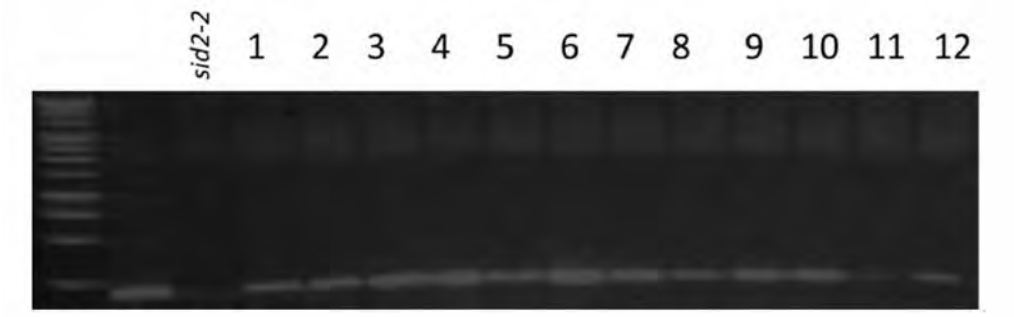

Fig. 3. F3 progeny derived from the *fad7-1gl1 X sid2* cross were screened by polymerase chain reaction (PCR) with primers for the wild-type *SID2* allele in order to to select for a homozygous line that carries the *fad7-1* mutation but not the *sid2* mutation, and that can be used as a control in experiments to analyze the effects of the *fad7-1*, *gl1*, and *sid2* genes singly and in combination. From the left, the first lane contains molecular weight markers, the second contains PCR products amplified from a *fad7-1 gl1* sample, the third is from a homozygous *sid2* mutant, and the remaining lanes (labeled 1 through 12) are from 12 separate T3 progeny collected from the same F2 parent, which was previously confirmed to be homozygous for the *fad7-1* mutation. All F3 progeny had the wild-type *SID2* allele. This gives a high probability that no segregation is present, and that the line is homozygous for the *SID2* allele, and therefore *fad7-1* single mutants.

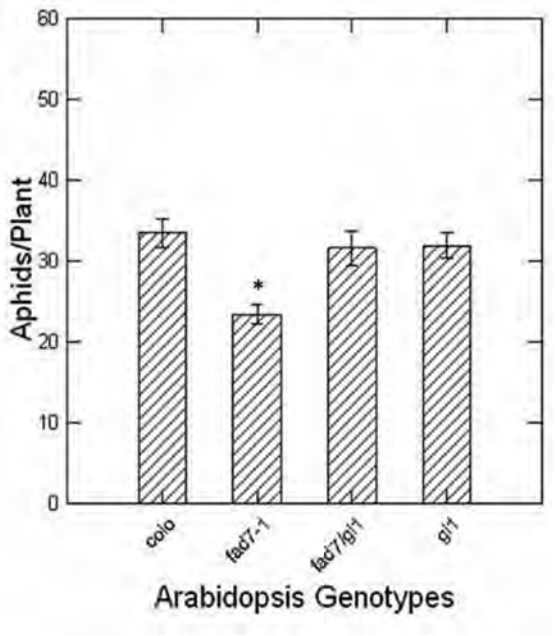

Fig. 4. Aphid population growth on different Arabidopsis genotypes, including wild-type plants (Col0), single mutants (*fad7-1, gl1*) and a double mutant with both the *fad7-1* and *gl1* mutations (*fad7/gl1*). Plants were inoculated with 3 aphids per plant, and then total aphid numbers were counted 72h later (n = 32). The *fad7-1* single mutants had significantly lower aphid numbers relative to the other three genotypes, according to a Kruskal-Wallis test with pairwise comparison ($P < 0.001$).

Because of the significant genetic resources found in Arabidopsis along with its short life cycle and hardy disposition, the development of a *fad7-1* single mutant allows for more efficient research into increased salicylic acid accumulation due to loss of FAD7. The SNP-based PCR screening along with glabra-1 phenotypic screening successfully identified homozygous *fad7-1* mutants without the *gl1* mutation in Arabidopsis. The screening methodology involved was efficient and relatively inexpensive, and can be used in further genotype tracking among plants including developing double mutants carrying the *fad7-1* and the *ssi2* mutations. Identified single *fad7-1* mutant lines will open the opportunity to test the effect of fatty acid desaturation on plant-aphid interaction in Arabidopsis.

The aphid reproduction bioassay was performed to test the effect of the *fad7-1* mutation on aphid reproduction and mortality in Arabidopsis, without the interfering effect of the *glabra1* (*gl1*) mutation. The *fad7-1* single mutant exhibited significantly decreased numbers of aphids relative to the other three genotypes after the 72 hour period. This result confirmed the *fad7-1* line in Arabidopsis to be aphid resistant relative to other genotypes as seen in tomato (Fig. 4).

Further research using single mutant *fad7-1* Arabidopsis will yield information into the mechanism that confers aphid resistance in FAD7 deficient plants through increased accumulation of salicylic acid. The epigenetic silencing of the *FAD7* gene also yields adverse effects to plants that include vulnerability to caterpillars and drought. The ultimate goal is to discover the primary catalyst of increased SA accumulation in plants and to maintain this effect without the loss of function of the FAD7 protein. Aphid resistant crops would lessen the formidable burden of agricultural pests to farmers.

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LITERATURE CITED

Avila, C.A., L. M. Arevalo-Soliz, L. Jia, D. A. Navarre, Z. Chen, G. A. Howe, Q. W. Meng, J. E. Smith, and F. L. Goggin. 2012. Loss of function of Fatty Acid Desaturase 7 in tomato enhances basal aphid resistance in a salicylate-dependent manner. Plant Physiology. 158:2028-2041.

Goggin, F.L. 2007. Plant-aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology. 10:399-408.

Kasajima, Y., N. Ide, H. Ohkama-Ohtsu, T. H. Yoneyama, and T. Fujiwara. 2004. A protocol for rapid dna extraction from *Arabidopsis thaliana* for PCR analysis. Plant Molecular Biology Reporter. 22:49-52.

Korbie, D.J. and J.S. Mattick. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature Protocols. 3:1452-1456.

Xia, Y., K. Yu, D. Navarre, K. Seebold, A. Kachroo and P. Kachroo. 2010. The *glabra1* mutation affects cuticle formation and plant responses in microbes. Plant Physiology. 154:833-846.