IDENTIFICATION OF CANDIDATE RESISTANCE GENES FOR BASIL DOWNY MILDEW VIA TRANSCRIPTOMICS

Annelise Vieira

INTRODUCTION + BACKGROUND

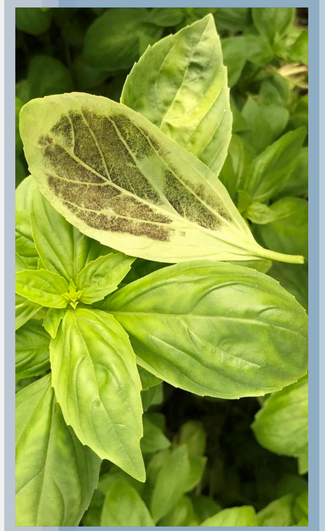


Figure 1: A basil leaf infected with downy mildew [1]

- *Ocimum basilicum*, or sweet basil, is one of the most important culinary herbs in the world and its production comprises a $300 million market in the United States [4].
- Basil downy mildew, a disease caused by fungus-like pathogen *Peronospora belbahrii*, has become extremely detrimental to the basil industry throughout the country since its introduction in 2007 [4].
- A basil cultivar with genetic resistance to downy mildew was developed at the University of Florida via an interspecific cross between *Ocimum basilicum* and *Ocimum americanum* followed by backcrossing. *O. americanum* is a wild African species of basil that is naturally resistant to BDM but lacks many of the commercially favored traits of *O. basilicum* [1].
- At this time, little is known about the genetic control or biochemical mechanism of the observed resistance, but understanding its genetic basis is important for improving models of plant pathogen response systems, enabling breeding for resistance in the face of pathogen evolution, and allowing the introduction of valuable genes into other susceptible plant species via breeding or genetic engineering.
- **HYPOTHESIS:** Analyzing transcriptomes of downy mildew resistant and susceptible basil plants for identification of differentially expressed genes and resistance gene analogs will facilitate selection of candidate resistance genes.

RESULTS

- Of the 280,536 transcripts evaluated within the transcriptome, 5,100 transcripts were identified as resistance gene analogs via DRAGO2.
- A range of domains were identified, including kinases, leucine rich repeats, transmembrane domains, coiled coils, Toll/interleukin-1 receptors, and nucleotide binding sites.
- 133 DEGs were identified between SI and SC and 199 DEGs were identified between RI and RC.
  - Of the RI/RC DEGs, 102 were identified as resistance gene analogs.
- Organized excel files were generated containing all identified transcripts, domains identified and coordinates.

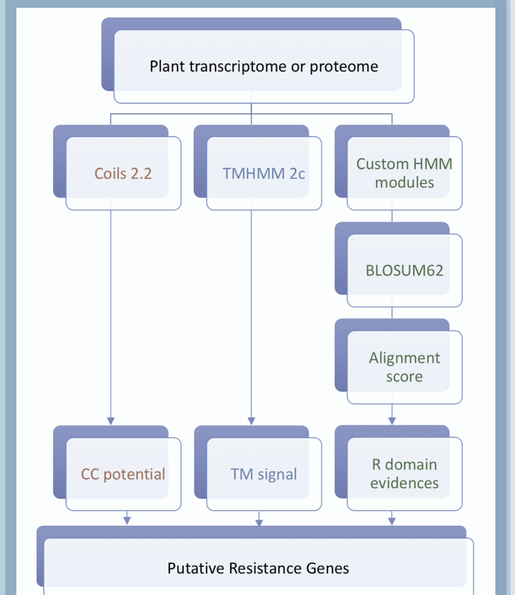
Figure 3: A portion one of the Excel files

	A	B	C
1	TRANSCRIPT IDENTIFIER	START-STOP.	DOMAINS
2	DN806_c0_g1_i1	97-126.	L.LRR.
3	DN901_c0_g1_i1	228-244.	N.NBS.
4	DN901_c0_g2_i1	56-64.	N.NBS.
5		99-115.	N.NBS.
6	DN1010_c0_g1_i1	16-27.	TRAN.TM.
7	DN1458_c0_g1_i1	14-28.	N.NBS.
8		48-58.	N.TM.
9	DN1718_c0_g1_i1	92-126.	CN.CC.
10		176-195.	CN.NBS.
11		253-268.	CN.NBS.
12		304-322.	CN.NBS.
13		331-355.	CN.NBS.
14		170-185.	CN.TM.
15		1-12.	CN.TM.
16		347-359.	CN.TM.
17	DN1718_c0_g2_i1	12-31.	N.NBS.
18		140-158.	N.NBS.
19		167-191.	N.NBS.
20		89-104.	N.NBS.
21		183-195.	N.TM.
22		5-21.	N.TM.

METHODS

- RNA was extracted from four samples of basil leaves:
  - Resistant inoculated (RI)
  - Resistant control (RC)
  - Susceptible inoculated (SI)
  - Susceptible control (SC)
- Transcriptomes were assembled for each sample and Differential Gene Expression analysis was performed using DESeq2 from Bioconductor to identify differentially expressed genes (DEGs) between samples.
- The DRAGO2 pipeline was used to search the RI vs RC transcriptome and the DEGs for common plant resistance gene motifs [2,3].

Figure 2: A representation of the DRAGO2 RGA prediction pipeline workflow [2]



CONCLUSION + FUTURE DIRECTIONS

- **TAKE HOME: The present study was successful in isolating a manageable number of genes of interest from the transcriptomic data, thereby generating a useful dataset for further study of resistance gene candidates.**
- To gain more insight into the mechanism of the observed downy mildew resistance, further study should involve
  - further bioinformatic analysis of the identified genes of interest
  - proposal of a mechanism of resistance based on the genes involved
  - quantification of expression of candidate genes in downy mildew resistant and susceptible basil varieties for experimental validation of transcriptomic data

REFERENCES

1. Ben-Naim, Y., Falach, L., & Cohen, Y. (2015). Resistance Against Basil Downy Mildew in *Ocimum* Species. Phytopathology, 105 (6), 778-785. doi:10.1094/phyto-11-14-0295-r
2. Osuna-Cruz, C. M., Paytuvi-Gallart, A., Di Donato, A., Sundesha, V., Andolfo, G., Cigliano, R. A., Sanseverino, W., & Ercolano, M. R. (2018). PRGdb 3.0: a comprehensive platform for prediction and analysis of plant disease resistance genes. *Nucleic Acids Research*, *46*(D1), D1197-D1201. doi:10.1093/nar/gkx1119
3. Sekhwal, M. K., Li, P. C., Lam, I., Wang, X. E., Cloutier, S., & You, F. M. (2015). Disease Resistance Gene Analogs (RGAs) in Plants. *International Journal of Molecular Sciences*, *16*(8), 19248-19290. doi:10.3390/ijms160819248
4. Wyenandt, C., Simon, J., Pyne, R., Homa, K., McGrath, M., Zhang, S., Raid, R., Ma, L., Wick, R., Guo, L. and Madeiras, A. (2015). Basil Downy Mildew (*Peronospora belbahrii*): Discoveries and Challenges Relative to Its Control. Phytopathology , 105(7), pp.885-894.
5. Zhan, X. Q., Yang, L., Wang, D., Zhu, J. K., & Lang, Z. B. (2016). De novo assembly and analysis of the transcriptome of *Ocimum americanum* var. pilosum under cold stress. *Bmc Genomics*, *17*, 12. doi:10.1186/s12864-016-2507-7

UF | IFAS Research
UNIVERSITY of FLORIDA