Title
Drought and the Microbiome: Advancements in Agriculture

Journal
Berkeley Scientific Journal, 24(1)

ISSN
1097-0967

Authors
Awasthi, Shevya
Das, Doyel
Harari, Emily
et al.

Publication Date
2019

DOI
10.5070/BS3241046910

Undergraduate

DROUGHT AND THE MICROBIOME:

Advancements in Agriculture

Interview with Faculty Member, Dr. Peggy Lemaux

By Shevya Awasthi, Doyel Das, Emily Harari, Ananya Krishnapura, Erika Zhang, and Rosa Lee

Dr. Peggy Lemaux[1]

***Dr. Peggy G. Lemaux** is a Cooperative Extension Specialist in the Department of Plant and Microbial Biology and the lead faculty member for The CLEAR (Communication, Literacy, and Education for Agricultural Research) Project at the University of California, Berkeley. She is the head of the $12.3 million EPICON (Epigenetic Control of Drought Response in Sorghum) project funded by the U.S. Department of Energy. Her research utilizes biochemical, transcriptomic, and genomic methods to investigate and improve the quality and hardiness of crop plants, especially in response to environmental stressors. In this interview, we discuss the EPICON project and her findings thus far concerning drought response in sorghum and its microbiome in large-scale field experiments.*

BSJ: Much of your research centers on sustainable agricultural practices and food security. What first drew you to these topics, and what has fueled your passion throughout your career?

PGL: I grew up on a farm in northwestern Ohio, so I understood how hard it is to produce food. Because of the hard work, I wanted to get as far away from the farm as possible. My brothers were both engineers. I also wanted to be an engineer, but my high school advisor said, "Oh, a lot of hard math; women can't handle that," and he actually directed me to home economics. I was a home economics major for two years, but then I was given a biochemistry book, and it was very thin. I thought, "This is not right. I know there's more to biochemistry than that." So, I switched over and became a microbiology major. My idea was that I wanted to help people, so I got my undergraduate, master's, and PhD degrees in microbiology, with an eye toward improving people's health. For my first postdoc, I was at the Stanford Medical School, and I got to see those efforts firsthand. It was not the altruistic approach that I had envisioned. However, walking around on the campus, I stumbled upon the Carnegie Institution, which focused on plant biology research. I made the switch and have never looked back. Having a background on the farm, I have always appreciated the difficulties of producing enough food—especially with the challenges of population expansion and climate change. I imagined what I could do in agriculture with what I learned in my first postdoc about genetic engineering technology. That is why I took this position at Cal—to try to help agriculture in California.

BSJ: You conducted a large-scale experiment with sorghum investigating the effect of drought conditions. What led you to use *Sorghum bicolor* as a model for testing plant drought response?

PGL: My focus on sorghum started with my involvement with the Africa Biofortified Sorghum project for the Gates Foundation. Our goal was to nutritionally enhance a crop like sorghum, which serves as the sole food source for hundreds of millions of people in Africa. We worked on increasing digestibility of sorghum with Bob Buchanan, also of the Plant and Microbial Biology (PMB) Department here at Berkeley. Because of my involvement with this project, I had the opportunity to visit South Africa during a period of drought. I got to see firsthand the difference in sustainability of a crop like corn, as compared to sorghum. It was striking. I learned that sorghum is very tolerant of drought and waterlogging—both hallmarks of climate change weather conditions. Sorghum and corn are closely related, so I thought if we could learn how sorghum can achieve these tolerances, we could enable other plants to gain those tolerances. This was the inspiration for our $12.3M Department of Energy (DOE) project, EPICON (Epigenetic Control of Drought Response in Sorghum).

BSJ: How did you simulate drought conditions for EPICON, and how did you confirm this treatment induced the intended stress response?

PGL: One of the great things about doing drought research in California is that in the summer we can conduct our experimentation without having to worry about rain, which isn't good for drought experiments! From early May to early November, there is no significant rain. For EPICON, we partnered with two Cooperative Extension Specialists. The University of California has nine Research and Extension Centers around the state. Of these Centers, one is directed by a sorghum expert, and another is directed by a drought expert, so it was perfect. The fields we used to grow our sorghum were equipped with drip irrigation lines for each row in the field so we could control how much water each plant received. The drought expert was able to calculate transpiration rates, the amount of water given off by the plants during growth, so that each week we could supply an amount of water equivalent to what was lost by the plant the previous week. We used three watering conditions—control, pre-flowering drought, and post-flowering drought. Sorghum has different ways of dealing with drought pre- and post-flowering. Pre-flowering drought is when we don't water a plant until it flowers, and post-flowering drought is when we stop giving a plant water after it flowers. We were able to confirm drought conditions by looking at upregulation of genes that we know are triggered by drought. We also directly measured the effect of the drought treatments on plant performance by measurements of the crop water stress index, which serves as an approximation for reductions in levels of active leaf transpiration. These measurements showed that both drought treatments led to increases in plant stress.

BSJ: Does drought have different effects on the microbiome in different stages of its development? If so, how did you account for this factor?

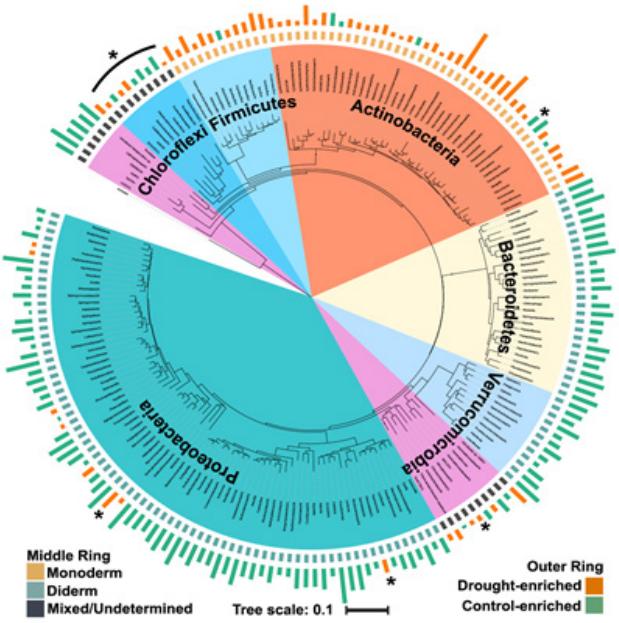

Figure 1: Phylogenetic tree of bacterial genera enriched and depleted in pre-flowering drought root samples. *The middle ring indicates whether the genus is categorized as a monoderm or diderm. The outer ring displays the relative log2-fold enrichment (red) or depletion (blue) of each genus in drought-treated roots as compared with control roots, indicating that monoderms are enriched in drought conditions.*[2]

PGL: The microbiome—both bacteria and fungi—react very rapidly to drought. In the first year, we only looked at responses six to seven days after resumption or cessation of watering. We realized that at that time point, a lot of responses, especially in terms of transcriptional responses, had already happened. The second year, we took samples eight, 26, and 50 hours after water conditions changed, and microbes were found to respond within hours.

BSJ: Could you briefly summarize the difference between the root, rhizosphere, and soil environments?

PGL: We have known for a long time that there are a lot of microbes in the soil, but we didn't really know what they did or if they had any real benefits for plants. For EPICON, we took weekly samples of soil, roots, leaves, and rhizosphere—each of which has a distinct community of microbes that we were able to characterize. This community is quite diverse under standard watering conditions. The rhizosphere is the thin region of soil surrounding the plant roots. When we took samples of roots, we removed the rhizosphere so we could distinguish between microbes inside the roots and microbes inside the rhizosphere.

BSJ: Which bacteria dominate the plant rhizosphere under normal conditions, and how does that composition change in drought?

PGL: Under normal conditions, the plant rhizosphere hosts a diverse community of bacteria and fungi, and these populations vary from one field to another in terms of the precise levels of microbes. When drought is applied, over time the diversity of those populations reduces to just a subset of those microbes—predominantly to Gram-positive, or monoderm, bacteria, like *Actinobacteria* and *Femicutes* (Fig. 1). When water is reapplied, the population quickly resumes the population profile it had prior to drought.

BSJ: How did you quantify relative abundances of different bacteria?

PGL: This is one of the great advances in being able to study and understand complex microbial populations. Through the use of genomic tools, like 16S and ITS metagenomics, it is possible to determine what types of bacteria and fungi are present in a complex sample like that of the soil or the rhizosphere. By taking weekly samples, you can see the dynamics of these populations and how they respond to drought and watering.

BSJ: What are some potential reasons that these bacteria are better able to survive under these conditions?

PGL: This is something we don't fully understand yet. Devin Coleman-Derr is another Principal Investigator here in the PMB department and also at the Plant Gene Expression Center (PGEC) in Albany. His group has speculated that the bacteria that hang around have a thicker cell wall and lack an outer cell membrane, which perhaps protects them from water loss. These are some possibilities, but we don't really know yet.

BSJ: You performed a gene ontology enrichment analysis to investigate molecular functions that may be increased in the microbiome under drought conditions (Fig. 2). Which functional gene categories were enriched?

PGL: Under drought conditions, plant metabolism is altered, resulting in the plant roots releasing certain carbohydrates and amino acids, along with a concomitant increase in certain types of transporter genes in the bacteria that are capable of taking up those metabolites (Fig. 3). So in a sense, the plant and microbes are talking to each other!

BSJ: You found that much of these augmented functions were in categories belonging to *Actinobacteria*. How did you determine whether the enriched gene categories you observed were simply due to increased numbers or an actual change in gene expression levels?

PGL: This was work carried out in Devin's lab at the PGEC. You can use a technique called quantitative PCR (qPCR) to determine how many microbes were actually there, in order to determine relative levels of specific bacterial types. They were able to show that there was an increase in numbers of bacteria during drought. Then, they quantified the relative abundance of *Actinobacteria* transcripts associated with these specific gene categories in order to account for abundance.

BSJ: You found that gene categories associated with carbohydrate and amino acid transport and metabolism were enriched in drought conditions. Was this enrichment associated with actual changes in sorghum root metabolism?

PGL: Yes. Using metabolomic analyses, our collaborators at the Pacific Northwest National Laboratory (PNNL) were able to detect metabolites in the soil and rhizosphere. Some of those metabolites really stood out in terms of amount, and they correlated with related transporters in the bacteria.

BSJ: How important is microbial diversity to plant health? In your studies of sorghum under drought conditions, how do *Actinobacteria* and other enriched phyla in the microbiome affect sorghum development and growth?

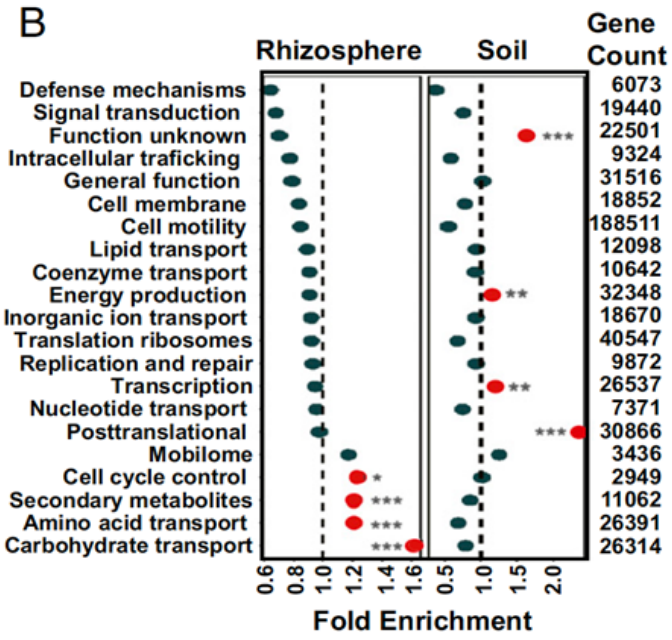

Figure 2: Gene ontology analysis of genes enriched in drought conditions in the rhizosphere (left column) and soil (right column). *On the x-axis is the relative fold enrichment of gene expression, normalized to total percentage of genes in that category represented in the dataset. Red data points indicate a p-value of <0.05 by hypergeometric test. Functional gene categories relating to metabolism, notably secondary metabolites, amino acid transport, and carbohydrate transport, were enriched.*[2]

PGL: This is a relatively new area of investigation. I think we are just now beginning to understand the importance of both bacteria and fungi in the growth and resilience of plants to abiotic stressors, like nutrient deficiencies and drought. Now there are companies popping up that are attempting to take advantage of this relationship, by either performing microbial community analyses or selling particular microbes that they believe will benefit the farmer in growing his/her crop—by having to use less fertilizer or perhaps less water! However, if I did the same experiment in a different place (which Devin actually has done in Albany), we would probably find that while there may be the same classes of microbes, like the monoderms, they're probably going to be slightly different. So, you're probably going to have to go to each specific field and find out what microbes are there in order to figure out which ones will be beneficial in that situation.

BSJ: You not only conduct original research, but you are also deeply involved with communicating it to the general public. What is the CLEAR project, and what inspired you to begin this initiative?

PGL: I was hired to interact with the public on issues like agriculture, food, and technology. I wanted to pass on the lessons learned to the next generation of research scientists so they are not afraid to talk to the public about what they do. CLEAR, or the Communication, Literacy, & Education for Agricultural Research Project, has offered that platform.

In 2015, the President of University of California came up with something called the Global Food Initiative. She asked every campus to come back and tell her what food initiatives were happening on their campus. A colleague at UC San Diego was approached by his Chancellor and was asked, "What are you doing in terms of science communication related to food?" So he called me and said, "I don't do anything in that arena, but I know you do. Help me out." And I said, "Okay, I'll call Pam Ronald at UC Davis because she does a lot of outreach." Together, we secured $450,000 in funding, which is amazing for outreach. I've been running CLEAR for four years since then. I don't even have to ask people to participate—they just volunteer. The real turning point was after the 2016 presidential election. One of the main things that students mentioned at that time was that the general public doesn't listen to scientists. They don't even know who we are. They know who a pharmacist is. They know who a dentist is. They know who a doctor is. But they don't know any scientists. That's how we came up the slogan we put on our T-shirts: "Talk to me, I'm a scientist." We want to be out there, so people can see us and go, "Oh, you're not so weird. You're a regular person." So we do outreach events at bars, zoos, libraries, the farmers market, etc. One of the students in CLEAR works at the Innovative Genomics Institute. He started a program at a local high school to teach people about CRISPR—not just the technology, but also the ethics of it. In one year, we reached 700 students in the Bay Area and Los Angeles, just by going out and giving talks at high schools. All of this was his idea. I'm just a cheerleader. I help people like him develop his presentation, questions, and activities. I love it, actually. I'm having a good time.

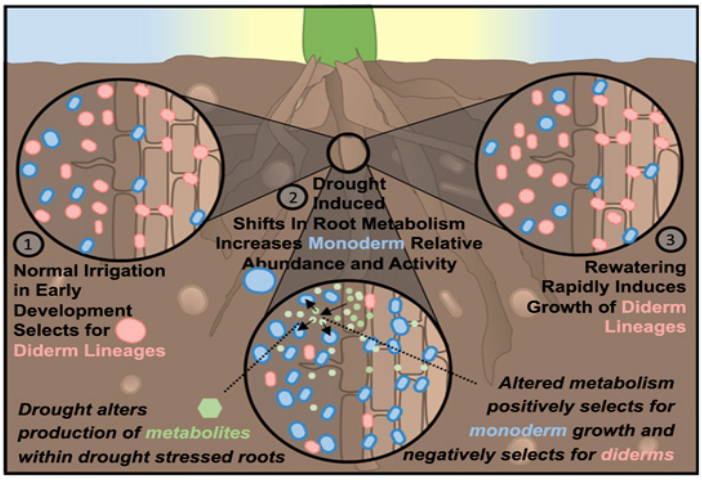

Figure 3: *Proposed scheme for changes in microbiome makeup before, during, and after drought conditions.*[2]

BSJ: You were able to convince many California dairy farmers to grow sorghum, which is a more sustainable alternative to typical forage crops, like corn. How did you establish these connections in the community? Did your experience in science communication help you in delivering this message?

PGL: I am a Cooperative Extension Specialist. Because of that, I have connections to growers, and they depend, in some sense, on advancements coming out of the university. Growers are economists, and they must make money in order to be successful business people. When the drought was in full force and they had to pay a lot for water, we could show them that sorghum required a lot less water than the other preferred forage for dairy cattle. It was an easier sell to convince them to convert over to sorghum, as long as you could show them the data that said, "Yes, you're going to get more for less money." In some areas where sorghum acreage was maybe only 1%, there were increases to 30%. Now that the drought is over, I don't know if they have continued to grow sorghum for forage. But when the next drought comes, and it will, they will hopefully remember sorghum and turn to that crop again.

REFERENCES

1. Peggy Lemaux [Photograph]. Retrieved from https://plantandmicrobiology.berkeley.edu/profile/lemaux.
2. Wheat Field image. Retrieved from https://www.pinterest.com/pin/835558537086916640/
3. Xu, et al. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *PNAS 115*(18), E4284-E4293. doi: 10.1073/pnas.1717308115