

An Overview of Biofortification of Zinc in Rice



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A Brief Background

My name is Katelyn Fritz; I grew up on a small farm just east of Des Moines, Iowa where we mainly had a corn, soybean crop rotation system. I attended the Global Youth Institute October of 2014 during my senior year at Southeast Polk High School, presenting my paper on governmental issues in Nepal. During high school I developed a passion for global agriculture and solving hunger related issues internationally. While writing my paper on Nepal and then attending the Global Youth Institute my passion grew even stronger. I then realized that I wanted to spend my life fighting these issues, though at the time I still wasn't sure what my piece to the puzzle was going to be. Figure one is a photo of me at the Global Youth Institute talking to the 2014 intern to Nepal about her internship, and comparing what I had learned through research to her experience.



Figure 1.

I wanted this internship more than I had ever wanted anything in my life. I knew I would grow from it immensely, and I really wanted to learn where my passions lie inside of the overarching issues. I was interested in a huge array of different things from fieldwork to lab work to going directly and working with the farmers. I had faith that my summer abroad would teach me just that.

There were a few things about me that made the internship a little more challenging. Being from Iowa I had never really experienced many accents, or not being able to communicate with someone because they speak a different language, and especially not every person for two months straight. Also, I come from a tall family. Growing up I always thought that being six foot tall for a boy was short. I, at five foot eight inches, was short for my family. But then I arrived in the Philippines and was significantly taller than every single person. Figure 2 shows one of the Filipino interns that wanted so badly to be taller than me in the picture that he actually got a step stool to stand on.



Figure 2.

About my Internship Facility

When I was told I was going to be spending two months in the Philippines I definitely was not disappointed, but I knew nothing about the country. I wanted to work in the southeastern Asia region and there were a few countries I was particularly fond of, but I had never thought about going to the Philippines. As soon as I landed in the country my opinion immediately formed, and an everlasting love developed. I was amazed at the number of people walking around on the streets at 12:30 pm. I was embraced by the people as they all tried to help me in any way that they could. The hour car ride to the research facility is a ride I will never forget. It was where my first impressions formed -- love started and then only kept on growing.

IRRI was founded in December of 1959 “as an organization to do basic research on the rice plant and applied research on the phase of rice production, management, distribution and utilization” (Our History). IRRI now does research on genetic diversity, better rice varieties, rice and the environment, value-added rice, policy and markets and knowledge and capacity building. It is located in Los Baños, Philippines, which is known as a science city and has a lot of international residents. IRRI is right next to the University of the Philippines, Los Baños (UPLB), known for being one of the top agricultural colleges in southeastern Asia.

During my first week at IRRI they were going through layoffs, which was very hard for everyone. IRRI gets a lot of their funding from the Consultative Group for International Agriculture Research (CGIAR) and they had recently just changed some of their policies. The change of policy upset some of the donors causing CGIAR to have fewer funds to distribute to research facilities. IRRI had already had their budget set for the next year based on the amount they were expecting to get from the CGIAR, but then when they only got about half of what they were expecting they ran into a huge problem. They cut expenses everywhere they could, but still layoffs had to be done.

□Where I Was

Going into this internship I wasn't sure what I wanted to do and I really wanted to gain a large range of experiences. Because of that, instead of having one specific lab and project that was entirely mine or to work on the entire two months, I was able to move around and gain more of a broad overview of many of the different things IRRI does. I mainly stayed on the subject of biofortification of zinc in the various labs I went to, but I was also able to work in a few other places. I spent one Saturday in the C4 lab, a day touring the facilities for Golden Rice, a week in the Genetic Transformation Lab, some time was spent with the sensory evaluation, and about a week doing farmer interviews focusing on women who manage the farm and the innovations they are using.

Within the subject of biofortification of zinc I was able to work in four different labs: Soil Chemistry, Plant Breeding, Grain Quality Service and Grain Quality Research. The main group I was with was the Soil Chemistry Lab under Dr. Sarah Beebout. Figure 3 shows a photo of all the scientists that I grew really close to and worked with in the soils group. The one on the far left is MJ, the main person I was with and job shadowed.



Figure 3

Goals/ Mission

There are three different types of malnutrition one being calorie deficient, where someone doesn't take in enough food to survive. Another type is being protein deficient, where someone eats and has a full belly but they are not getting the protein required to live a healthy life. The last type is micronutrient deficiencies, and just as you may assume that is where the person is eating, but isn't getting a sufficient amount of micronutrients. The International Rice Research Institute's (IRRI) main focus is eliminating micronutrient deficiencies through rice.

IRRI also has goals of producing rice varieties that are drought resistant, creating varieties that can grow in saline soils, creating rice that uses C4 pathways instead of C3, and many other goals that are trying to be accomplished in the social science department.

The People

In general the Filipino people were some of the happiest, nicest and funniest people I have ever met. They never took anything seriously including the layoffs I had mentioned earlier. I specifically remember the day before the layoffs were going to happen a few of the guys from the soil chemistry lab were talking in Tagalog and laughing with each other. I was curious about what they were saying, so I asked, and they told me they were joking that each other was going to get “an envelope” tomorrow during the meeting, meaning that they were being laid off. I found it so strange that they would say to their good friends that they would lose their jobs and that they were all laughing about it.

In the Philippines the schooling is done in English so if the people have had an education, I was able to communicate with them especially around Los Baños. In Nueva Ecija though it wasn't entirely the same. For the first time on the trip I was not able to talk to people I was meeting, and I felt very out of place. Every person in the town stared at me as I passed amazed by both my height and my skin color. I heard “matangat” and “ganda mo” everywhere I went meaning “tall” and “you're beautiful” respectively. Never in any way did I feel unsafe, just in a way that they were all very curious of me.

The children in Nueva Ecija were the most curious. I spent hours outside with them just staring at me. Most of them knew no English and the ones that did were much too scared to say anything. They would just circle around me being very careful to keep a safe distance. Every once in awhile a little boy would push his friend closer and they would all squeal because the safe distance was no longer kept. Figure 4 shows one of the times the school children were surrounding me as I just looked around almost as amazed by them as they were of me.



Figure 4.

While I was at IRRI I met many other amazing interns from all around the world. We would all get together on the weekends and travel to the beach, or climb Taal Volcano, or any other tourist attraction that was near us that we could find. I grew very close to many of them, but especially four that were from Illinois. Going halfway around the world I didn't expect to make friendships

that would last even after I left, but I did. The other interns I met at IRRI will forever have a special place in my heart. Figure 5 shows a photo of the four interns from Illinois and a few of our Filipino friends as we were climbing Mt. Makiling.



Figure 5.

Dr. Sarah Beebout was my supervisor during my time at IRRI, but more than just being my supervisor she became my second mom. The Beebout family invited me to family meals, birthday parties, church events and really anything their family did they were sure to include me in and give me a place where I felt I belonged. It was so nice to have someone there from the same state as me and who understood each bit of culture shock I was going through and being able to explain it to me. I grew very close to each of them but especially to their two daughters. Ate Katelyn, Ate being literally translated to older sister, quickly became my favorite thing to be called and especially by those two little girls. I miss the Beebout family dearly and I look forward to every time I am able to Skype them and talk to them. Figure 6 is a picture of us on my last weekend in the Philippines, at a beach in the Batanges.



Figure 6.

What I Did

With the soil chemistry group I was able to do both fieldwork and lab work. One of the things I did in the lab was testing zinc concentration in the plants at three different stages. At 30 days we test both the shoots and the roots. At flowering we test the panicle, stem and leaves and finally at harvest the stem, panicle, upper three leaves and other leaves are tested. We tested by drying the samples and grinding each one into a powder. Next we weighed out one gram of each sample and added it to a molar solution of HCl. Once the samples have sat and been filtered, we ran each individually through the Atomic Absorption Spectrometer (AAS) to give us data on the zinc concentration. Figure 7 is a photo of one of the Filipino interns and I running the AAS to get results for the experiment.



Figure 7.

The other thing I did in the lab was root scanning. Root scanning is done at two different stages of plant development, and is the main method we are using to test the crown root hypothesis discussed later. To do the root scanning we spread out all of the roots onto a tray and then put it through a scanner. The images are then put through a processor that counts the number of roots. We are trying to see if the varieties with the greatest number of crown roots then correlate into having a larger concentration of zinc in the grain once it is developed. The week I spent doing root scanning was when I ultimately decided that I did not want to work in the lab for the rest of my life.

In the field I was able to do planting, pulling, transplanting and soil sampling. I worked in two of the three areas where IRRI has plots. The bay field was the most interesting and was a wetland before they turned it into a rice field. There was so much water in the field that there were actually eels that lived in the field. Figure 8 is a photo of two of the main guys I did field work with and I at the IRRI field after my first day in the field.



Figure 8.

Looking at biofortification of zinc I spent a week in the plant-breeding lab. There I was able to go on many tours where I learned the basics on plant breeding and how IRRI's plant breeding lab's method differs slightly from other methods. I was also able to experience another way of testing samples for zinc content. I was able to take one sample that was my own and go through de-hulling, sorting, polishing, sorting again, and then running the sample through the x-ray machine. It was very interesting to be able to fully run two different samples two completely different ways but get the same outcome. Figure 9 shows me as I was de-hulling my sample with the plant-breeding lab.

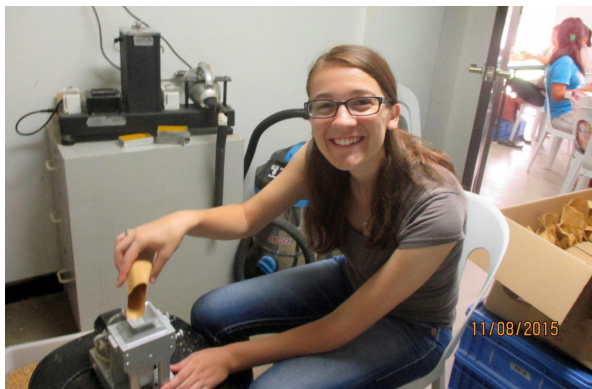


Figure 9.

One week I spent in the two Grain Quality Labs. The service lab gets samples from all over IRRI and is paid to do quality tests on them. The research lab has their own experiments and use their own samples. I started out in the service lab where I was given a tour and then taught how everything is done. I then worked on doing viscosity tests of the grains to simulate how it will be when you are eating the rice. I then went on to do grain scanning. Grain scanning was not nearly as tedious as root scanning, but it was the same concept. We were scanning the grains and then putting the data into a processor to measure the length and width of each one.

I then went to the research lab. In the research lab I helped the scientists work on their experiments by doing little jobs. For example, I helped one lady by counting germinated seeds and cutting the root from the rest of the seed. I did a lot of weighing of samples for people and I read a book on rice production while working with that lab. But my favorite thing I did was helping to grind samples with liquid nitrogen. We would get the samples and the containers freezing cold with liquid nitrogen and then place them into a shaker machine. When they came out we had a fine powder of sample. I felt like a mad scientist while doing it, it was a lot of fun.

One of my closest friends at IRRI was in the C4 lab and she was having a lot of problems with her experiment, so she had to go in and work on Saturday. Since she was there and I had nothing to do, I went with her to try and help her finish sooner and to learn another new thing. Rice is a C3 plant meaning that the first stable product of photosynthesis is 3-carbon. Plants whose first stable products though are 4-carbon are considerably more efficient in CO₂ fixation under current low atmospheric CO₂ concentrations. The lab is trying to take genes from corn plants and insert them into the rice to give them the C4 pathway, therefore making them more efficient.

One of my weeks was in the Genetic Transformation Lab. There I was able to learn about the process of how specific genes are located and the steps they take in inserting them. I did things like working with rice embryos, tested a new huller and did polymerase chain reaction (PCR) in the lab. But the thing I found the most interesting was when I was able to do Southern Blotting. Before this experience I had never heard of Southern, Northern or Western Blotting. Southern Blotting is a technique used to detect a specific DNA sequence, Western is used to detect protein sequences and Northern is used to detect RNA sequences. It was very interesting to go through the steps of the process and then being able to see the results and our DNA strands at the end. Figure 10 is me in the Genetic Transformation lab as I was working on separating embryos.



Figure 10.

One of my coolest overall experiences was being able to do a crossing on a rice plant. Rice is a self-pollinating plant so crossing them can be a little more difficult and very, very rarely do they cross in nature. I started one day by doing the emasculation. Emasculation is done by first removing the flag leaf. You then remove the upper and lower portions of the panicle because the upper may have already pollinated itself and the lower may be too young, but the middle is at the

perfect stage. Next you very carefully cut the lemma and palea at an angle to avoid cutting the pistils. If the pistils are cut the stigma may be damaged. After that you use a tiny vacuum to remove the pistils being sure not to damage the stigma. Figure 11 is me cutting the lemma and palea at an angle.



Figure 11.

Two days later I went back to the greenhouse to do the pollination. You know the plant is ready to be pollinated when the pistils come out. Plants are viable for pollination 24-48 hours after emasculation and the pollen is viable in the air for a few hours and is done in a closed room for contamination reasons, but you can still only use one pollinator per day. To do the pollination process you first need to remove the pollen from the panicles you are not going to use by flicking them to remove the chance of them pollinating the panicles we are going to pollinate. You then take the plant into the closed room and gently shake the pollen from the panicles onto the other panicles. Figure 12 is me as I was doing the pollination.



Figure 12.

One day I was able to spend touring the facilities IRRI has for Golden Rice. Figure 13 shows Golden Rice compared to normal white rice, and from there you can see how it got its name. Golden Rice has been genetically modified to have Vitamin A in it although it is not normally

present in rice grains. Vitamin A is one of three micronutrients that many people are lacking in their diet and this new rice is hoping to be the cure. When growing, Golden Rice and regular rice cannot be told apart as shown in figure 14. It is not until you see the grain that you can see the difference. It is currently in the testing phase and still has a few more years before it will be approved for the market.



Figure 13.

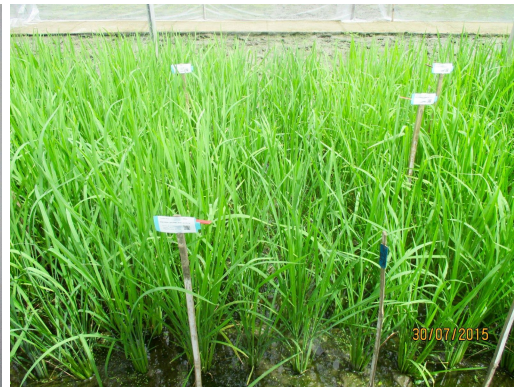


Figure 14.

While at IRRI I was able to listen to a presentation about sensory evaluation given by a lady named Rochi. The presentation was part of the process of her applying for a job, which she did get, and the presentation gave me a really good overview of everything the sensory evaluation group evaluates. Previously, I had never thought about the consumer's preferences when creating a new variety. There are so many things that they look at to see if consumers will like it. It amazed me to learn about the process.

My absolute favorite part of the entire experience was being able to do go to Nueva Ecija for three days to do farmer interviews with women who managed the farm. Nueva Ecija was about five hours north of Los Baños and was an entirely different experience. I talked with some of the most inspiring women I have even met and I learned each of their stories of how they overcame the obstacles in their life and became as successful as they are now. The first interview I went to was my favorite, a photo of us is shown in figure 15. She was so humble and gave back to others in the community because she was now one of the wealthier. She started with such a poor beginning and worked her way to the top and I will never forget the respect I had for her sitting and listening to her story.



Figure 15.

During the group interview the subject of abusive relationships came up and it amazed me to hear that almost every person said that husbands abuse at least one out of every ten wives in their village. Growing up in Iowa I had never heard of anyone being abused and now they are saying that the odds are that in this group of the ten ladies I'm sitting with, one is in an abusive relationship. I was heartbroken.

How it Will Help Food Security

There are three main interventions to micronutrient deficiencies: change of diet, vitamin supplementation and fortification. Fortification is adding a nutrient to a product as it is being produced to increase the nutritional value of it. An example would be adding iodine to salt. The problem with using fortification with rice though is there is little between harvest and consumption other than it being milled. Many farmers mill and eat the rice they grow, making fortification virtually impossible. Instead IRRI looks at using biofortification, or increasing the nutritional value of the plant as it is growing, to fight the problem. The three main nutrients that are commonly linked with micronutrient deficiencies are: iron, vitamin A and zinc.

IRRI works to find solutions for all three, the biggest success so far being golden rice, which was mentioned before. Again, the focus of the groups I was mainly involved with was biofortification of zinc. A past panel screening showed potential for zinc to go through a breeding process to obtain the ideal level, so as of now they are working to find the solution without genetic modifications. The soil chemistry department got involved because in field tests they were finding that different types of soil produced different levels of zinc. Our study is to figure out why.

The two main hypothesis we were testing were: 1) the more crown roots the plant has, the higher the zinc concentration will be; and 2) the rhizosphere processes change the soil chemistry of soil directly adjacent to the root making the zinc more readily available. Zinc Sulfide precipitation and Zinc Carbonate precipitation are the two best explanations why zinc is unavailable in some soils. The crown root hypothesis is based on carbonate and sulfide reactions not occurring in the

top layer of soil because of the gas exchange leaving the zinc available. So, if there are more roots in that part of the soil, more zinc can be absorbed leading to a higher concentration of the nutrient in the grain. The rhizosphere processes hypothesis refers to plants omitting acid, phytosiderophore, or oxygen each of which has their own way of helping the root absorb zinc more easily.

IRRI works with three different test plots. One with a high concentration of organic matter and sulfur, one high in carbonate and one located at IRRI which is just average soil in the Philippines, and can be used as a control. Having the different plots allows us to see how seeds will grow in different soils and work toward getting a plant that will produce consistently high concentrations of zinc in all soil types.

So far the results have shown some uncertainty with the root scanning. The larger the number of crown root will usually result in a higher zinc intake, but not always at the same point in development meaning that it won't always lead to a higher concentration in the grain. Further analysis is needed to show why it happens at the different stages. Also, there is something in some genotypes that cause the zinc to become mobile in the plant and move to the grain. More work is needed to pinpoint what gene causes it. If we are able to get results that tell us why some plants have higher zinc content in the grain than others, we will be able to breed for that trait and develop a variety that could potentially end the lack of zinc in diets.

Overall Experience

It's hard to even come close to putting my experience into words. I looked at every day, every challenge, every good and bad thing as an experience, and that's truly what it was. I literally and figuratively climbed mountains while I was in the Philippines. Never before had I been anywhere without my mom, or had I gone more than a day without being able to talk to my family. It was definitely one of the hardest things I have ever done. I spent the whole first half of my first day in tears because I already missed everyone at home, and I knew it would be eight more weeks before I would be able to see them. After that though I changed my attitude and remembered how this was a once in a lifetime experience, and I needed to make the most of it, or I would regret it for the rest of my life.

I came in knowing I would learn a lot about science and I definitely did. I came from not knowing what a rice plant even looks like to being able to tell you just about anything you want to know about the plant, but I also learned a lot about myself. I learned that it's okay to be scared of new things, but still try them because you may end up loving it. I learned that I can be independent, and I can make new friends in a place where I know no one. Learning who I am and how to be independent prepared me so much for college, and I often look back now at who I was and how much easier this transition has been because of my time abroad.

I miss the Philippines every single day. The first month after I got home it was all I talked about. I found a way to relate it into every conversation I had. I miss the food and now crave rice. The

comfort style food with loads of meat, rice and a sauce was second to none. I must've gained ten pounds being there just because I could never get enough of the Filipino meals. Then even if I didn't feel like having a Filipino meal the rice that they served at the cafeteria was delicious. I never knew you could have low quality rice but coming back to the U.S. and craving rice and it not hitting the spot has been really hard.

I miss the people probably the most of all. I miss needing to leave for work early because I knew that every person I passed would want to say hello. I miss the way everyone made me feel like they were genuinely happy to know me and would do anything to help me. There were numerous times just walking around IRRI's campus that people would offer me rides back to my dorm, although I never took one; I knew that they genuinely were just trying to help. Or all the times people would run after me offering me their umbrella because I was in the sunlight and my skin was exposed or it was sprinkling rain and they didn't want me to get wet. I miss the conversations we would have over meals talking about the work we are doing in the lab and being able to learn about everyone else's projects and seeing how they all fit together to reach one large goal.

IRRI gave me an experience that shaped my career path and showed me what I want to do. Being able to do lab work, fieldwork and work directly with farmers all in the same vicinity of time showed me what I really do like to do. I didn't mind any of the work, but I fell in love with the people and learning about them and their cultures and life stories. I went from wanting to probably work in the lab to now looking at doing more hands on outside work on people's farms getting to know them and their operations.

I started out not too sure about the decision for me to go to the Philippines, but by the time I left I felt as at home there as I did in Iowa and I cried the entire way to the airport, not knowing when I would be able to return. This was the place where I learned more in two months than I think I had learned in my 13 years of school thus far. This was where I met some of my best friends, and had actual intellectual conversations with them instead of just meaningless nonsense. The Philippines was where I found myself and I learned where my passions truly lie. I changed as a person, and matured more than I expected to. I left home as an 18 year old kid, but I came back as an adult.

I can't thank IRRI or the World Food Prize enough for giving me this experience. Both organizations went above and beyond to make my summer one I will never forget. I went in as a normal high school graduate, but became above average from learning all the things I did in that one summer. I am humbled that I was chosen to go on this internship and hope that I lived up to the expectations that were set for me. I can't wait to see what the future has in store for me now and I owe it all to everyone who has helped me along the way.



Bibliography

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