Science and Sightseeing: A Working Summer in Mexico

By

Jerome Jacobsen
2015 Borlaug-Ruan Intern
Mahtomedi, Minnesota

THE WORLD FOOD PRIZE
World Food Prize Foundation
Des Moines, Iowa, United States

CIMMYT MR
International Maize and Wheat Improvement Center
International Maize and Wheat Improvement Center
El Batán, State of México, México

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Personal Introduction

I can hardly believe that I am already a senior in high school and that I've had so many amazing experiences during my school years, to which the World Food Prize Foundation has been critical.

I live in Mahtomedi, Minnesota, a northern suburb of the Twin Cities. Agriculture was not on my radar before my experiences with World Food Prize Foundation. I'd never been on a farm, and I had never thought about food science or the immense importance of agriculture.

My experience with World Food Prize Foundation started with a paper. My teacher informed me of an opportunity to write a research paper about pressing global issues. I didn't know much about it beyond that, but I love learning new things, so I took her up on the offer. I delved into research about water scarcity in Yemen and became very interested. I finished up my paper and sent it off.

I did not think much about the paper until I was informed I was to attend the State Youth Institute. Sharing my paper and hearing the papers of others was very exciting. I was even more excited when I learned I would move on and go to the Global Youth Institute in October, 2013.

I attended the Global Youth Institute and was blown away by the amazing discussion between global leaders as well as other students. I learned the critical importance of fighting food insecurity. After the Institute, when the opportunity to apply for an internship arose, I jumped at the chance. I had been inspired to learn more about scientific research purposed to combat malnutrition, and was amazed that the World Food Prize Foundation was providing students with the opportunity to experience research firsthand.

In the summer of 2014, I was a Wallace-Carver Fellow at the U.S. Meat Animal Research Center in Clay Center, Nebraska. Working on a research project was an amazing project; I loved learning about genetics and how it was used in the context of animal research, and I desired more experience.

The next year, I applied to the Borlaug-Ruan Internship and was accepted. Overjoyed, I couldn't wait to explore not only plant science and biofortification, but an entirely new culture as well.

CIMMYT

The International Maize and Wheat Improvement Center is a non-profit research and training institution dedicated to both the development of improved varieties of wheat and maize, and introducing improved agricultural practices to farmers located in El Batan, Mexico, northeast of Mexico City.

During my stay, I worked with the Global Maize Program. The Global Maize Program strives to provide diverse, high-yielding maize varieties that withstand infertile soils, drought, pests, and diseases; conduct research to help farmers exploit the full potential of improved seed while conserving soil and water resources; explore new market opportunities for small farmers; and provide training opportunities in maize breeding and crop management research (Maize Research).

I worked with Dr. Gordon Huestis and Dr. Martha Hernandez Rodriguez while at CIMMYT. Dr. Huestis is from California and recently joined CIMMYT. Dr. Hernandez has worked at CIMMYT for many years and is from Mexico. Both of my supervisors taught me so much about science and were instrumental to making my internship as fantastic as it was.

Research

250 million preschool children are vitamin A deficient worldwide. Further, vitamin A deficiency causes blindness in an estimated 250,000 to 500,000 children each year with half of these children dying within a year of losing their sight (Micronutrient Deficiencies). Biofortifying staple crops such as maize would thus be a socially and economically sound way to combat vitamin A deficiency on a global level (Yan et al.).

Some carotenoids, including B-carotene found in maize, are converted to vitamin A through animal metabolism. In Zea mays, three polymorphisms in the crtRB1 gene are associated with carotenoid variation. Favorable alleles in the crtRB1 gene increase the proportion of B-carotene, which would therefore increase the amount of converted vitamin A in those consuming the maize (Yan et al.).

It is necessary to develop a high throughput automated crude prep DNA extraction method in maize to genotype samples quickly and accurately in order to screen breeding lines for the favorable high B-carotene alleles to ensure that maize with higher provitamin A activity is available to alleviate the acute worldwide vitamin A deficiency in children.

We are working to develop an automated maize crude prep DNA extraction method using a Beckman Coulter Biomek FXP Laboratory Automation Workstation. Using NaOH to free DNA from a seed chip sample, the solution is then neutralized with Tris-HCl and diluted in water to prepare for a PCR reaction. After the PCR reaction, samples are read using the BMG Labtech PHERAstar FS HTS Plate Reader and analyzed using LCG Kluster Caller software to determine each sample's genotype includes the favorable crtRB1 allele.

Through changing such variables in the DNA extraction process as NaOH molarity, NaOH and Tris-HCl quantity and proportion, incubation temperature, incubation duration, size of sample, and PCR program, we aim to find a reliable method that yields highly accurate results. After developing the best method, we will automate the process using the Beckman Coulter Biomek to accommodate large numbers of samples.

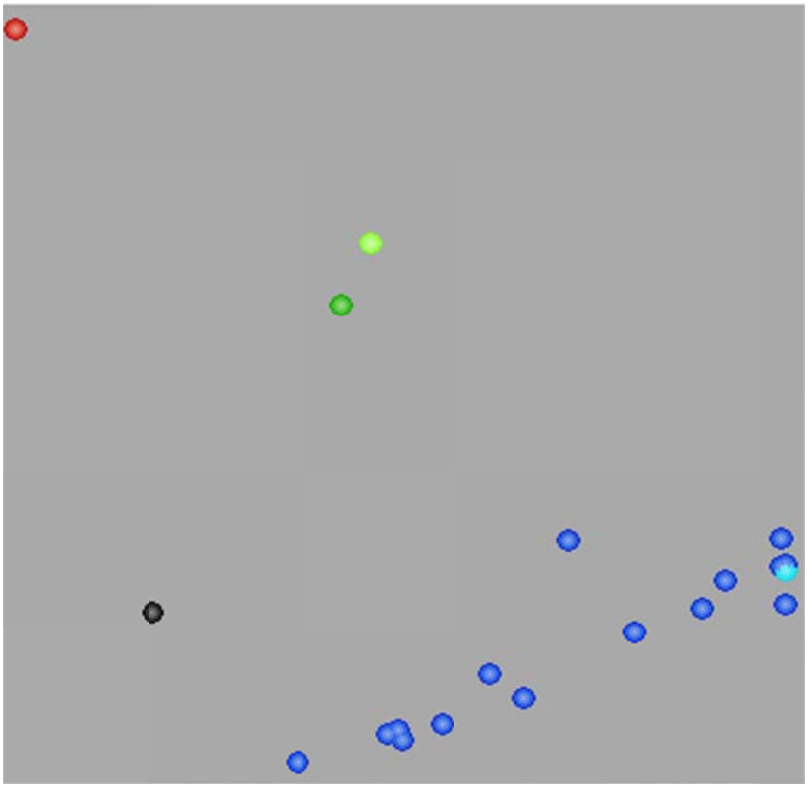
This automated DNA extraction and analysis process will allow for fast analysis of many lines; consequently, breeders will be able to know which lines contain the favorable allele for provitamin A activity and plant those seeds. Thus, this automated crude prep DNA extraction method ensures that micronutrient rich maize will reach children worldwide and alleviate vitamin A deficiency.

So, I spent the majority of my summer developing a process to assist in the process of screening seeds for selection in breeding. Every day, I would change one of

the steps in the DNA extraction protocol to see whether or not the end result worked better or worse with the genotyping test. Results at the beginning were unsuccessful. I developed after several weeks, however, a protocol that yielded successful DNA genotyping results. The final protocol was as follows:

1. Sample approximately 10-20 mg of seed chips into collection tubes.
2. Pulverize samples in TissueLyser for 15 seconds
3. Add 64 ul of NaOH .10M to collection tubes. Mix Well.
4. Incubate in water bath for 10 minutes at 65 degrees Celsius
5. Add 52 ul of Tris-HCl pH 7.8 .20M. Mix Well.
6. Incubate for 5 minutes at room temperature.
7. Add 384 ul of H_2O. Mix Well.
8. Centrifuge at 3750 rmp for 20 minutes.

This protocol worked the best of all experimental protocols. Using other DNA extraction and tests, I compared the results from this specific protocol and test to ensure the results obtained were accurate. This method yielded accurate results on par with longer, more expensive methods.



Analyzing the results using Kluster Caller software shows the separation of the samples by genotypes into three distinct groups: the homozygous favorable, unfavorable, and heterozygous. In the graphic above, the blue dots represent the unfavorable, green the heterozygous, red the favorable, and black the water. With the above protocol, there is

successful amplification of the SNP in question with low amplification of the water samples. In this plate of samples, there are many more unfavorable samples than favorable, which is in line with the breeding population.

After developing this method, I developed a program for the Laboratory workstation so that this extraction can be done almost entirely by the machine without the need for human intervention. This automated process will save time in the lab and allow many more samples to be analyzed than before. This will reduce the time needed to develop higher provitamin A lines of maize.

The automated DNA extraction method optimized for SNP KASP assays will dramatically reduce costs for the laboratory as the sampling and entire extraction is completed in the same tube. Further, the reagents used in this extraction are cheap, easy to obtain, and not as dangerous as some reagents used in other extraction methods. The automated nature of this extraction method will free time for technicians to focus on other projects in addition to genotyping. This crude extraction method will shorten the time needed to identify line genotypes and thus decrease the time to develop maize with higher provitamin A content, meaning this maize will be distributed to those in need in the developing world more quickly. I am proud and excited to have contributed to this important project in human nutrition.

Other CIMMYT Experiences

In addition to my main research project, I had several other professional experiences at CIMMYT.

My first week at CIMMYT, the center was hosting a Science Week, a conference in which scientists and researchers from research centers from around the world came to attend research presentation, poster sessions, and to learn about new practices at CIMMYT-Mexico. It was an awesome week, and I had the opportunity to meet many interesting scientists and learn about their work and the impact it has.

One of the most memorable events at CIMMYT Science Week for me was a small group session about improving the inequality of women in Southeast Asia. Hearing passionate scientists working together to develop solutions to a pressing world issue was absolutely inspiring.

I am so grateful I got take part in the Science Week conference because it was the perfect opportunity to learn more fully about CIMMYT's goals and meet the scientists I would work with for the summer.

I was tasked with a secondary development project during my internship. CIMMYT had recently purchased a fluorescence reading machine. After purchasing the machine, however, it was never used and never set up and all the operating information and passwords were lost. My supervisors asked me to try to make the machine operable and develop a DNA Quantitation Protocol optimized for small reaction volume.

I spent my first day with the machine looking it up online and calling the company to see how to activate it. It was quite an adventure to try and contact the correct person. Eventually, I found the technician who had brought the machine and set it up at CIMMYT. In addition to talking with the technician, I was able to locate the manual for the machine.

I learned that the correct software was not installed on the computer, so I had to track down the correct software, install it, and reconfigure the FLUOstar Omega machine. It was a lot of frenetic work, but I managed to get the machine up and running.

I spent the next few days becoming acquainted with the machine, learning how it worked and figuring out what kinds of things it could do. After I had familiarized myself with the layout of the machine, I began to work on reducing the reaction volume of a DNA Quantitation protocol.

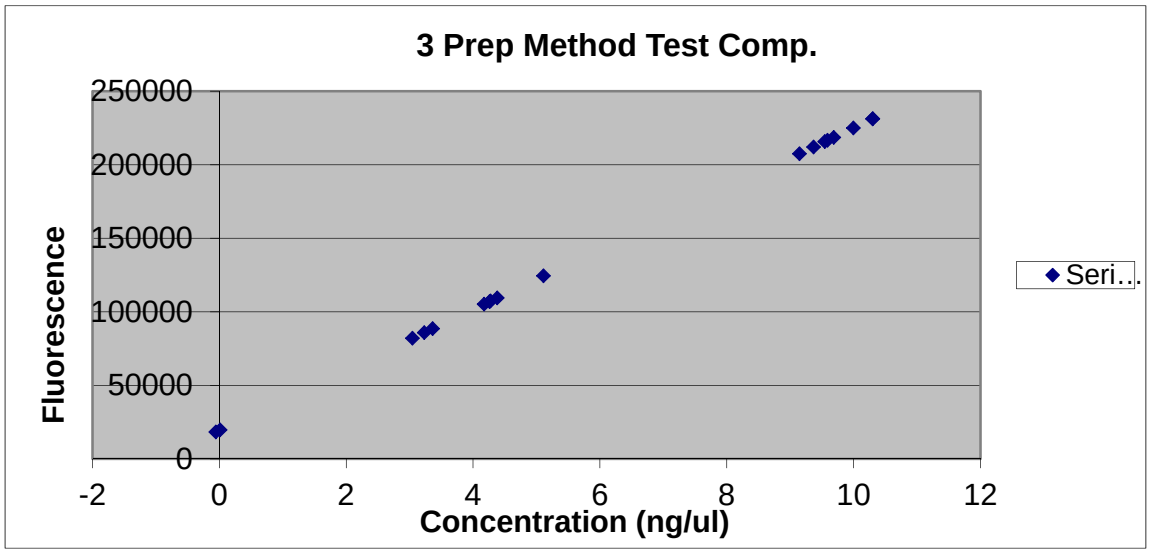
I used a quantitation protocol optimized for 2 mL reaction volume as a base and began to experiment with lower reaction volumes. In DNA quantitation using fluorescence, a dye is added to DNA and a buffer. Unknown samples are compared to a standard curve generated from known DNA concentration standards. My aim was to

determine how small the reaction volume could be without losing accuracy of the quantified concentration. In order check for accuracy, my unknown samples were experimental DNA samples from maize seeds that had been quantified using absorbance, a less reliable quantification method, and had a known theoretical concentration.

I started experimenting with the protocol and FLUOstar machine and through many trials of reducing the input volumes, I found that calculated concentrations were similarly accurate down to a total reaction volume of 100 ul. This means that samples can be quantified at a reaction volume of 100 ul and the results will be as accurate as a reaction volume of 2000 ul, the original protocol amount.

By reducing the reaction volume, the cost of quantitation is hugely reduced. With a 2 mL reaction volume, the cost per sample is around 250 pesos. With a reaction volume of 100 ul, however, cost is reduced to around 12 pesos per sample, which makes using this method in lab much more cost effective and feasible.

The table below shows a graphical representation of how samples are assigned a concentration based on how much they fluoresce in relation to the fluorescence levels of known standards.



This was a fun side project. I really enjoyed that both of my research projects this summer dealt with the development of protocols. Development is an interesting part of research in that it is necessary to increase the rate at which research can take place. Further, I learned that development has challenges that can be very different from research, and much of it is very repetitive, though it feels awesome once a functioning protocol is developed that people will use. I had so much fun teaching the lab technicians how to use the machine and the protocol I'd worked on.

Cultural Experiences

While my major role during the summer was to learn more about scientific research in a lab setting and contribute to a project, the truth is that I was in a different country for two months, and I was bound to explore and be affected by my new surroundings. While I was in Mexico, I lived at the CIMMYT research campus, which is gated and contains apartments, houses, a community center, a restaurant, a gym, a pool, and sports facilities. It was an amazing place to live for eight weeks. I had my own apartment, which was a first for me. It felt very strange to live completely by myself, but I busied myself with studying and taking advantage of the amenities at the research center so that I didn't feel so lonely.

With such a pretty view from my deck, it was hard to feel too sad. While there weren't any students near my age at the research center while I was there, I did become good friends with my supervisor Dr. Martha Hernandez Rodriguez. In addition to directing me at work, Martha would take me to visit cool tourist spots with her family.

I went to downtown Mexico City several times during my internship, and it was amazing every time I went. There are so many historic buildings, museums, and restaurants that I could have spent every day there and never have run out of things to explore. There are also so many people in Mexico City that simply traveling on the subway was an amazing adventure.

Above is a crowded street in Mexico City, in between historic buildings. Below is the cathedral in Mexico City's central plaza.

In addition to downtown Mexico City, I was also able to explore the preserved Aztec city of Teotihuacan, which is one of the most well preserved sites and home to one of the world's largest pyramids. It was completely awe-inspiring to climb up the Pyramid of the Sun and bask in the sunlight on top of the pyramid and take in the views of the rest of the buildings in Teotihuacan. During my stay, I also visited several museums in Mexico City, Frida Kahlo's house, a firefly sanctuary, small villages, a town that shuts down and becomes a clothing market on the weekends, and many other unforgettable, uniquely Mexican locations.

While it was amazing to visit places in Mexico, the experiences that most deeply impacted me during my internship were my volunteering endeavors. Every Saturday, I would go into the city of Texcoco and serve meals to the homeless. It was a truly humbling experience to realize how much of an issue poverty and food insecurity truly is. In my hometown, I had never been exposed to the true horrors of homelessness and hunger, but in Mexico, it was apparent. There were homeless people on every street struggling to survive. It was a no-brainer to me that I should volunteer to give back to my adopted community.

Each weekend, I would wake up early in the morning, take the bus into Texcoco with another volunteer from CIMMYT, and we would purchase fruit to bring to the meal kitchen. When we arrived at the location, we would work with the other volunteers to prepare meals. Then we would open the doors and serve those in need of a meal. I spent a lot time with the homeless who came to the meal kitchen over my internship, and I became friends with many of them. I enjoyed hearing their stories and getting to know them. I can say that they became my friends, and it broke my heart that these wonderful

people who tried so hard to make a living were trapped in poverty and homelessness. I know that giving them one meal may not have done much to improve their lives, but I believe that working with the homeless and showing them that people care about them improved their outlook as much as it improved mine. The picture below shows me in the center surrounded by a few of the people I spent time with every weekend.

I also spent many of my weekends volunteering at an orphanage near the research center. Children from only a few months old to their teens lived at this orphanage, and I was touched by their positive attitudes. Spending time reading and playing with the children, I got to know them and I was amazed at their aspirations. One young girl wanted to be a doctor, a young boy wanted to be a police officer, and one child simply wanted a family of his own to love when he grew up. I wanted to as much as I could for these children, so I organized a clothing and toy drive for them at the research center. The support from those at the center was overwhelming, and we collected many toys, a lot of clothing, and extra food and bath supplies as well. Working with the children was an amazing volunteer experience, and I shall hold it near to my heart forever.

In addition to these volunteer experiences, I also taught English to some of the workers at CIMMYT. Knowing English is a valuable skill, so I had a blast teaching several people basic English. Everyone was so apt to learn that it was a thrilling experience, and not too stressful. I also volunteered at several fundraising events for scholarships for the children of low-income workers at CIMMYT.

My experiences with volunteering in Mexico had more impact on me than anything else I did in Mexico. I saw that poverty and hunger are real problems for real people. I tried to help those in need in the small ways I was able, but meeting these amazing people who strive to achieve better lives has empowered me to fight for those who cannot fight for themselves.

Conclusions

The Borlaug-Ruan internship has had a profound impact on me. I discovered so much during my time in Mexico, not only in a research setting but interpersonally and culturally as well. I am forever grateful to the World Food Prize for making this opportunity available to students.

The research experience I gained during my internship has inspired me to continue exploring and studying science in college. I have discovered a passion for science research, especially in genetics and human health and will study this in college. The Borlaug-Ruan Internship gave me the opportunity to discover what I am truly passionate about and what I am interested in studying further in college.

Beyond the research, the people and experiences I had are what really stand out. My volunteering experience with the homeless and orphans are ingrained in my mind. The people I spent time with were not merely people in need; indeed, they became my friends, and great friends at that. Hearing the life experiences of elderly people at the meal kitchen and listening to the aspirations of the children in the San Bernardino Orphanage showed me how diverse the lives people lead can be, yet how ultimately similar all people are. Further, the friends I made at CIMMYT I shall hold on to forever. Whether it was Thursday night dinners at various restaurants in town or spending the day with my supervisor and her family, the people at CIMMYT accepted me into their lives and shared their favorite parts of Mexico with me and made my internship a truly transformative event.

Through the Borlaug-Ruan Internship, I was able to live immersed for two months in a vibrant, multi-faceted culture. I learned about the ancient civilizations in Mexico both firsthand at a preserved Aztec city and the famed National Anthropology Museum, the colonial history exemplified in the architecture of Mexico City, and the realities of Mexico as it is today. I have gained respect for Mexico and a desire to delve more deeply into the rich culture and language of this amazing country in the future.

My summer internship has broadened my horizons and fostered my development as a global citizen. This experience has allowed me to understand more not only about the world beyond my hometown but also about who I am as an individual and what I want to do for the world in the future. The Borlaug-Ruan International Internship was one of the defining experiences of my life so far, and I know that The World Food Prize, Mexico, and human nutrition will continue to influence my life moving forward.

Acknowledgements

Wow. There are so many people who made my experience possible that I would like to thank. First of all, I would like to thank the World Food Prize Foundation and all the amazing people there for providing this opportunity and selecting me as one of this year's interns. I would like to say a huge, huge thank you to Lisa Fleming for making every step of this internship go as smoothly as possible and for providing continuous support not only while I was in Mexico, but before my trip and after my trip. A big thank you to Ambassador Quinn, Keegan Kautzky, Libby Crimmings, Jacob Hunter, and everyone else from the World Food Prize Foundation I've met throughout my journey with the World Food Prize.

I need to thank my mother, first for letting me go to Mexico for the summer, as well as supporting me in all of my academic and creative endeavors. I would not have been able to explore my interests without her support.

I want to thank all the amazing people I met at CIMMYT. Thanks to my supervisors Dr. Gordon Huestis and Dr. Martha Hernandez Rodriguez for welcoming me to their project and making my stay in Mexico extremely enjoyable and interesting. I'd like to thank Kate Dreher for accompanying me on all of my volunteering outings. Thanks to everyone else I met at CIMMYT!

I'd like to thank all of my friends I met in Texcoco volunteering on Saturday as well as all of my young friends. You guys truly made my summer unforgettable!

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