ANALYZING THE COMPONENT TRAITS OF POTATO ROOT SYSTEM ARCHITECTURE IN RELATION TO DROUGHT TOLERANCE CHARACTERISTICS

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2014 BORLAUG – RUAN INTERNATIONAL INTERN

INTERNATIONAL POTATO CENTER

LA MOLINA, LIMA, PERU

14 JUNE 2014 – 12 AUGUST 2014

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Acknowledgements

I would like to extend my deepest gratitude to the World Food Prize Foundation, to the Ruan Family for their generous support of this extraordinary opportunity, and to Ambassador Kenneth Quinn for his tireless dedication to continuing the legacy of Dr. Norman Borlaug. I am forever grateful to the late Dr. Borlaug because his life and work inspired me to pursue plant breeding as a way to make a meaningful difference in our world.

A special thanks to Ms. Lisa Fleming and all of the other staff for selecting me as an intern and for the countless hours and dedication that went in to making this such an unforgettable experience.

Thank you to Dr. Barbara Wells, the Director General of the International Potato Center, for allowing me such an incredible research opportunity. Thank you to Dr. Awais Khan, leader of the Adaptation and Abiotic Stress Genetics Group, for mentoring me throughout this internship. And I am grateful to all the other staff at the International Potato Center that facilitated my internship and helped me throughout my time in Peru.

And finally, I would like to thank my family for providing continual love and encouragement throughout this experience, and for supporting all my aspirations and endeavors.

INTRODUCTION

The International Potato Center

In 1971, the International Potato Center (known by its Spanish acronym CIP for el Centro Internacional de la Papa) was founded as a research-for-development institute. CIP focuses on roots and tubers, with much of the early research dedicated to potato research and development. Over the years, CIP has become more globalized as a member of the CGIAR (Consultative Group for International Agricultural Research) and has expanded its offices to over thirty countries throughout Asia, Africa, and Latin America [3].

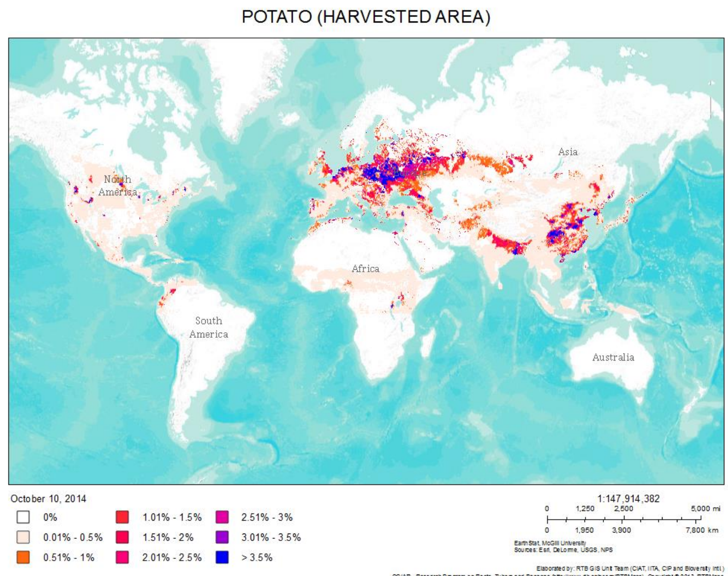
The vision of CIP is to improve the lives of the poor through roots and tubers. CIP works extensively with partners worldwide "to achieve food security, well-being, and gender equity for poor people in root and tuber farming and food systems in the developing world…through research and innovation in science, technology, and capacity strengthening." These partnerships extend across the agricultural spectrum from the laboratory research to the marketplace [3].

Recently, the leadership at CIP has developed a new strategic plan for the next ten years. This plan lays out the goals for research and efforts that seek to build on the achievements at CIP while looking to the future to maximize its global impact. The organization focuses on six strategic objectives outlined in the strategic plan that encompass early and late-stage research and development of roots and tubers as well as the continued commitment to preserving the genetic resources of roots and tubers in the CIP Genebank for conservation and utilization in the coming decades [3].

At CIP, there are several research divisions, and my internship was within the Germplasm Enhancement and Crop Improvement Division under Dr. Merideth Bonierbale. My internship mentor was Dr. Awais Khan, who led the Adaptation and Abiotic Stress Genetics Group within that research division. This group focuses mainly on addressing the growing issue of drought and its impact through improved varieties of drought resistant potatoes

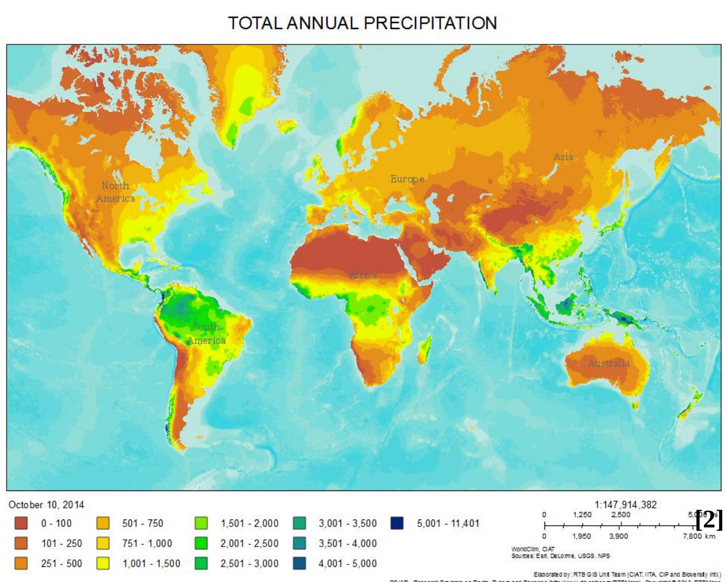
Potatoes and Drought

Worldwide, potatoes are the third most important food crop and they outstrip all other food crops in the developing world [4]. Potatoes are grown in over 125 countries they provide a significant source of carbohydrates for hundreds of millions of people in developing countries [5]. Farmers growing potatoes can produce more food faster on less land and in harsher climates than any other major food crop [5]. With over 95% of the global population growth occurring in developing countries, potatoes are becoming increasingly important for global food security [4].



[1]

Globally, growing populations and food consumption is rapidly increasing the demand for fresh water. However, these pressures as well as the changing climates strain water resources and cause shortages. Approximately 40% of the world's population suffers from serious water shortages, and that is projected to worsen in the coming years [6]. Also, agriculture accounts for more than 85% of the global fresh water usage, which must be reduced through the development of crops that are more efficient in their water use [6].



[2]

Although potatoes do use water much more efficiently in comparison to other crops, they are still susceptible to abiotic stresses such as drought [6]. This sensitivity causes detrimental impacts to the plant and reduces annual potato yields. Such losses are crippling to smallholder farmers, especially in developing countries. In order to address this growing problem, CIP has begun to research and develop potato varieties that minimize the potato losses due to drought stress, most notably through the Adaptation and Abiotic Stress Genetics group.

Research

Introduction

During my internship at CIP, I focused on two concurrent research projects with the Adaptation and Abiotic Stress Genetics group under Dr. Khan. The purpose of these projects was to understand the role of root system architecture (RSA), agro-morphological and physiological traits in a plant's response to drought stress. Both projects sought to characterize the RSA of different potato varieties and determine its relationship to drought tolerance at one critical developmental stage, the tuber initiation stage, with one project in a traditional greenhouse and the other in an aeroponics greenhouse.

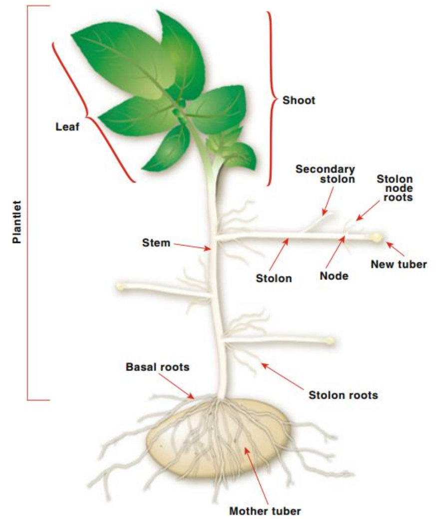
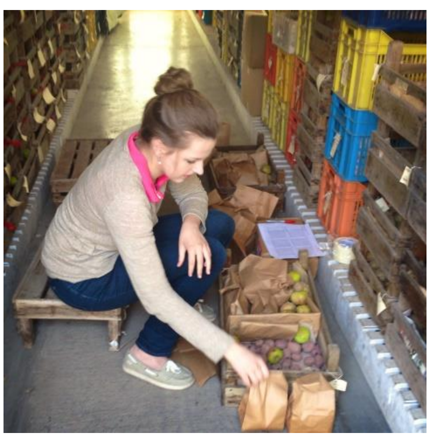


Diagram showing key aspects of potato plant anatomy [7]

Greenhouse Project

Project Plan

The research project in the traditional greenhouse sought to compare the effects of drought on the RSA of six different potato varieties in order to identify the root features associated with drought tolerance. Each of the six potato varieties had thirty plants for a total of 180 plants. These potatoes were divided equally into two treatments, well-watered and water-deficient.



Selecting the seed potatoes for planting

The potatoes were grown in individual pot filled with a sand substrate to allow for ease of examination and processing after harvest. The pots were arranged in the greenhouse in six sets of thirty. Each of these sets represented one treatment (either well-watered or water-deficient) and there were five plants per variety in the sets. One set of each treatment was harvested at 30, 60, and 90 days after planting. By harvesting at three different times, we were able to analyze the plants and their RSA at three distinct points as they matured.

Planting the seed potatoes

Because the project focused so heavily on drought and its effects, it was very important to carefully calculate and control the amount of water for the plants. We used the CropWat software to determine the optimal timing and amount of irrigation for the plants based on the crop, the substrate, and other climactic factors measured by sensors in the greenhouses. Also, to simulate drought conditions, we halved the optimal amount of water for the water-deficient sets at 40 days after planting. At this time, the plants were in the critical tuber initiation stage, and we supposed that drought conditions in this stage would have the most dramatic and detrimental impacts on the plants.

Measurements and Evaluations

At determined intervals, plants were evaluated for the following characteristics:

- **Emergence** – checked at seven, ten, and fourteen days after planting. After fourteen days, the plant was discarded if it had not yet emerged.
- **Height –** measured about every fifteen days with a standard ruler to the highest point on each plant.
- **Stem number –** counted about every fifteen days
- **Stem diameter** – measured with a digital caliper that calculated the diameter at the base of the largest stem for each plant about every fifteen days
- **Chlorophyll content** – measured about every fifteen days with a SPAD meter at the tip of the apex leaflet of the fourth fully formed compound leaf (or the most mature fully formed compound leaf if there were not yet four) six times and averaged to find the value [8].
- **Fluorescence –** measured about every ten days starting 25 days after planting, using a chlorophyll fluorometer and the Fv/Fm dark-adapted test that required clips to be placed over the tested portion of the leaf for about fifteen minutes before measurement
- **Flowering** – checked every five days between 40 and 60 days after planting

Measuring stem diameter with a digital caliper [shown here in aeroponics]

At each harvest, the selected sixty plants were destructively harvested and analyzed through a series of measurements and evaluations, including:

- **Leaf fresh and dry weight** – the compound leaves were removed from the stem and weighed, then they were placed in a special oven to dry before being weighed again
- **Stem fresh and dry weight** – all the above-ground stems were removed and weighed, then they were placed in a special oven to dry before being weighed again
- **Tuber number** – the tubers were counted
- **Tuber fresh and dry weight** – the tubers were removed from the stolons and weighed, then they were placed in a special oven to dry before being weighed again
- **Root number** – the roots were counted

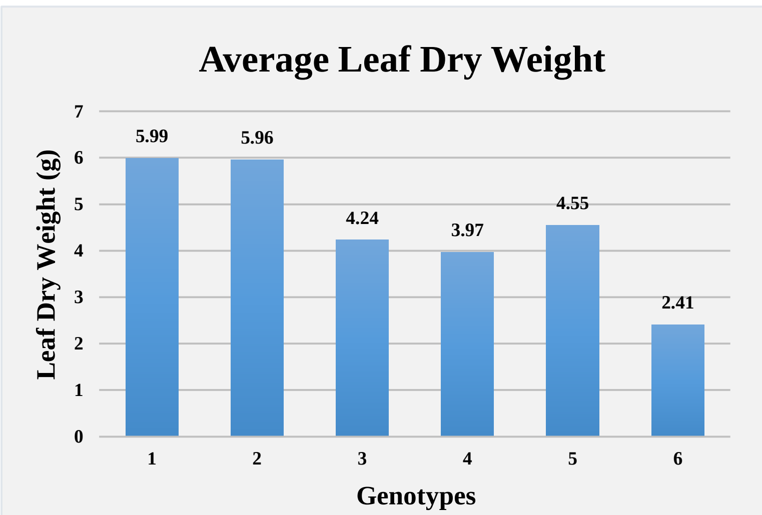
Harvested potato plant

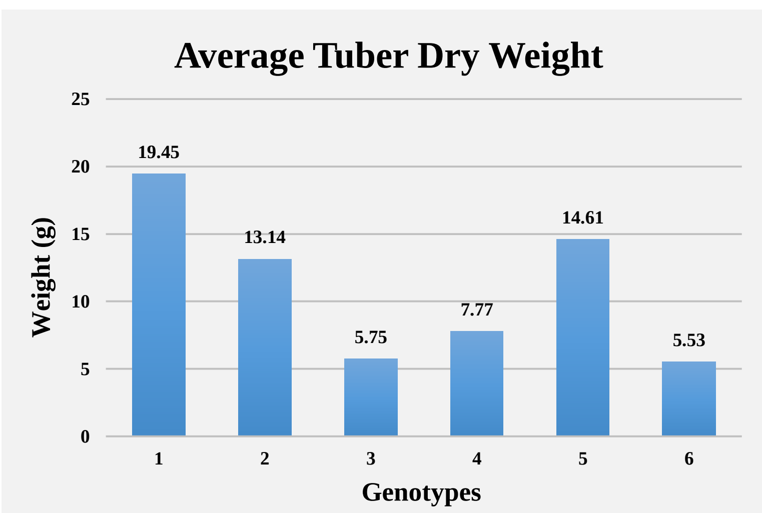
- **Root fresh and dry weight** – the roots were separated from the stolons and weighed, then they were placed in a special oven to dry before being weighed again
- **Stolon number** – the stolons were counted
- **Stolon fresh and dry weight** – the stolons were weighed and then they were placed in a special oven to dry before being weighed again

These measurements were determined to be most important in the characterization of drought responses based on subject literature and previous experiments carried out by the Adaptation and Abiotic Stress Genetics group.

Results

In previous studies, decreased tuber dry weight and leaf dry weight indicate drought stress. One of the earliest and most visible indicators of drought stress in potato plants is reduced expansion of the leaves. This reduction is due to the fact that water powers leaf cell expansion, and with reduced water intake, the leaves do not fully expand and they grow to be markedly smaller.

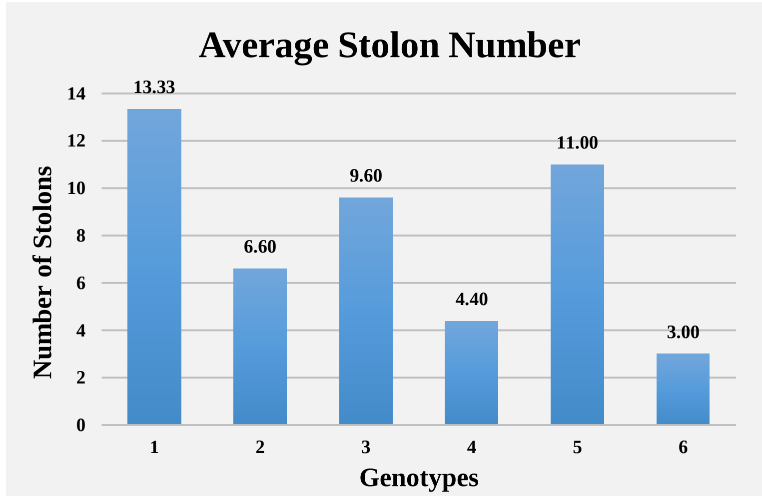




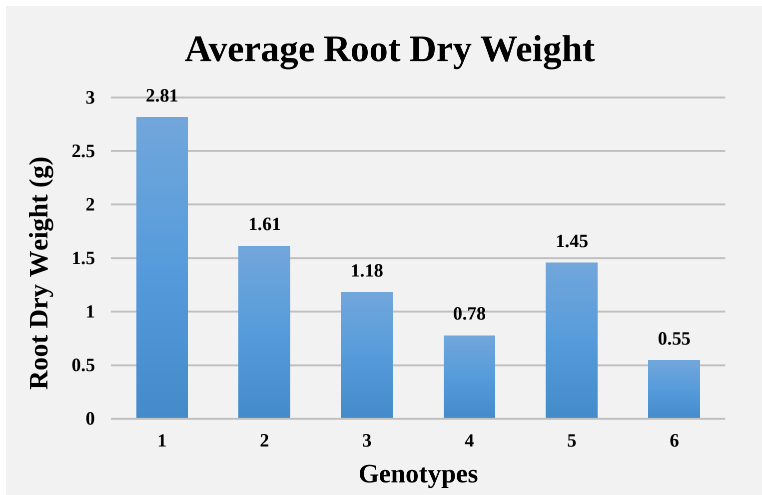
This reduced size translates to a reduction in leaf dry weight, which was measured in this experiment. Also, the reduced leaf size means less photosynthesis, which directly impacts tuber dry weight because not as much starch is produced and stored in the tubers. Thus, tuber dry weight was also measured as an indicator of drought.

For this experiment, Genotype 1 had the highest tuber dry weight and leaf dry weight compared to the other five varieties. Because these two characteristics are considered to be indicators of drought, it can be inferred that Genotype 1 is comparatively the least affected by this level of water deficiency.

Because Genotype 1 performed the best with the drought indicators of leaf dry weight and tuber dry weight in this experiment, I focused on that variety for analysis. I looked for other measured characteristics that had similar trends for the varieties as the leaf dry weight and stolon dry weight. In this way, I was able to try and find patterns of characteristics related to drought tolerance.



The highest drought tolerance in Genotype 1 corresponded to the highest average number of stolons for this experiment. This suggests an association between stolons and plant productivity under drought conditions. Such a correlation is reasonable because the more stolons on a potato plant, the higher number of tubers possible, as the tubers form at the ends of the stolons.



Also, Genotype 1 had the highest average root dry weight for all the varieties. This implies that Genotype 1 had the most extensive root system of the selected varieties. By having a more extensive root system, this genotype was better suited for water and nutrient uptake in unfavorable drought conditions.

Aeroponics Project

Project Plan

The second project, in the aeroponics greenhouse, focused on the characterization of six different potato varieties in this novel system. The main objective of this project was to test whether aeroponic greenhouse system could be used to characterize the differences in root structure in potato and to develop a high throughput root phenotyping system.

There were six different varieties selected for this experiment, with two native varieties, two Tuberosum varieties, and two of CIP's improved varieties. There were nine plants per variety, for a total of 54 plants, and three plants per variety were destructively harvested at each of the harvest times – 20, 40, and 60 days after transplant. These plants were started by planting seed potatoes in trays containing sand substrate. Once the nine plants per variety had grown to about four centimeters tall, they were washed and transplanted after the seed tuber was removed.

Preparing the plants for transplant

The plants were inserted into small holes in the top of the aeroponics tables with the aboveground plant growing out the top of the table and the roots hanging into the chamber below. The aeroponics tables had wood beams that formed the outer structure, and the top and bottom of the tables were covered in black plastic. For the sides, there were removable side panels that were also covered in black plastic. This plastic was to simulate the dark, soil environment so the roots would grow normally. Also, the plastic-lined panels could be removed, so we were able to view the root systems of the plants without disturbing their normal growth and development. The scales affixed to each side of the 'window' on an aeroponics table allowed for consistency in our root length measurements.

A section of the aeroponics table

Measurements and Evaluations

This project had a series of evaluations of the plants and their roots every week, including:

- **Plant height –** measured with a standard ruler to the highest point of the plant
- **Number of leaves –** counted all the fully matured compound leaves
- **Chlorophyll content -** measured with a SPAD meter at the tip of the apex leaflet of the fourth fully formed compound leaf (or the most mature fully formed compound leaf if there were not yet four) six times and averaged to find the value [8]
- **Stem diameter** – measured with a digital caliper that calculated the diameter at the base of the stem
- **Root length** – removed the side panel and took a picture, which was inputted into a program with the attached measurement scale on each aeroponics table used to calculate the root length

Taking SPAD measurements

At each harvest, the following measurements were taken:

- **Leaf area** – the compound leaves were removed from the stem of the plant and scanned. This scan was evaluated using a program that identified the leaves based on their green color, and calculated their area
- **Leaf fresh and dry weight –** the compound leaves were removed from the stem and weighed, then they were placed in a special oven to dry before being weighed again
- **Root length** – the roots were laid out and measured to the longest point
- **Root number** – the roots were counted
- **Root fresh and dry weight** - the roots were separated from the stolons and weighed, then they were placed in a special oven to dry before being weighed again
- **Stolon number** – the stolons were counted
- **Tuber number** – the tubers were counted
- **Tuber fresh and dry weight** - the tubers were removed from the stolons and weighed, then they were placed in a special oven to dry before being weighed again
- **Stem fresh and dry weight -** all the above-ground stems were removed and weighed, then they were placed in a special oven to dry before being weighed again

Discussion

Regrettably, I was not able to see this project to completion and the data was not shared with me. However, while I worked on the project, I noticed definite trends in the results, with the CIP improved varieties outperforming the Tuberosum varieties, and the native varieties falling far behind them both. This was shown most clearly by the root systems of each variety, as the improved variety had more roots that were longer and had higher root hair density than the roots of the native varieties.

Comparison of native variety roots (left) and improved variety roots (right)

Also, the aeroponics system worked very well for root characterization as it allowed continual access to the roots. Also, we could harvest the roots without damaging them at all. This could be especially useful when studying more delicate characteristics, like root hairs, that would are damaged by traditional harvesting from substrate.

Other Research Work

During my eight week internship, I focused mainly on the two projects outlined above, but I also had several opportunities to assist in research work with other groups at CIP. These experiences gave me a better understanding of the wide range of research performed at CIP. Also, I had a chance to travel to a CIP research stations and assist with the work at that site.

Potato Virus Testing

One of the other groups at CIP was doing research on improved potato varieties and their resistance to Potato Virus X and Potato Virus Y. These two diseases can cause tremendous damage to a potato crop. In order to test for resistance, I helped infect the selected seed tubers with Potato Virus Y.

Dozens of aphids on the aphid-attracting plant

Potato Virus Y must be directly infected by grafting or by pests. So in order to infect the selected tubers, they had grown a plant that attracted enormous numbers of aphids. These aphids were then transferred to potato plants that were confirmed carriers of Potato Virus Y, and by feeding on the leaves the aphids carried the disease with them. My job was to help transfer these aphids to the selected seed tubers by vigorously shaking them off the infected leaved into the bags with the tubers.

Taking leaf samples for virus testing

A few weeks later, I also helped this group test for Potato Virus X and Potato Virus Y in a large population of potato plants. I assisted in the preparation of the leaf sample packets, and then took samples of several hundred leaflets.

Huancayo Research Station

One of the CIP research stations in Peru is located in the mountainous city of Huancayo, east of Lima. I traveled here with a researcher who manages some CIP's early-stage breeding programs. Because of her role, I was able to assist in many of the aspects of breeding.

Emasculated flowers

Performing crosses is important for the development of new varieties with desirable characteristics. Potatoes have perfect, complete flowers meaning that they have all the male and

Pollinating a potato flower

female parts in the flower, which means that they mostly self-pollinate. So in order to perform crosses, I emasculated the flowers a day before pollinating them.

First, I identified a clump of flowers with at least three almost mature flowers. Then I took a tweezers and carefully split open the petals and removed the anthers with the pollen. The next day, the flowers were ready for pollination, so I would use the pollen of the desired cross and carefully apply it to the stigma.

Also, I harvested and processed some of the potato berries for their seeds. The berries of successful crosses had grown and were ready for harvest sixty days after pollination. I snipped the stem of these berries and secured the bag for each pollination set. Then I placed the harvested berries in a container to be stored and to ripen for a few weeks.

Harvested potato berries

Macerating mature potato berries

In order to remove the seeds from the potato berries, I processed the ripened berries that had been harvested and stored. First, I macerated the berries into a container of water, mixing the seeds of all of the berries from one plant. After that, I strained and rinsed the seeds several times to remove all the pulp before wrapping them in a waxed paper sleeve to be dried and stored.

Sampling leaf tissue

Chloroplast Staining

One of the lab research experiences that I had involved staining and counting the chloroplasts of the stomatal guard cells. I did so by carefully peeling away the top layer of the underside of the leaf and placing it in a staining solution on a labeled microscope slide. After preparing several of the slides, the staining would be complete and I began to observe the slides under the microscope. I identified, counted, and averaged the number of chloroplasts in the stomatal guard cells of the samples. The purpose of counting the chloroplasts was that the number corresponds to the ploidy of the potato plant, which is important for the plant breeders to determine because potatoes have different levels of ploidy.

Cultural Experiences

This internship not only allowed me to gain quality research experience, but I also had the chance to experience the culture of Peru.

With a young Peruvian girl in traditional dress

For my original World Food Prize Youth Institute paper, I selected Peru as my focus country so I had some background knowledge of the country. However, my ideas and expectations of life in Peru before this internship paled in comparison to the lively, vibrant Peruvian people I encountered.

An artisan market near Cusco

At the city government building for Lima

This was the first time I had traveled to South America and it was an incredible opportunity to truly immerse myself in the language and culture of Peru.

Hiking Machu Picchu

I also had the chance to travel to the mountainous region of Peru, to Huancayo and to Cusco, as well as to Arequipa in southern Peru.

This internship was a truly unforgettable experience.

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