Maize in Mexico

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CIMMYT: International Maize and Wheat Improvement Center

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Abstract

On June 17th, 2006, I left the familiar people and community of Des Moines, Iowa, on an adventure that would transform how I saw myself, my nation, and the world. I had the incredible opportunity as a Borlaug~Ruan International Intern to work at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico for eight weeks. I worked with the Diversity Group in the biotechnology labs, observing and assisting with a variety of lab techniques including DNA extraction and quantification, polymerase chain reactions (PCR), and agarose gels. Outside the lab, I had the chance to visit field sites and to explore many fascinating historic and cultural sites. Visiting the fields and speaking with the Mexican farmers gave me a new insight into the food security issues faced by small farmers in Mexico and in developing communities throughout the world.

My work gave me a new understanding of genetics, molecular biology, and laboratory techniques, but more importantly, the experience gave me a love for the people of Mexico and a desire to commit my life and career to improving livelihoods around the world.

Acknowledgements

I sincerely thank all of the wonderful people of CIMMYT for making my internship a phenomenal, life-changing experience. Your kindness, patience, and generosity touched me deeply.

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My deepest gratitude goes to the World Food Prize Foundation for providing this incredible opportunity. I am grateful for the vision of Norman Borlaug and John Ruan, and the leadership of Ambassador Kenneth M. Quinn that made the World Food Prize Youth Institute and the Borlaug~Ruan International Internship a reality. I am also indebted to Lisa Fleming for all of her hard work and for watching out for me during my internship.

My most heartfelt thanks goes to my family and friends. Their support, messages, and prayers gave me the strength and courage to boldly face each new challenge.

INTRODUCTION TO THE INTERNSHIP

My introduction to the World Food Prize was the announcement posted in my 9th grade classroom. "STUDENT WANTED," it said, to write a paper for the Youth Institute of the 2003 World Food Prize Symposium. Despite living in Des Moines, I had only a vague knowledge of the World Food Prize and Norman Borlaug. I was a typical high school student living in an Iowa city surrounded by corn and soybeans. However, I was looking for a research opportunity, so I eagerly agreed to research and write a paper and to represent Johnston High School at the Symposium.

The Symposium opened my eyes to the issue of food security. Nothing had prepared me for the complexity and extent of world hunger or for the dedicated efforts around the world aimed at its eradication. I was inspired to make a difference and to fight against hunger and poverty.

I continued to study hard in school, but I took a greater interest in world issues and in my Spanish and science classes. I started working in the seed physiology lab at Pioneer Hi-Bred International Headquarters in Johnston, Iowa, where I discovered the beautiful complexity of corn research. Finally, I saw my opportunity to get involved in the international research community through the Borlaug~Ruan International Internship. I carefully prepared my submission materials and then strove to excel in the interview and selection process.

I was ecstatic when I learned that I was the intern selected to travel to CIMMYT, the International Maize and Wheat Improvement Center in El Batán, México. I left the Des Moines International Airport early on the morning of June 17th ready to work, learn, and overcome challenges. I was ready for my adventure - and what an adventure it was!

THE ROLE OF MAIZE IN MEXICO AND THE WOLRD

The people of Mexico have a powerful connection to maize, the crop that was domesticated by their ancestors and has fed the people of Mexico for centuries. My internship gave me a love for the people of Mexico and a passion for this crop which is such an integral part of their history, culture, and identity. From maize motifs carved into stone by the ancient Aztecs displayed in the Museum of Anthropology, to images of maize in the colorful murals of Mexican artists, to the stalks of maize growing in the planters in the concrete barricades at the ever-present highway tolls, maize is an essential part of Mexico's past, present, and future. During a day of field visits, I discussed the role of maize in Mexico with national Antonino Sebastian Gutierrez. His statement "Maize is as important as God" still rings powerfully true for Mexico.

The domestication of maize in Mexico was vital to the rise of early Mesoamerican civilizations. Maize was domesticated between 4000 and 3000 B.C. from the wild grass teosinte in the Mexican Sierra Madre. By 1400 B.C., maize cultivation had spread to both coasts and had enabled the rise of the early Mesoamerican civilizations. Maize cannot survive naturally in the wild; both maize and the people of Mesoamerica needed each other for survival. The importance of maize is reflected in the Mayan creation myth; after failing to create human beings out of first mud and then wood, the gods create human flesh from maize dough. Not only does maize play a central role in many religious ceremonies and stories, it also has a prominent role in secular celebrations and daily life. With hundreds of uses for the grain, and many more for the stalk, leaves, and roots, the role of maize is central and irreplaceable to traditional culture and ways of life in Mexico.

Maize plays a crucial role, not just in Mexico, but around the world. By 2020, the demand for maize in developing countries is expected to surpass both rice and wheat, the two other major cereal crops. The global demand for maize is expected to rise 50% from 558 million tons in 1995 to 837 million tons in 2020. In order to meet this rising demand, international maize research organizations like CIMMYT are committed to developing and implementing improved varieties and farming practices to improve yields while preserving natural resources.

CIMMYT HISTORY

In 1943, a partnership of the Mexican government and the Rockefeller Foundation created the Cooperative Wheat Research and Production Program for the purpose of bringing agricultural improvements to the farmers of Mexico. The program was committed to using scientific research in genetics, plant breeding, plant pathology, entomology, agronomy, and soil science to address the wheat shortage in Mexico. Years of persistent and virulent rust strains combined with reduced soil fertility meant that Mexico produced less than half of the wheat that the country consumed in 1944, the year that geneticist and plant pathologist Norman Borlaug joined the program. After years of research as head of the wheat breeding program, he developed a rust-resistant, dwarf variety with higher yields and better response to irrigation and fertilizers that was well adapted for Mexico’s diverse climate and soil conditions. The short plant stood up under the weight of the additional grains, which would cause traditional tall wheat varieties to lodge (fall over) before harvest. As a result of the improved variety, Mexico gained wheat self-sufficiency in 1956.

During the early 1960s, the world took notice of Norman Borlaug’s achievements with dwarf wheat varieties in Mexico, and other countries looked at the Cooperative Wheat Research and Production Program as a model to create their own agricultural research programs. With the help of the Rockefeller Foundation and the World Bank, genebanks were created around the world to collect, store, and study the major food crops. In 1966, the Rockefeller and Ford Foundations and the Government of Mexico transformed the Cooperative Wheat Research and Production Program into the International Maize and Wheat Improvement Center. Known by its Spanish acronym CIMMYT

(Centro Internacional de Mejoramiento de Maíz y Trigo), the new center was moved to El Batán in central Mexico. CIMMYT would research and preserve wheat and maize landraces as an extension of Borlaug's earlier work. This newly transformed program would have a broader focus on both wheat and maize and links to breeding programs around the world.

In the late 1960s, Pakistan and India faced extreme wheat shortages. In a bold step, both nations imported the improved Mexican dwarf hybrid seed and dramatically increased wheat production. Pakistan became self-sufficient in wheat by 1968 and in India, the wheat yields of 1979 had increased 300% from harvests of the early 1960s. Shortly afterward, China also adopted the improved wheat varieties. No longer was Norman Borlaug's improved variety merely helping to improve Mexican agriculture, it was helping to feed the world. As a result of his work to reduce hunger, Norman Borlaug was awarded the Nobel Peace Prize in 1970.

In 1971, international organizations and donors created the Consultative Group on International Agricultural Research (CGIAR) to fund and support agricultural research institutions like CIMMYT. There are 15 CGIAR member centers located throughout the world, each committed to preserving the diversity of the world's major food crops and addressing a different aspect of food security. CIMMYT is CGIAR's primary maize and wheat research center and is focused on creating solutions to improve agricultural systems to reduce hunger and poverty while preserving natural resources.

OVERCOMING CHALLENGES

With over 815 million people still suffering from hunger, CIMMYT continues to face huge challenges in improving food security around the world. With four experimental stations in Mexico, and others in Colombia, Kenya, Zimbabwe, Ethiopia, Turkey, Georgia, Kazakhstan, Afghanistan, India, Nepal, Bangladesh, China, Australia, and the Philippines, CIMMYT works to address the issues faced by farmers in developing countries around the world. CIMMYT's goals are clearly outlined in their mission statement: "Through strong science and effective partnerships, we create, share, and use knowledge and technology to increase food security, improve the productivity and profitability of farming systems, and sustain natural resources."

CIMMYT researchers continue to apply the lessons learned during the early years of the Green Revolution. Scientific advances will be slow to impact the farmer unless implemented with the help of local governments and international organizations. Advances will be ineffectual unless they are applied with consideration for the farmers' needs. CIMMYT works to understand the entire system, particularly the needs of the farmers, to create and implement improvements.

CIMMYT uses a number of tools to develop improved varieties of maize and wheat, including those developed in the Diversity Group whom I worked with during my internship. The Diversity

Group focuses on characterizing genetic variation in maize populations from around the world and comparing this diversity to modern breeding lines and cultivars in order to help the Genebank preserve the most diverse range of populations and to help maize and wheat breeders use the genetic diversity to create improved varieties.

DIVERSITY

Understanding of global diversity is essential to preserve genetic variation, a vital characteristic that protects species from extinction. A homogeneous population is more susceptible to extinction by pathogens or other environmental conditions. With more genetic variation, some individuals are likely to have genes that code for proteins that provide resistance. Genetic variation is essential as the driving force behind evolution and plant improvement by breeders. Eliminating worldwide genetic diversity would inhibit plant improvements through natural, farmer, and breeder selection.

Researchers study populations to characterize and quantify diversity. Populations, or landraces, are defined by the Diversity Group as a group of individuals that share a common gene pool and have the potential to interbreed. Unlike an inbred, which is composed of genetically identical individuals, populations contain a variety of genetic characteristics. While the progeny of an inbred are practically clones, each generation of a population produces new combinations. These new combinations are much like the genetic recombination that creates unique children. Every child (with the exception of identical twins) is genetically unique, even though siblings may share a common gene pool contributed by the parents. Traditional populations of maize cultivated and preserved over generations are also known as farmer’s varieties, and they form a significant source of diversity that is slowly disappearing, corresponding with global urbanization. Populations can also be created by breeders through planned crosses to create breeding populations that are used to develop new cultivars (cultivated varieties).

A population can be quantified by identifying the frequency of specific alleles, a genetic characteristic unique to each population. Population studies by the Diversity Group are based on the Hardy Weinberg Principle which states that a population with random mating will achieve equilibrium of allelic distribution. Therefore, although individuals may change with each generation, allelic frequencies within the population will be maintained. As a result, the variety within a population will be maintained in the same proportions as long as there are no forces to shape genetic diversity.

Dynamic populations, however, are a challenge to characterize. Populations are rarely static; instead they are constantly influenced by forces such as mutation, migration, recombination, selection, and drift. Mutation is the slowest yet most important cause of change because it is the only source of new genetic material into the species. Migration, recombination, selection, and drift just rearrange or alter the frequency of the existing genes. As the frequency of alleles is changed, the frequency of genes and their corresponding phenotypes, or physical characteristics, are changed.

Another challenge of characterization is the large sample size needed to accurately characterize a population. No single individual can represent the genetic diversity of the entire population because a population is composed of differing individuals. As a result, the average population cannot be accurately characterized with less than 15 samples. Analyzing a bulk sample created by combining equal amounts of DNA from 15 individuals from a landrace dramatically reduces the cost and time needed to characterize samples compared to analyzing the 15 plants individually. By using markers such as single sequence repeats (SSR) and specially designed analysis programs, researchers can determine the frequency of alleles in a population based on the frequency of alleles in the bulked sample.

Single sequence repeats (SSR), also known as microsatellite markers, are repeated patterns of nucleotides that are often located between sequences that occur only once in the genome. For example, dinucleotide repeats such as CA and GA are found dispersed throughout most eukaryotic genomes. SSRs are incredibly useful markers due to the high level of polymorphism, or the number of forms that an allele or specific part of DNA can take. This polymorphism is a result of the relatively high frequency of "mistakes" made during replication in these regions compared to the rest of the genome. Every few generations, one of the "mistakes" will be incorporated into the genetic material of the offspring, thus producing a new allele that differs from the original allele by the number of repeats. By using several polymorphic microsatellite markers found throughout the genome, researchers can characterize both individuals and populations.

The Diversity Group is currently working to understand the pattern of maize migrations and introductions around the world and to complete the characterization of global maize diversity. An earlier study by the group characterized samples from Europe and the Americas, identifying the points of origin and patterns of introduction. Dendrograms are used to graphically represent the relationships between traditional landraces collected from Europe and the Americas. The branches represent divisions between the landraces that occurred as the varieties were adapted for new regions and were placed under new environmental pressures and farmer selection.

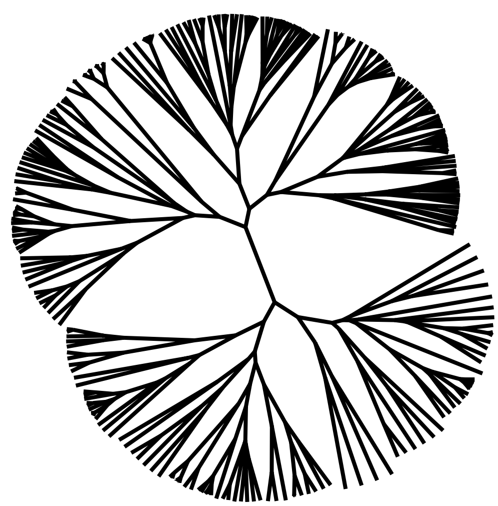


Figure 1: Dendrogram representing relationships between European and American Maize Populations.

This earlier study had insufficient time and funding to create a global view, so the new project will complete the picture to create a powerful tool for the Genebank and maize breeders. The Genebank is responsible for maintaining the genetic diversity of maize and wheat populations "in trust for humanity." However, because of limits in staff, space, and financial resources, the Geneback cannot store every population. Instead, the Genebank preserves the maximum diversity for the breeders and humanity by using the Diversity Group's studies to pinpoint populations that are unique from the existing collection. The maize and wheat breeders can use diversity characterization to identify populations with desired characteristics for crosses.

Although hybrid breeding takes advantage of the phenomenon called heterosis, the principle that crossing two unrelated maize inbreds will create an offspring that can out-yield either parent, this principle cannot be extended indefinitely. Breeders must cross within a range of genetically similarity; lines that are too similar will not create heterosis, and genetically distant varieties often produce hybrids that exhibit a lower performance than both parents. For example, crossing temperate and tropical maize varieties, the two main maize divisions, will often produce offspring that are not adapted to either climate. However, by only crossing genetically similar populations, breeders are reducing diversity. By identifying new breeding material that contains new genes within an otherwise similar genetic makeup, breeders can successfully incorporate new, desirable traits such as drought tolerance into improved varieties.

I had the opportunity to work with many amazing people associated with the Diversity Group and the biotechnology labs. Brian Love, a graduate student supported by the University of Alberta in Canada and the Smithsonian Tropical Research Institute in Panama, directed my work for the first two weeks of my internship. His research analyzes Panamanian maize and rice systems to create a detailed picture of this complex center of diversity. By understanding patterns of seed exchange and the farmer selected traits of the landraces, Brian Love hopes to understand how new, hybrid varieties are affecting the diversity of the native landraces. After two weeks, Senior Scientist Marilyn Warburton, a molecular geneticist from the United States and the head of the Diversity Group, returned to CIMMYT and began directing my research.

The other members of the Diversity Group were great to work with and always offered assistance and advice as I mastered my laboratory techniques. Susanne Dreisigacker, a post-doctorate researcher who will start her new position as Associate Scientist in January, offered useful advice and helped me prepare the lab procedure for my research project. Technicians Claudia Bedoya and Ana Lidia Gomez were infinitely patient teachers who were always willing to answer my questions worded in stumbling Spanish. Santiago Ramírez, a Masters student from the University of Hohenheim in

Germany and a native of Mexico City, was always willing to translate when my Spanish (frequently) proved inadequate, and he offered the sympathetic advice of a fellow student whenever my experiments did not go as planned. Assistant Hugo López kindly let me watch him whenever he skillfully prepared a gel for the sequencer, extracted DNA from leaf tissue, or performed some other technical lab task with apparent ease.

The CIMMYT staff proved valuable, not only in demonstrating lab techniques and sharing genetics knowledge, but also in developing my understanding of the role of researchers who study maize populations. The Diversity Group is preparing to study Asian and African maize populations in order to better understand how the maize of the world is related. In order to gain this broader, worldwide understanding, the Diversity Group will compare the samples from Asian and African landraces with the stored samples from the American and European maize populations. However, first the Diversity Group must determine the quality of the stored DNA samples.

PROJECT DESCRIPTION

My assignment at CIMMYT, in addition to learning and assisting the Diversity Group of the Biotechnology Labs, was to determine the quality of DNA samples stored in the Gene Bank that have experienced evaporation. The Diversity Group handles many maize and wheat samples, and proper storage is always important. The samples are put in 96 well DNA plates and then sealed before freezing for storage. However, the seals that were supposed to prevent evaporation of a group of samples failed, and as a result, many of the DNA samples dried out. It is important to determine if this DNA can still be salvaged. If not, it is worthless and should not be kept, and DNA extraction must be repeated for several hundred samples.

Both maize and wheat DNA were tested. The wheat samples consist of landraces, cultivars, and synthetics that were used in diversity studies. Synthetic wheat varieties are created when scientists replicate the original cross between durum wheat and wild wheat varieties, producing breeding material with greater resistance to conditions such as disease and drought. The maize set of DNA samples was used in the Diversity Group’s earlier study on the European and American landraces. Now that the group wants to complete the picture of global diversity by incorporating samples from Asia and Africa, the group will need the earlier samples as a basis for comparison. The current study will use some new markers, different from the SSR markers used in the earlier study. In order to relate the new data to the existing population studies, the new markers should be run on the old DNA samples as well. If the DNA will not amplify the study will have to be redesigned.

I designed and implemented an assay to optimize DNA amplification and to view the results using an agarose gel. Unlike a DNA quality gel, which is a relatively fast and easy way to determine DNA quality and quantity, my assay would determine if the DNA amplifies, which was the primary concern about the specific maize and wheat samples selected for testing. Using the standard CIMMYT laboratory protocols as a guide, I developed a procedure to re-suspend the DNA and then to optimize and identify DNA amplification using polymerase chair reactions (PCR) and agarose gels.

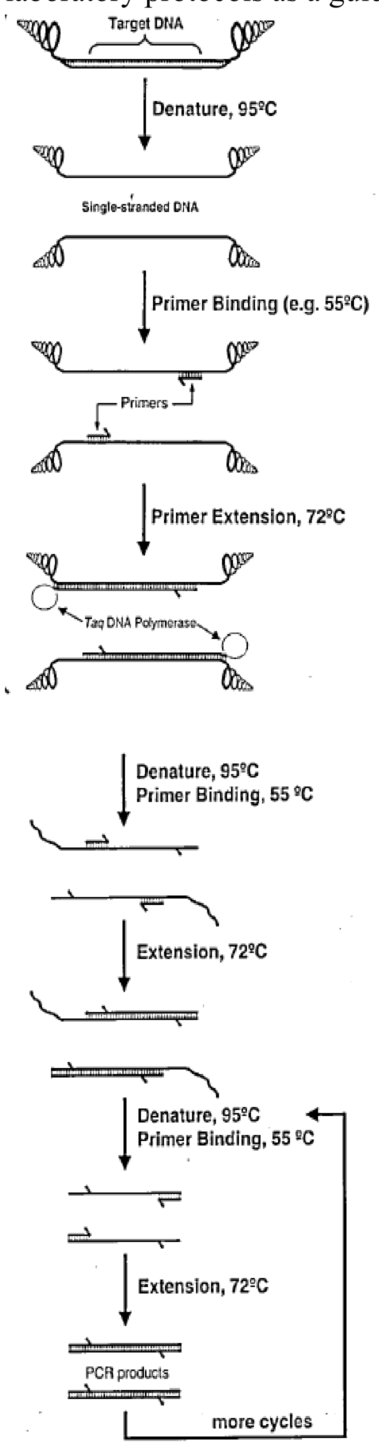


Figure 2: PCR diagram

TECHNIQUES AND PROCEDURES

The technique of PCR with an agarose gel to view the results is the ideal assay to determine if the re-suspended DNA samples amplify. PCR, or polymerase chain reaction, amplifies specific portions of DNA based on the primers chosen for the experiment. The process takes place in a thermocycler, a programmable heater set to cycle through the conditions necessary for each of the three steps in PCR: denaturing, annealing, and elongation.

When double stranded DNA is separated into two single-stranded parts, it is said to be denatured. This process occurs at a high temperature between 94 and 96 degrees Celsius. Next, the primers bind, or anneal, to the single-stranded DNA. The annealing temperature is determined experimentally for each primer. Primers are short, synthetic pieces of DNA that target a single locus, or a location with a specific sequence. Elongation is the next step after annealing. Taq polymerase is the enzyme responsible for connecting nucleotides to the single stranded DNA, thus elongating the new strand of DNA. Taq is named after the heat-loving bacteria *Thermus aquaticus* from which the enzyme was first extracted. While most polymerase enzymes located in other organisms degrade at high temperatures necessary to separate the double-stranded DNA during denaturing, Taq polymerase is not damaged, and in fact works best at approximately 72 degrees Celsius (about 162 degrees Fahrenheit).

These three steps are repeated thirty-six times to produce a huge number of the desired DNA fragments. Each time the steps are repeated, the number of short, desired fragments is doubled. After 36 cycles, there are more than 100 billion fragments which is enough to see by eye.

One of the challenges faced by researchers is selecting the optimum primer for the polymerase chain reaction. Primers are designed to be specific to a specific locus (location with a specific sequence) in the genome. Research groups from around the world have identified thousands of primers and their corresponding loci. By accessing online databases such as the United States Department of Agriculture supported GrainGenes and Maize Genetics and Genomics Database (MaizeGDB), researchers can find loci within or near the gene of interest.

For my assay, however, I was not interested in identifying a specific locus within the genome; I was merely concerned with amplification. As a result, I selected primers for my maize and wheat samples that amplified consistently based on past experiments done at CIMMYT. I then used Graingenes and MaizeGDB to determine the approximate fragment lengths. Although the fragment lengths are usually not listed on MaizeGDB for the maize primers, approximate lengths can be determined by using the ladder, or size standard. A number of size standards are available commercially, but CIMMYT produces the phi (φ) ladder for the use of its scientists.

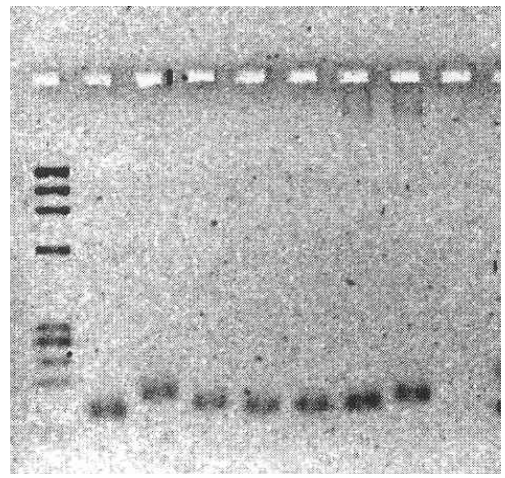
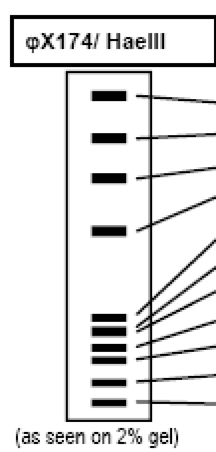


Figure 3: The first column of the agarose gel is the phi ladder. By comparing the known base pair lengths of the phi bands with the amplified DNA fragments, approximate lengths can be determined.

Agarose gels are an efficient and effective way to compare amplified DNA fragments. The gels are prepared by mixing agarose, in powder form, with TBE buffer solution. The mixture is heated and stirred until all of the agarose has dissolved. While the gel is cooling, an electrophoresis mold is prepared for the gel by taping off the ends and inserting the combs. Then the gel is carefully poured in the mold to a depth of 4 mm. After the gel has set, it is placed in the electrophoresis machine that has been filled with a solution of TBE buffer, and the combs are removed. The products of the PCR amplification are combined with a blue dye, and then each sample is loaded into a separate well in the gel. An electrical current is applied, causing the DNA fragments to travel across the gel



band	PhiX/ HaeIII	% of total
1	1353	25
2	1078	20
3	872	16
4	603	11
5	310	6
6	281	5
7	271	5
8	234	4
9	194	4
10	118	2
11	72	1
total	5386 bp	

Figure 4: Phi (φ) ladder with the base pair lengths of each fragment indicated in the chart.

toward the positive terminal because DNA has a net negative change. The longer the fragment of DNA, the slower it moves through the matrix of the gel. After the fragments have traveled across the gel, they are stained with ethidium bromide, which binds to the DNA. When photographed while exposed to ultra-violet light, the ethidium bromide glows, and the bands become visible. Clear, distinct bands indicate successful amplification.

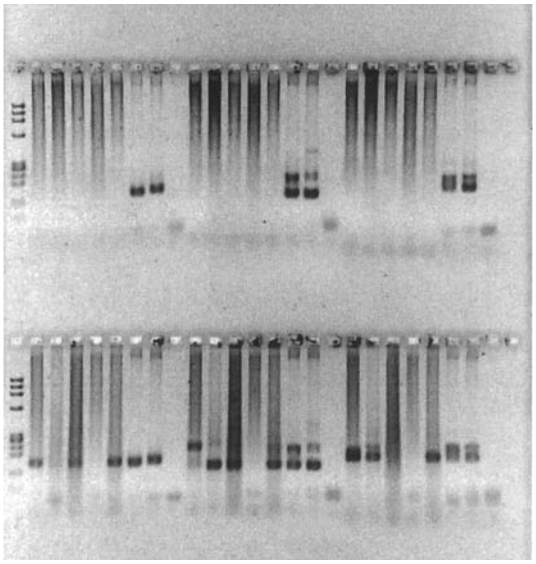


Figure 5: Wheat DNA with WMC primers.

Laboratory science offers many complications that interfere with amplification and the creation of distinct bands. Figure 5 demonstrates the results of overloading the gel with too much DNA. When too much DNA is added to the reaction, the gel will show smears instead of distinct bands. The sharper bands represent the positive controls, indicating a successful PCR. The clear lanes represent the negative controls, demonstrating the reaction was not contaminated. The phi ladder indicates a successful run on the agarose gel. Examining the wells of the gel reveals dark spots indicative of starch or sugar contamination, which in large amounts can interfere with the agarose gel.

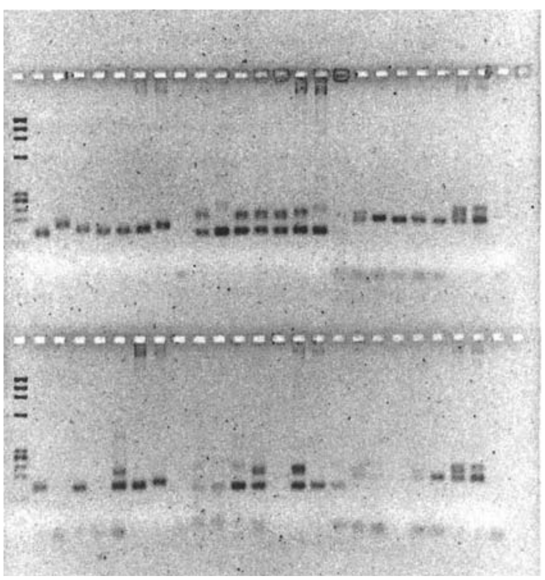


Figure 6: Wheat DNA with WMC primers.

Figure 6 demonstrates the clear, allelic bands that resulted when the same reaction was completed after diluting the DNA sample by a factor of 1 to 100. With less DNA on the gel, the bands become apparent. Note the differences in amplification between the two wheat plates demonstrated by the stronger amplification present on the top half of the gel compared to the bottom half. The second wheat plate has experienced more fragmentation than the first. However, the amplification was successful overall, indicating that the wheat DNA samples remain intact.

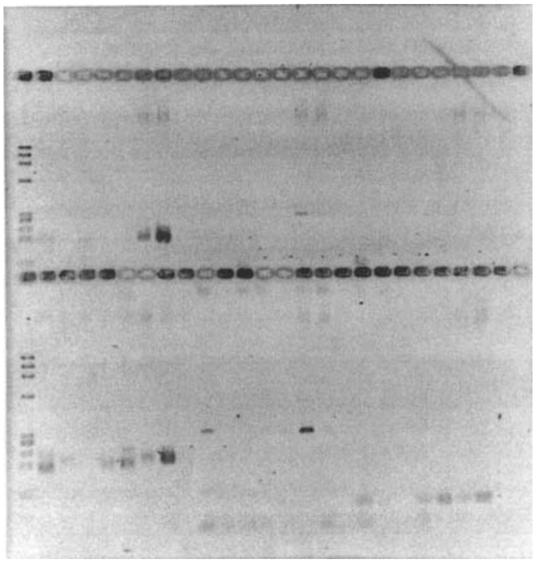


Figure 7: Maize DNA with BNLG primers

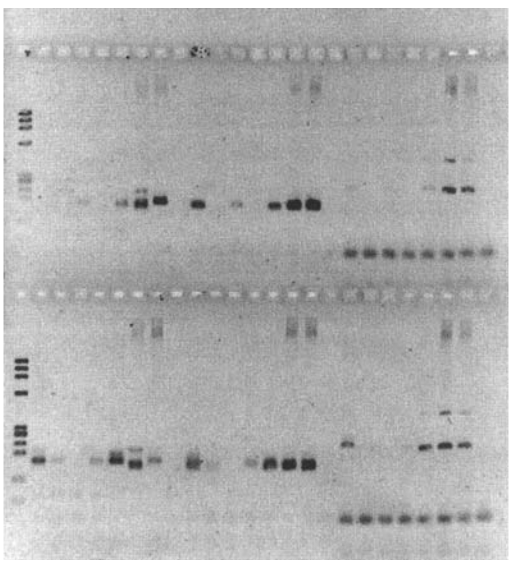


Figure 8: Maize DNA with PHI primers.

Figure 7 demonstrates poor amplification of Maize DNA samples. While the positive controls did amplify for the first two primers (see dark bands on gel), the third primer shows no amplification. Because the phi ladder indicates that the gel was run and stained according to protocol, the PCR was the source of the unsuccessful results. The BNLG primers used in the reaction are not standard primers used in the diversity laboratory, and therefore, the primers could be the cause of the unsuccessful reaction.

Figure 8 demonstrates amplification of the maize DNA samples with the PHI primers. The amplification of the maize DNA is not as distinct as the amplification of the wheat DNA in Figure 6, indicating the maize samples degraded more than the wheat samples. Notice the row of primer dimers formed on the right third of the gel that are noticeably less dark than the remaining bands. The primer dimers are created when bonding occurs between the primers, and then this undesired artifact is amplified during the polymerase chain reaction. A determination that the bands are primer dimers and not the desired product can be made based on their size and presence in the negative control. The fragments are smaller than the shortest visible phi ladder fragment. Also, the PHI primers are expected to create fragments between 150 and 210 base pairs in length, depending on the specific primer used, and consistent with the size of the dark bands on the gel. Finally, the bands are present in every well, including the negative control, indicating that they cannot be the desired DNA amplification.

Developing and implementing the assay presented many challenges. Although the concept is simple, executing the precise techniques necessary to complete the reaction successfully without contamination required much practice. Determining the correct amount of DNA to add, when the original sample was at an unknown concentration due to evaporation, was an additional challenge that had to be addressed for each plate. Testing the undiluted DNA produced smears like those in Figure 5, so then the DNA was combined with 99 parts ultra-pure Sigma water to 1 part DNA sample. This concentration produced the clear bands present in Figure 6. As I perfected my procedure and technique, I observed the importance of controls throughout the experiment. Negative controls were important to reveal the presence of contamination or "primer dimers" (undesired PCR artifacts formed by the primers) such as those present in Figure 8. Successful amplification of the positive controls demonstrates the reagents and conditions for the reaction were proper; therefore, when the samples fail to amplify, it indicates poor DNA quality, not a failed reaction.

TEST RESULTS AND CONCLUSIONS

The results of my tests showed that although the DNA had degraded somewhat, a carefully executed PCR can produce successful amplification for many of the samples. The plates will be kept, but researchers will have to check for amplification before attempting to sequence or characterize the samples. My tests also indicate that lypolizing, or freeze-drying the DNA, is a favorable way to store the genetic material, and that DNA is resilient to non-ideal storage conditions.

PIONEER IN MEXICO

Pioneer Hi-Bred International provided the opportunity to visit a number of field sites near CIMMYT. I was able to see how Pioneer is working in Mexico and the connection between research and implementation that is crucial in the fight to improve food security. Pioneer employees Antonio Gutierrez and Jesus Gonzalez picked me up early one morning, and we started our tour examining the Pioneer test plots located in the CIMMYT fields. After weeks of working in the lab, I was excited to finally get into the fields and to compare the Pioneer varieties developed for cultivation in Mexico with those I was familiar with from my pollination work at Pioneer in Johnston, Iowa. As I examined the stalks of healthy green maize, I recognized the vigor and uniformity that is characteristic of Pioneer varieties. The varieties were taller than many of the hybrids I had pollinated in Johnston, causing me to feel sympathy for the ingenieros responsible for pollination. I was excited to see purpling, an unusual trait that I had encountered in the seed physiology lab where I worked at Pioneer, but never in its native environment.

As we examined the maize plants, Jesus and Antonio discussed Pioneer's "High Valleys" program to develop improved varieties for fields in Mexico where the elevation is greater than 2000 meters (6562 feet). Pioneer recently entered the high-elevation market and they have already made great strides in developing and distributing new varieties. The Mexican market is challenging for many reasons including the variety of altitudes and soil conditions that exist throughout the country. The challenge is two-fold: first, developing varieties that will thrive under the different growing conditions, and second, identifying the right variety for the farmers in each area. If farmers are given the wrong seed for their growing conditions, it will damage both their yield and their view of Pioneer.

Next we drove to Rancho San Jose, a large dairy farm that grows corn for feed and silage at an elevation of approximately 2200 meters (7218 feet). Although the farm is quite modern (relying on hybrids, inputs, and mechanization), the fields showed poor weed management.

With just 10% of the local farmers growing improved hybrid varieties according to Pioneer statistics for the sales region, the opportunities for growth are balanced by the challenge of educating the farmers about hybrid varieties and cultivation techniques. The large farmers with more capital are better equipped to adopt improved field management techniques that preserve soil quality and produce better yields, and as a result, are easier markets than small farmers who produce maize mainly for self-consumption. However, Pioneer still faces many challenges in demonstrating the benefits of Pioneer hybrids to the large farmers. For example, Pioneer distributed seed during the spring 2006 planting season to 10 large farms like Rancho San Jose so the farmers could observe the benefits of Pioneer's improved varieties. However, instead of planting a row or a plot with the Pioneer varieties, the farmers mixed the bags of sample seeds with the rest of the seed in the planter. Of the 10 farmers that received Pioneer seed, only Rancho San Jose kept the seed separate. As a result, Pioneer is using Rancho San Jose as a sample plot to demonstrate the benefits of Pioneer's varieties to other farmers from the area.

The next farm we visited belonged to Señor Roberto Hernández, a wealthy Mexican farmer who was proud of his large, well-managed fields. The vigor of the Pioneer varieties and the effective weed control contrasted sharply with the poor crop management characteristic of small Mexican farms. As Jesus and Antonio spoke with Roberto, a picture of a life so different, yet fundamentally similar emerged. He demonstrated the same educated concern with the rain, weather, and soil displayed by my farming relatives from Iowa. Yet, looking around at his farm, I saw how different it was from the large operations characteristic of Iowa farms. He was dependent on a pump for irrigation and manual labor for much of the field work. As we chatted, a young field worker came over to meet us. He introduced himself and told us he planned to study Agronomy at the University in Chapingo in the fall. I was impressed by his hard work in the field, and his dedication to improve his education.

We next visited an alfalfa field belonging to Señor Victor Mazzuti, a dairy farmer who cultivates alfalfa for animal feed. Usually alfalfa is planted in the winter month of November when it is just warm enough for the alfalfa to germinate and grow but too cold for the weeds to become established. However, because this field had been planted in June (perfect weather for weeds), the alfalfa was fighting with a number of noxious and invasive weeds for soil and water.

Large parts of central Mexico can be very dry for parts of the year, so irrigation is important to ensure a healthy crop. Most irrigation is done with "black water," the waste water from Mexico City. This alfalfa field, however, was irrigated with "white water," clean water from an underground well.

The widespread use of black water for alfalfa irrigation is a health problem for both humans and animals. Because alfalfa is fed to directly to animals, the waste can make them sick. Also, alfalfa is sold in Mexico City to make a popular fermented beverage, causing people to ingest the wastes and pollutants from the black water.

Our last stop was a small, suffering, demonstration plot in Texcoco owned by Señor Juan Hernández. The poor land management characterized by inadequate use of irrigation, herbicide, and fertilizer was in marked contrast to the fields from earlier in the day. Yet, it was this last field that represented the challenges faced by most Mexican farmers. As I looked to the nearby homes occupied by families who clung to the farming traditions despite the extreme challenges, the larger goal of food security became more personal and less abstract.

Improving the livelihoods of people like the struggling farmers of Texcoco is the reason that international research organizations strive to develop improved varieties and techniques. Farmers in developing regions often cannot afford to buy improved hybrid varieties from commercial seed producers or the inputs and irrigation systems needed to successfully grow these varieties. Instead, these farmers rely on their own seed supply or subsidized seeds provided by the government or organizations like CIMMYT, and then they cultivate the seed using traditional methods. As my supervisor Marilyn Warburton explained it, “If we can help people improve their livelihoods to the point that they can afford Pioneer seeds and the higher levels of input these seeds require [irrigation, fertilizer, ect.] we will put ourselves out of business … and this will be the ultimate sign that we have done our job right!” While commercial agriculture will be an important part of improving agriculture in Mexico, developing regions will still rely on non-profit research organizations such as CIMMYT to develop and implement improved varieties.

The challenges of improving agriculture go beyond providing farmers with inputs such as improved maize varieties, fertilizer, and herbicide. Pioneer, along with other commercial, governmental, and international organizations, is working to educate farmers about proper crop management. Herbicides and insecticides can improve yields, but ignorant usage can harm the plants, the farmers, or the environment. Farmers need to learn when to apply the products, how much to apply, and how to protect themselves and the environment. Moving away from traditional cultivation methods to improved techniques such as two line planting and reduced tillage planting can improve yields, preserve the soil, and reduce labor. The traditional cultivation technique of mounding the soil over the base of the plant was used to prevent lodging. However, this labor-intensive technique is unnecessary with new hybrids that are shorter and have stronger root systems that resist lodging.

On the way back to CIMMYT, I discussed the challenges of incorporating new maize varieties into Mexican markets. Antonio said that to many people in Mexico, “Maize is as important as God.” Maize is such an integral part of the history, identity, and survival of Mexico, that changing it can be like asking them to change their religion. Asking a farmer to give up a traditional variety that has been in his family for generations is like asking him to give up a son. Such is the challenge faced by researchers who want their advances to be implemented by the farmers.

PERSONAL REFLECTIONS

My travels around Central Mexico gave me a love of Mexico’s rich culture and wonderful people, as well as a greater understanding of the contrasts that define Mexico. I saw both extremes of development: the modern buildings and international business of Mexico City and the small, undeveloped communities found throughout Mexico. Yet, no matter where I went, I was impressed by the kindness of everyone I met.

During my visit to the CIMMYT tropical field site Agua Fría, Mexican farmers in Poza Rica told me of the challenges they face every day. We gathered in the school house of the village, and as soon as the meeting began, a senior member of the group stood up and started listing problems he faced everyday in raising his maize, from poor soil quality, to disease and insects, to bad seed. Even though I struggled to understand his rapid and colloquial Spanish, it was apparent from his voice and demeanor, as well as from the reactions of the other members of the group, that he was a well-respected member of the community and many shared his views. As the other members of the village spoke, a picture of the harsh realities of farming life in rural Mexico formed. With little or no savings, the villagers are dependent on the food they produce for survival. Although their homes, clothes, and bellies spoke of having sufficient food for the present, their eyes showed fear for the future.

As the farmers outlined their concerns and the views of CIMMYT, they revealed many of the challenges faced by international agricultural research organizations. Yield was not the most important maize characteristic to the farmers; they were more concerned with characteristics such as flavor and color that depend on regional preferences. A collective complaint of the farmers was the unpleasant flavor of the CIMMYT varieties. Others were unwilling to adopt CIMMYT varieties because of the failed attempt of another farmer. This prejudice echoes the challenge faced by commercial seed distributors like Pioneer who must identify the correct variety for the farmers’ field conditions or risk damage to the company’s reputation. A single farmer’s bad experience with an agricultural organization like CIMMYT can affect the rest of the village, preventing the entire village from successfully integrating improved varieties and technology.

While the farmers of Poza Rica demonstrate one view of Mexico, a true picture of the country could never be complete without a visit to Mexico City. During my internship, I visited Mexico City several times, and although it would take a lifetime to explore the whole city, I was able to see many incredible places. The Historical District offered the opportunity to see many of the contrasting faces of Mexico in one place. The Zócalo, the heart of the Historical District, is Mexico City's main square, and is second in size only to the Red Square in Moscow. Centrally located among culturally and historically significant sights such as the Catedral Metropolitana, the Templo Mayor, and the National Palace, the Zócalo serves as an important symbol for Mexico and a place for political and cultural demonstrations. During my second visit to Mexico City, I saw a large tent where supporters of Lopez Obrador and the PRD party had set up a protest demonstration on one side of the square and a group of traditional dancers performing with feathered headdresses and shell jewelry on the other side. Nearby the Zócalo, vendors sold anything and everything you could eat, drink, wear, watch, or use, shouting out their prices to the crowds of people trying to maneuver through the narrow stalls.

One side of the Zócalo is dominated by the Catedral Metropolitana, a beautiful church built by the invading Spanish on top of an Aztec temple, and then continuously modified to the present. The tremendous weight of the Catedral Metropolitana is causing it to slowly sink into the soft, clay soil on which its foundation rests. Next to the Catedral Metropolitana is the museum Templo Mayor where some of the ruins of the Aztec temple are visible. As I walked amongst the ruins of the ancient temple, I looked up at the Catedral Metropolitana and the Zócalo and I was awed by the harsh juxtaposition between the influences of the early Aztec civilization, Spanish colonization, and modern-day Mexico.

Across from the Catedral Metropolitana sits the Palacio National, the home of Diego Rivera's murals depicting the history of Mexico. One mural still haunts me. It depicts the rise of Spanish colonialism in Mexico, and the cruel brutality that came with it. Riviera distorted the features of the Spanish and exaggerated their pale skin, giving them a creepy, dead look. The mural is filled with Spanish injustices against the indigenous people - slavery, branding, hangings, and torture. But what haunted me more than all of the pain and suffering were the eyes of a baby. Near the center of the mural, there is an indigenous, dark-haired woman holding a green-eyed baby. Like the eyes of the Mona Lisa, the baby's eyes follow you from wherever you look. The eyes showed a deep sorrow, as if to say to the colonizing Spanish, "What cruel world have you created for me?"

While the Historical District is characterized by buildings and ruins representing some of the most important periods of Mexico's history, other parts of Mexico City are filled with international businesses, elegant restaurants, beautiful landscaping, and tall, modern glass buildings that would be at home in any upscale urban community in the United States. American culture was powerfully felt in many parts of the city, such as near the Paseo de Reforma where a Starbucks coffee shop sat among a number of other American businesses next to the American embassy.

Near one end of the Paseo de Reforma is Chapultepec Park, the home of a number of museums and attractions including the famous Museum of Anthropology. I visited the Museum twice, but even that was not enough to explore all it had to offer. Upstairs, the displays represented the variety of cultures that can be found throughout Mexico. Downstairs are the extensive exhibits on the history of Mexico dating back to the first inhabitants of the region.

Every day I was moved by the kindness, courtesy, and respect I received from everyone at CIMMYT and in Mexico. In the lab, the scientists and technicians were always patient and understanding. They listened to my stumbling Spanish, and encouraged me to keep trying, even when it seemed like I was making no progress. They were always willing to interrupt what they were doing (despite my insistences to the contrary) to answer a question or explain something to me. When I made mistakes, they kindly explained the source of my error and let me try again until I got it right. Outside the labs and around CIMMYT, I would greet and be greeted by everyone I knew (and many people who I did not know) with a friendly "¡*Buenos días*!" At meals, everyone was kind and welcoming, always willing to make room for one more at the table.

Some courtesies, however, took more time for my independent tendencies to get used to. When I received rides from CIMMYT drivers or staff, often someone would jump out of the car and open the door for me. Not only did the gentlemen in Mexico open doors, when passing through a doorway that did not have a door, I had to get used to the gentleman in front of me suddenly pausing and moving to the side to let me through first.

I had the opportunity to meet many fascinating people during meals at the *comedor*, but I will never forget Monday, July 10th. I headed to the cafeteria for my usual variation on an American breakfast consisting of hotcakes, *activia* yogurt, and orange juice. But my "usual" morning was to be wonderfully different. Norman Borlaug invited me to join him, Dr. Evangelina Villegas, and Villegas's nephew, for breakfast. I was sitting with one of the few Americans to win the Nobel Peace Prize and a woman who won the World Food Prize in 2000

Dr. Evangelina Villegas, Diane Brown, and Dr. Norman E. Borlaug jointly with her fellow researcher for their work with QPM: Quality Protein Maize. I was moved by their kindness, and their insistence that I should have company for breakfast.

That night at dinner I saw Norman Borlaug eating alone, so I asked if I could sit with him. He kindly agreed, and I had a wonderful time discussing a variety of issues related to Mexico and food security. He described the challenges of working with existing political systems worldwide in order to implement change. He expressed a concern that I would hear echoed by many people at CIMMYT over the rise of radicalism around the world. We also discussed the role of the *ingenieros,* trained Mexican technicians, in improving agriculture in Mexico. When Norman Borlaug came to work in Mexico, they had to train the local workers, who had no formal training or education in agricultural sciences, to perform the field and lab procedures. Norman Borlaug's respect for the hard work and intelligence of the Mexican technicians was apparent as he discussed their crucial role in CIMMYT's research, in the past, present, and future.

My internship experience had a powerful impact on how I view the world. I discovered the importance of communication and understanding in implementing technological improvements, as well as the powerful impact of international research groups make in developing regions around the world. I also developed a love for the people and culture of Mexico. However, most importantly, my experience strengthened my passion and commitment for implementing sustainable improvements to food security and participating in the global community.

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