Use of Doubled Haploids and Molecular Marker Assisted Selection to Expedite Breeding Processes in Maize

Abigail Pepin
World Food Prize Foundation
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Acknowledgements

I wish to send out my gratitude to all of the people who made my stay in El Batán, México possible. I wish to thank all of the people of CIMMYT for making my internship more than just a summer job but rather a rewarding learning experience. All of their guidance and generosity has been greatly appreciated.

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1- Introduction

1.1 Personal Remarks

Unlike many of the other 2013 Borlaug-Ruan interns throughout the world, I grew up with limited exposure to agriculture. I was raised in Colorado Springs where the drought prevented much growth and fires ravaged all that was living. When I moved to West Des Moines, Iowa, I gained more knowledge about the importance of agriculture to sustaining not only our country, but also the world. I knew about irrigation and about the processes through which plants utilize photosynthesis to produce ATP; however, I never fully conceptualized all of the efforts that go into ensuring that the food that we find in the supermarkets is of good quality and appropriate nutrition. This all changed when I wrote a paper on combating soil erosion and depletion in Malawi for the first Iowa Youth Institute in 2012.

My experiences at the Iowa Youth Institute were inspiring. I had never seen people so dedicated and passionate about truly making a difference when it comes to solving food security. I was fortunate enough to advance to the Global Youth Institute in October of 2012 where I was able to meet people committed to making a difference such as World Food Prize Laureate Dr. Daniel Hillel. Talking not only with people from scientists and professors but also with students who shared similar desires to combat issues with food security was an enriching experience. The many speakers at the Global Youth Institute who had dedicated their lives to combating hunger captivated my attention. I had never seen such a group of people truly dedicated toward one purpose. It was these devoted people that pushed me to apply for Borlaug-Ruan Internship during my senior year. I was a high school student who wanted to learn and had an intense desire to make a difference.

After submitting my application to the World Food Prize, I waited anxiously to see if I would be selected for the internship. I was jumping up and down with joy when Lisa informed me that I would be interning at El Centro Internacional de Mejormiento de Maíz y Trigo (CIMMYT) in El Batán, México where Dr. Norman Borlaug once did his research. The Borlaug-Ruan internship has completely altered my perception of agriculture and has cultivated my passion for learning more about the ways that we can combat food insecurity throughout the world. CIMMYT has worked hard to ensure that the crops that they provide have good nutritional quality to combat malnutrition. The institution has made an inspiring number of advances to help farmers and malnourished people all around the globe. I am so fortunate as to be able to have spent two months of my life here among such dedicated professionals.

1.2 The History of El Centro Internacional de Mejormiento de Maíz y Trigo (CIMMYT)

CIMMYT has a long history of producing different varieties of wheat and maize to fend off starvation. The institution was begotten out of a pilot program created by the Mexican government and the Rockefeller Foundation dubbed the Office of Special Studies (OSS) that sponsored the research of The Cooperative Wheat Research and Production Program. The goal of the pilot program, dubbed the Office of Special Studies (OSS), was to increase Mexico's farm productivity. What the institute grew into was much more ("CIMMYT and Mexico").

During the 1940s, OSS committed research to plant breeding, entomology, agronomy, and genetics to help combat rust diseases that were plaguing the wheat varieties and causing shortages throughout Mexico. When Dr. Borlaug joined the OSS team in 1944, he worked with researchers to develop varieties of wheat that had resistance to the many rust diseases that were plaguing the country. By using introducing dwarf wheat varieties and using new growing techniques whereby wheat was grown in Obregón (in a hot, dry region of Mexico) and then shipped to Toluca (in a wet, cooler region of Mexico), Dr. Borlaug achieved an amazing amount of success in allowing the wheat to be able to sustain great environmental stress. Breeding dwarf plant varieties with tall wheat varieties resulted in more energy expenditure on creating seeds. Through his research, the wheat varieties become resistant to rust as well as many environmental stresses. Collectively, this led to increased production of grain throughout Mexico. Researchers like Borlaug at OSS helped Mexico to achieve self-sufficiency by the 1950s. OSS began to expand to India and Pakistan where they helped to bring new record levels of harvests to the farmers. This was all under the pilot program ("CIMMYT and Mexico").

In 1960, Mexico established the National Institute for Agricultural Research that became responsible for conducting research on Mexican agriculture. In 1966, inspired by the success of the OSS program, the Rockefeller and Ford Foundation and the Mexican government funded the creation of what came to be known as CIMMYT, an international non-for-profit agricultural research institute based in Mexico. The Cooperative Wheat Research and Production Program had morphed into something much larger. Many developing countries that faced challenges of insufficiency of grain began to look at CIMMYT and Borlaug's new strains of wheat as the panacea to poverty, famine, and hunger within their countries. Consequently, CIMMYT began to spread rapidly.

Today, CIMMYT has developed into an international organization based in El Batán, México with centers in Turkey, India, China, Iran, Kazakhsan, Kenya, Zimbabwe, Colombia, Bangladesh, and Afghanistan. CIMMYT seed varieties are grown in over a hundred countries around the world, and CIMMYT has helped to train 10,000 researchers who are helping farmers throughout the world. CIMMYT has a strong history of research in both maize and wheat.

CIMMYT is active in attempting to improve food availability and security throughout the world. CIMMYT truly embodies their mission statement: "Through strong science and effective partnerships, we create, share, and use knowledge and technology to increase security, improve the productivity and profitability of farming systems, and sustain natural resources" ("CIMMYT Intellectual Privacy Policy"). This statement holds true in every project that CIMMYT researchers are working on. Here, amongst some of the greatest scientists in history, I have spent my two months.

1.3 CIMMYT's Global Maize Program (GMP)

Millions of people go hungry everyday; however, even many of those that have food suffer from nutrient deficiencies. Lack of and insufficiencies of substances such as vitamins and minerals can stunt growth and cause a myriad of serious disorders that will affect the world's population in many years. No longer is it sufficient to say that everyone must have something to eat; we need to ensure that people all around the world are getting adequate nutrition to be able to maintain a healthy life. Expansion of the world's population has resulted in an increased demand for food of desirable nutritional values.

CIMMYT's Maize Quality and Nutrition lab is dedicated to fulfilling such demands in maize through research and analysis. This is where I had the opportunity to work during my 2013 Borlaug-Ruan International Internship under the supervision of Dr. Natalia Palacios Rojas. The idea of combating malnourishment has always been of interest to me because it is of vital importance to ensure that people around the world are able to live life free from diseases caused by inadequate supply to food or poor micronutrient diversity.

1.4 The People of CIMMYT

During my two months here in CIMMYT Mexico, I had the privilege of working with some of the most dedicated scientists in the world. Being around such impassioned people who enjoyed doing what they do was one of the highlights of my trip.

My advisor, Dr. Natalia Palacios, was always there to guide me in whatever way necessary despite her busy schedule. She acted as a teacher and was always willing to provide relevant articles regarding what she was researching. She also answered any questions or concerns that I had. I have been encouraged by her dedication to her work and enthusiasm for coming into work every day.

I had the opportunity to work in the biotechnology laboratory where I worked with Martha Hernandez who assisted me in running tests for close to 50 SSR markers along the Y1 locus of interest. She ensured that I was exposed to a variety of different techniques around the laboratory from DNA extraction to seed chipping to gel electrophoresis using both acrylamide gels and agarose gels. Rafael Venado offered much useful advice and helped to train me on various techniques in the laboratory. Alberto Vergara assisted me in extracting DNA and was always willing to answer my questions.

Dr. Vijaya Chaikman proved to be extremely helpful to me during my stay here in CIMMYT with my work with doubled haploids. As a researcher on doubled haploids, Dr. Chaikman taught me the principles through which he found the seeds and subsequently treated them. He also began directing my research with my germination trials, and always offered useful advice. Dr. Mike Olsen and Dr. Sudha Nair became my mentors on molecular markers and their applications to what Dr. Palacios's lab was attempting to accomplish with the crtRB1 allele for provitamin A.

The people at CIMMYT were more than willing to go out of their way to assist me in whatever way that I needed. They helped me to understand the role of researchers in the GMP program beyond just my project; consequently, I was exposed to a variety of different projects for training.

2 – Background

Pedigree breeding is a technique that plant-breeders employ during inbreeding of populations to develop homozygous lines with favorable traits. The plants with the favorable allele are selected for and breed in each successive generation until a homozygous line with such an allele is developed. This technique is highly used because unfavorable genotypes can be eliminated before lines are evaluated. Single-seed descent is another method to minimize genetic drift. An individual seed is taken from each plant to inbreed the population before testing; however, this method often takes longer. Still another breeding method to developing homozygous lines is to use a doubled haploid breeding scheme.

CIMMYT is looking to use molecular markers to select for haploid kernels from induced F1 plants. This would allow the researchers to save a generation in breeding. However, there are two primary obstacles to achieving this goal. The restraints include the viability of seed-chipped doubled haploid kernels over the time needed to extract DNA and analyze samples and also the ability to identify markers to differentiate between the favorable haplotype from unfavorable alleles from the population and the DH inducer allele. My project collectively is an attempt to explore the feasibility of individual haploid kernel selection through investigation of its constraints. This project has two separate components: investigating the viability of chipped haploid seeds and researching whether it is possible to differentiate between the favorable allele and the unfavorable allele from the inducer line and parents.

Through the investigation, CIMMYT will better optimize its deployment strategies of molecular markers in biofortification efforts in the Maize Quality and Nutrition Lab. Biofortification is the tool through which food crops (in this case maize) are breed to increase their micronutrients. Close to 190 million children and 19 million pregnant women suffer from vitamin A deficiencies (K. Pixley). As a result, many of these populations are suffering from morbidity and mortality. In order to combat such issues, CIMMYT's GMP team in the Maize Quality and Nutrition lab has been dedicated to the biofortification of maize with provitamin A carotenoids, which our bodies can convert into vitamin A. The lab has specifically been targeting the crtRB1 allele to increase the micronutrient density in maize kernels. Biofortification has become an important tool to prevent malnutrition throughout the world in recent years.

3 – Research

The broad objective of this study was to investigate the constraints against using markers to select for haploid kernels produced on induced F1 plants: haploid kernel viability and the ability to use molecular markers to distinguish the haplotype of interest.

The two main objectives this paper seeks to investigate are:

- To investigate the impacts on chipped haploids after several weeks of storage.

- To investigate the possibility of differentiating between haplotypes of interest from the unfavorable allele within the parent populations and the doubled haploid inducer lines.

In turn, CIMMYT will use this research to best optimize the deployment of molecular markers on large effect QTL or major genes.

3.1 Doubled Haploid Germination Trials

3.1.1 Introduction

Doubled haploid (DH) technology has become an increasingly important tool in recent years for breeders. Doubled haploid is a term given to haploid (n) cells that undergo spontaneous or artificially induced chromosome doubling. Doubled haploid lines are created through the induction of haploids through crossing of heterozygous plants with a DH inducer; identification of haploid kernels through a purple morphological marker in the endosperm and embryo; and chromosome doubling through colchicine treatment. This is a significant process because it can significantly accelerate breeding programs by reducing the amount of breeding cycles required to reach homozygosity. For example, from a heterozygous source population, a conventional inbred line would take approximately 6-8 generations to reach homozygosity while using doubled haploid technology it would take about 2-3 generations. (Prasanna et al.)

Besides the amount of time that it takes to develop a homozygous line, DH technology is also significant in that it helps to eliminate genetic drift during the inbreeding and selection process. Genetic drift can cause problems during generation testing schemes when even if a good F3 family is identified for a trait, random segregation can cause poorer performing F4 progeny. In order to avoid this, traditionally, breeders will minimize genetic drift on selection by delaying field testing until the progenies are inbred, a term that has come to be known as pedigree breeding. The drawback to using this method is that they take usually around a year longer than early generation testing. By comparison, DH technology allows breeders to have the same timeframe as early generation testing while minimizing the genetic drift. Breeders typically induce F1 because it saves a generation of selfing; hence, the timeframe is shorter and the breeder will minimize the genetic drift during inbreeding.

Further, haploids, by nature of their gene composition, offer researchers the opportunity to study mutations, gene-cytoplasmic and gene-environmental interactions (Georgiev). Each of these factors can be analyzed without influences from such factors as heterozygosity.

3.1.2 Hypotheses

The rate at which a root is growing, the germination rate, and the number of infected seeds (bacterial or fungal infection) are all factors determining a seed's viability. Heightened root growth will indicate viability while increased growth of fungus will represent decreased viability. By comparing the weekly data, determinations can be made on how the chipped seeds for several weeks will affect the viability of the maize plants. Hence, if these variables reflect weakened chipped doubled haploid seeds, then chipping will contribute to a loss of viability of doubled haploid seed in the long term.

3.1.3 Methodology

Seed chipping is a technique used commonly to extract tissue from the maize kernels. In this method, chipping scissors are taken at a 45° angle to the endosperm of the kernel. The tissue is cut into thin layers and put into plastic tubes in a 96-tube container using a funnel (**Figure 1**). Parafilm is used to cover the other tubes to prevent any "jumping" of tissue. Exactly half of two populations (Population 1 and population 2) were chipped. The seeds were

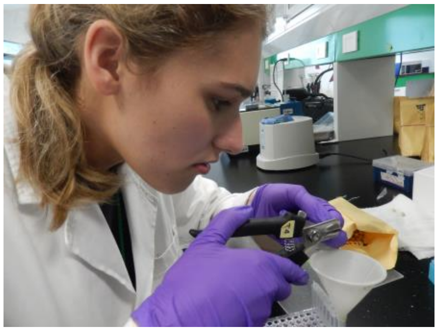


Figure 1. Seed Chipping Technique

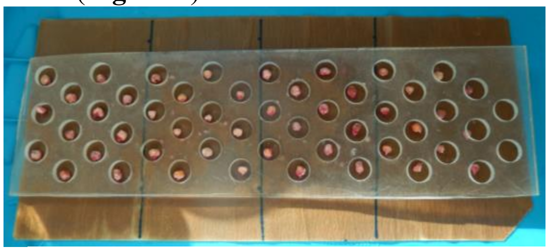


Figure 2. Formatting Bundle

separated into separate envelopes, each containing 48 seeds of chipped or 48 seeds of unchipped seeds. The envelops were then stored in a Cold Room at 4° in plastic bags.

Figure 3. Seeds on Bundle with Radicle Side Down

Figure 4. Rolled Bundle

Each week, a portion of the seeds was removed from the cold room (the amount of seeds depended on the number of bundles that needed to be run). Germination paper was cut at one of the corners and marked into four separate sections. The paper was moistened with bleach water (5 ml bleach water, 2.5 L distilled water). Two soaked germination papers were placed on top of each other aligning the cut corner. Using a temple, an equal number of seeds were placed into each marked quadrant (**Figure 2**). Seeds were placed embryo side touching the paper with the radicle side placed toward the cut end of the paper (**Figure 3**). Another sheet of soaked germination paper was placed on top of the seeds with the corners aligned. The paper was rolled from the uncut end to the cut end and tied with two rubber bands, one at each end (**Figure 4**). The rolled bundle is put with the cut ends facing down in a plastic tub with the thin layer of bleach solution. The plastic tub is wrapped in aluminum foil to prevent any sunlight from reaching the seeds. The plastic tub is placed in the incubator at 28°C. The seeds are allowed to germinate for 72 hours. After that time span elapsed, the bundles were unrolled, and measurements on root length, number of seeds infected with fungus, and number of seeds germinated were taken. The bundles were rerolled and rubber bands were placed at either end. The bundles were placed, cut side down, into the tub with bleach water. Then, the tub was placed back into the incubator at 28°C for 24 hours. At 24 hours, the bundles were taken out of the incubator and taken to the greenhouses. There the seeds were placed into Styrofoam containers with sterilized soil (**Figure 5**). The seeds were subsequently watered.

Figure 5. Seeds after Planting

Three different forms of these trials were run. The longest run trial consisted of placing untreated seeds all together. In one quadrant, chipped haploid seeds would be placed, and in the following quadrant, unchipped haploid seeds would be placed. In this way, the seeds would be alternating. For the next taco, the order was switched. Two tacos were run concurrently. The population used for these trials came from population 1 (see **Figure 6**).

Figure 6. Population 1

In another trial, all of the haploid seeds were treated with the mix to prevent fungus, shown in Appendix A. The same protocol was followed with alternating chipped vs. unchipped seeds. Two tacos of alternating order were run concurrently. The population used for these trials came from population 2 (see **Figure 7**).

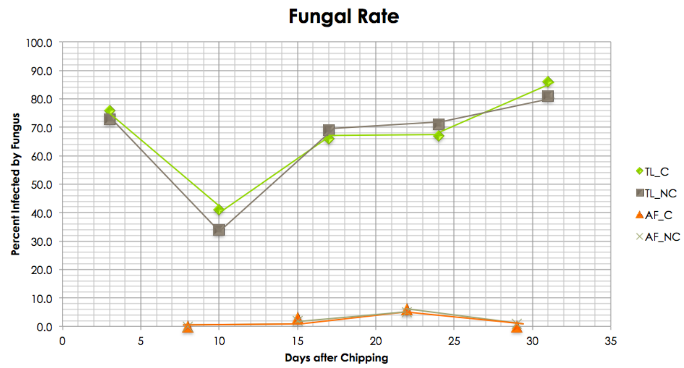
In the final experiment, the chipped and unchipped haploid seeds from the same population were mixed. Half of the population was treated while the other half remained untreated. The treated vs. untreated populations then alternated in the quadrants. Two separate populations were tested for this trial: population 1 and population 2.

Figure 7. Population 2

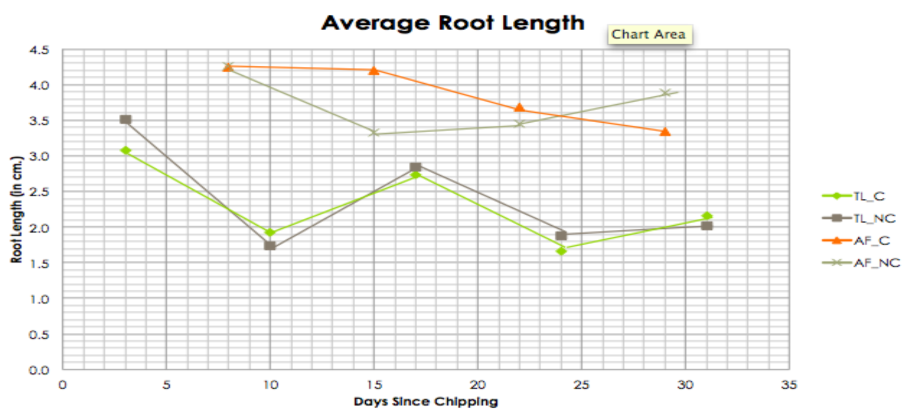
3.1.4 Results

The results of this experiment show that over the time span, chipping did not have a significant impact on both populations for the three variables tested (i.e. percent germinated, percent infected with fungus, and average root length). While the differences between the two populations were significant, it cannot be determined if this is due to the treatment or the population as these two variables are confounded. The LSMeans between the two separate populations is significantly different which explains the significance between populations. Still, when examining the LSMeans, the means between the chipped and unchipped populations within the same population are essentially the same, indicating there is no significant difference across the three factors examined that would indicate reduced viability (see **Table 1.1-1.4**). Further, biostatistical analysis shows that the days after chipping when used as a covariant is significant. This seems reasonable as each week the conditions changed slightly as I improved with the technique. Even so, when examining Graphs 1.1-1.3, it can be determined that the chipped and nonchipped populations follow closely to each other between each week. For all graphs and tables, see **3.1.5 Tables and Figures**. For the complete Biostatistical Analysis Report, see Appendix D.

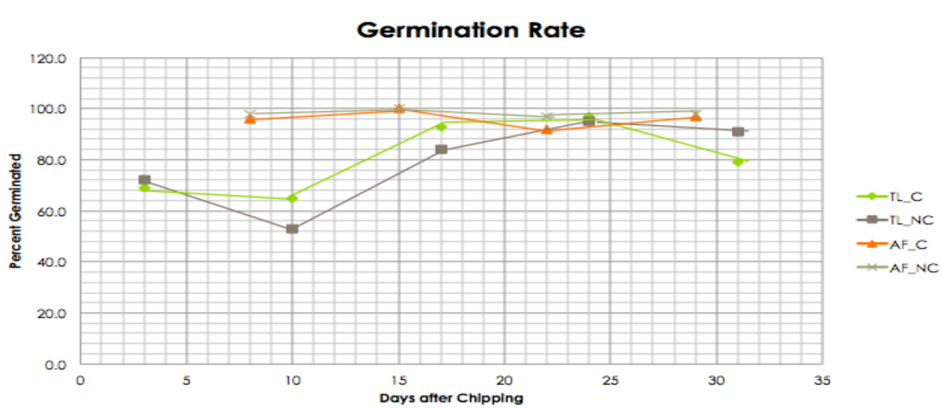
3.1.5 Tables and Figures



Graph 1.1 Percent Infected with Fungus. The green diamonds and the brown squares reflects data collected from population 1, while the orange triangles and the green x's reflect data collected from population 2. The green diamonds and the green x reflect the chipped seed, while the orange triangles and the brown squares reflect data from unchipped seeds.



Graph 1.2 Average Root Length. The green diamonds and the brown squares reflects data collected from population 1, while the orange triangles and the green x's reflect data collected from population 2. The green diamonds and the green x reflect the chipped seed, while the orange triangles and the brown squares reflect data from unchipped seeds.



Graph 1.3 Percent Germinated. The green diamonds and the brown squares reflects data collected from population 1, while the orange triangles and the green x's reflect data collected from population 2. The green diamonds and the green x reflect the chipped seed, while the orange triangles and the brown squares reflect data from unchipped seeds.

Population	Chipped	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
TL11B 6603-1	Chipped	0.80600000	0.67050000	2.31000000
TL11B 6603-1	Not chipped	0.79000000	0.65600000	2.40150000
AF13A 652-44	Chipped	0.96312500	0.02125000	3.86687500
AF13A 652-44	Not chipped	0.98375000	0.01937500	3.72687500
Combined	Chipped	0.87583333	0.38194444	3.00194444
Combined	Not chipped	0.87611111	0.37305556	2.99055556

Table 1.1 LSMeans for Three Factors. TLIIB 6603-1 reflects biostatistical analysis created from population 1 while AF13A reflect biostastical analysis created from population 2.

The GLM Procedure Dependent Variable: GERMINATION					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Population	1	0.48804110	0.48804110	32.81	<.0001
Chipped	1	0.00000139	0.00000139	0.00	0.9923
Days after Chipping	1	0.22059187	0.22059187	14.83	0.0003

Table. 1.2 GLM Procedure with Germination as the Dependent Variable.

The GLM Procedure Dependent Variable: FUNGUS					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Population	1	7.46610020	7.46610020	296.79	<.0001
Chipped	1	0.00142222	0.00142222	0.06	0.8128
Days after Chipping	1	0.13974188	0.13974188	5.56	0.0213

Table 1.3 GML Procedure with Fungus as the Dependent Variable

The GLM Procedure Dependent Variable: ROOT LENGTH					
Source	DF	Type III SS	Mean Square	F Value	Pr>F
Population	1	39.28261642	39.28261642	74.26	<.0001
Chipped	1	0.00233472	0.00233472	0.00	0.9472
Days after Chipping	1	6.64110750	6.64110750	12.56	0.0007

Table 1.4 GML Procedure with Root Length as the Dependent Variable

3.1.6 Discussion

The graphs and figures show that over a month's time, chipping largely does not have a significant difference. Collectively, both chipped and unchipped seeds of the same population experienced approximately the same LSMeans, indicating that on the whole, there is no significant difference. This can also can be seen in **Table 1.5** where the P values indicate there is no significant difference. Days after Chipping and Population both are significant. This seems reasonable as technique improved over the long term. Also, population 2 (AF13A 652-44) on the whole proved to be more vigorous than population 1 (TL11B 6603-1). This can be seen when comparing the LSMeans of each population. This explains the significance factor. Population 2 appeared to remain more constant over the timespan, but it cannot be determined if this is due to treatment or population. Additional trials need to be run to confirm this.

Further, in **Graph 1.3**, there appears to be a start of a divergence between germination percent among chipped versus nonchipped seeds in the TL11B 6603-1 population. More time and trials need to be run to confirm if this is significant in that the seeds will have reduced viability or if the result is insignificant.

On the whole, the results prove that over a month's time, there is no significant difference in haploid seed viability. Hence, in using the F1 deployment model, this does not pose a significant problem. The seeds remain viable even if they are chipped.

3.2 Molecular Marker Assisted Selection

3.2.1 Introduction

CIMMYT is attempting to develop a marker-assisted-selection strategy known as forward breeding native trait alleles. In this process, molecular markers are employed to select progenies that carry a particular favorable allele. For example, in the Maize Quality and Nutrition Department at CIMMYT, researchers are investigating β-carotene hydroxylase 1 (crtRB1), which is involved in the presence of provitamin-A compounds. This allele is considered favorable because activation will result in the accumulation of provitamin-A in the kernels. The prevalence of this gene in the kernels will allow for the conversion to vitamin A in the human body, reducing the prevalence of vitamin A deficiency. In order to ensure that all of the kernels contain the favorable gene, the progenies need to be fixed in a homozygous state.

There are two methods to best accomplish this fixed state. The first method is to take samples from the leaf tissues of F2 maize plants and analyze the DNA of each plant to determine the homozygosity. The plants that are homozygous for the favorable allele such as crtRB1 would be self-pollinated for the F3 plants. An alternate method is to use a method known as seed chipping, whereby slices of the endosperm are removed for DNA analysis. F2 maize kernels are chipped to determine the homozygosity. Those that are homozygous for the favorable allele can be grown out and self-pollinated. They also can be crossed with a DH inducer line. Both methods will produce plants that are homozygous for the selected trait.

3.2.2 Hypotheses

If using molecular markers, polymorphism will be detected between two populations: one with allele y1 (white seed color) and one with allele Y1 (yellow seed color). If the difference in alleles can successfully be distinguished using molecular markers, individual haploid kernel selection using molecular markers will be feasible.

3.2.3 Methodology

The first step was to identify a locus of interest. In my case, a haplotype that could be visually distinguished was chosen: the allele named y1 that causes a maize kernel to be white versus yellow. Because this haplotype was known to be on chromosome 6, SSR markers were identified that were adjoining to the y1 region. Using the primers adjoining the locus of interest, a test was designed to examine polymorphism between two of the parents of my population: CML 488 (a white parent) and CML 327 (a yellow parent). The template for the DNA was as follows:

	1	2	3	4	5	6
A	CML 488		CML 297		CML 327	
B	CML 488		CML 495		CML327	

Table 1.5. Template for DNA for determining polymorphism between two parental populations

CML 297 and CML 495 were used as controls. Then, a master mix was prepared (see Appendix C). The PCR was conducted with four separate programs to correspond with different melting temperatures (TM) levels. The programs that were run consisted of: SSR52, SSR55, SSR60, and SSR65. Finally, the samples were each run on 3-4% agarose gels. After staining with ethidium bromide solution, pictures were taken using a UV light Photodoc system. The PCRs were examined for those that showed polymorphism between the two parent populations. In total, approximately 17 polymorphic markers were identified along chromosome 6 (see **Figure 6 and 7** for results). From there, the DH inducer line, Tail 9, was also included. The template for the DNA was as follows:

	1	2	3	4	5	6
A	CML 488		TAIL 9		CML 327	
B	CML 488		TAIL 9		CML327	

Table 1.6. Template for DNA for fingerprinting including DH Inducer line, Tail 9.

PCR programs consisted of: SSR52, SSR55, SSR60, and SSR65. The samples were each run on 3-4% agarose gels. After staining with ethidium bromide solution, pictures were taken using a UV light Photodoc system. Finally, markers were identified that were 20 cM of y1. These included: bnlg1188, bnlg1538, y1ssr, and umc1006. It was identified that the best results came from the program SSR55.

After chipping a total of 80 seeds, the DNA was isolated from 20 seeds of each phenotype: yellow haploid seeds and white haploid seeds after removing tissue from the endosperm of each seed using the seed chipping method (see Appendix B for protocol). The seeds came from population 2 (AF13A 652-44). Using the stock DNA concentration, a PCR plate was prepared using 3 µl of DNA in this order:

	1	2	3	4	5	6	7	8	9	10	11	12
A	CML488	Y1	Y3	Y5	Y7	Y9	TAIL 9	W1	W3	W5	W7	W9
B	CML327	Y2	Y4	Y6	Y8	Y10	H_20	W2	W4	W6	W8	W10
C	CML488	Y11	Y13	Y15	Y17	Y19	TAIL 9	W11	W13	W15	W17	W19
D	CML327	Y12	Y14	Y16	Y18	Y20	H_20	W12	W14	W16	W18	W20

***Y denotes seeds with a yellow phenotypic color. W denotes weeds with a white phenotypic color.
***TAIL 9 is the DNA from the DH inducer line that represents the third parent.

Table 1.7. Template used for DNA extraction

The Master Mix prepared had the following reagents:
SIGMA water - 3.55 µl
Green Buffer 5x [1x] - 3.0 µl
$MgCl_2$ 1.2 µl - [2 mM]
dNTPs 1.2 µl - [2.5 µM]
Forward & Reverse primer [0.2 µM] - 3 µl
Promega Taq [0.5 units/µl] - 0.05 µl

The plate was run on SSR55, and the amplified solutions were run on polyacrylamide gels (See **Figure 6**). The results were then optimized. The modifications to the master mix are as follows:

SIGMA water - 3.85 µl
Green Buffer 5x [1x] - 3.0 µl
$MgCl_2$ [2 mM] - 0.9 µl
dNTPs [2.5 µM] - 1.2 µl
Forward & Reverse primer [0.2 µM] - 3 µl
Promega Taq [0.5 units/microliter] - 0.05 µl

Three microliters of the stock DNA of the samples were amplified with the PCR program of SSR55. Finally, the amplified solution was run on polyacrylamide gels. For results on each of the gels, see the **3.2.4 Results** section.

3.2.4 Results

The results of the CML 488/CML 495/CML 297/CML 327 gels demonstrate that there is polymorphism in some markers along chromosome 6 as seen in **Figure 7-9** (labeled yellow). Many of the optimized results similarly demonstrate polymorphism (see **Figure 10-13**). Out of the four markers that were within 20 cM of the Y1 locus (bnlg1188, bnlg1538, y1ssr, and umc1006), only three showed polymorphism. Y1SSR and BNLG1188 were run on polyacrylamide gels as seen in **Figure 14-16**. In **Figure 15**, the optimized PAGE results show polymorphism between the samples and the parents. The white population has bands that ran the same bp length as that of CML 488 (white parent) and Tail 9 (inducer line) while the yellow population has bands that ran the same bp length as CML 327 (yellow parent) and Tail 9 (inducer line). The differences between these lengths can be easily differentiated.

3.2.4 Tables and Figures

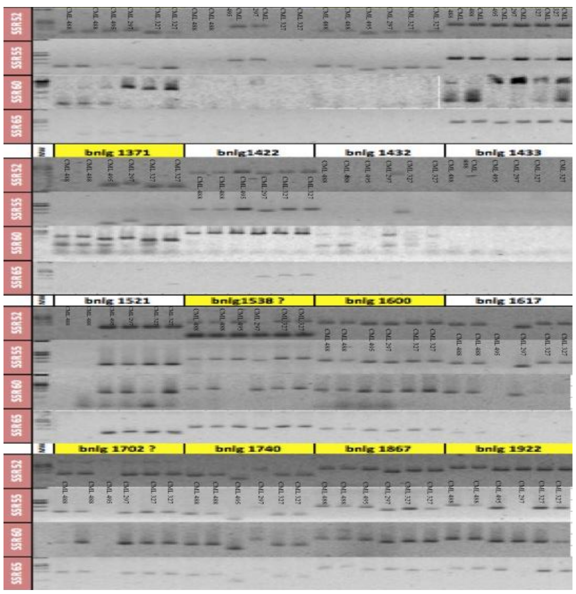


Figure 7. Agarose Gel Pictures with SSR Primers under Chromosome 6.

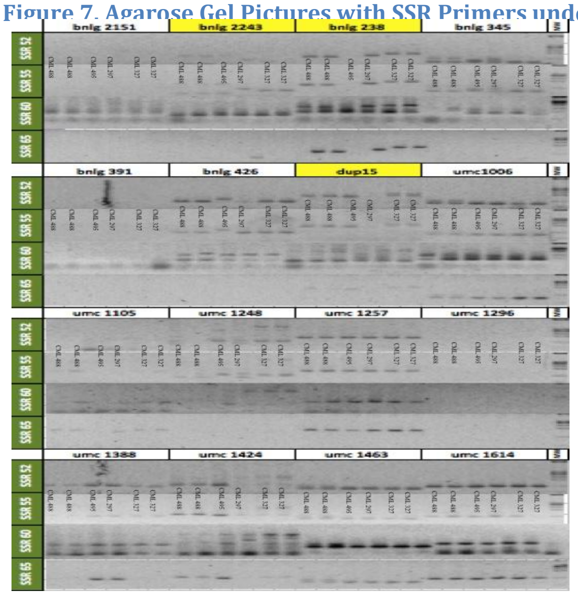


Figure 8. Agarose Gel Pictures with SSR Primers under Chromosome 6.

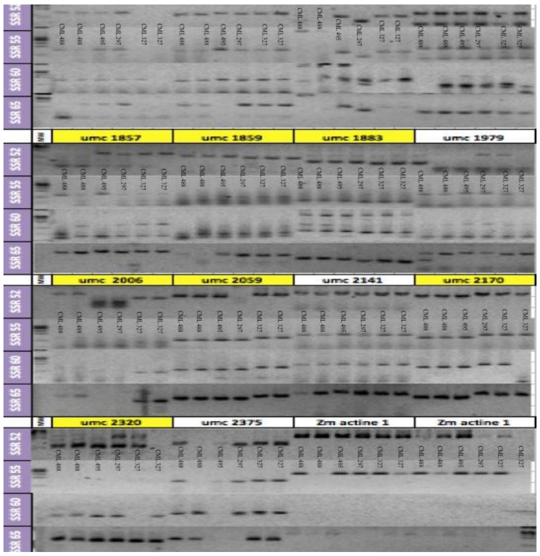


Figure 9. Agarose Gel Pictures with SSR Primers under Chromosome 6.

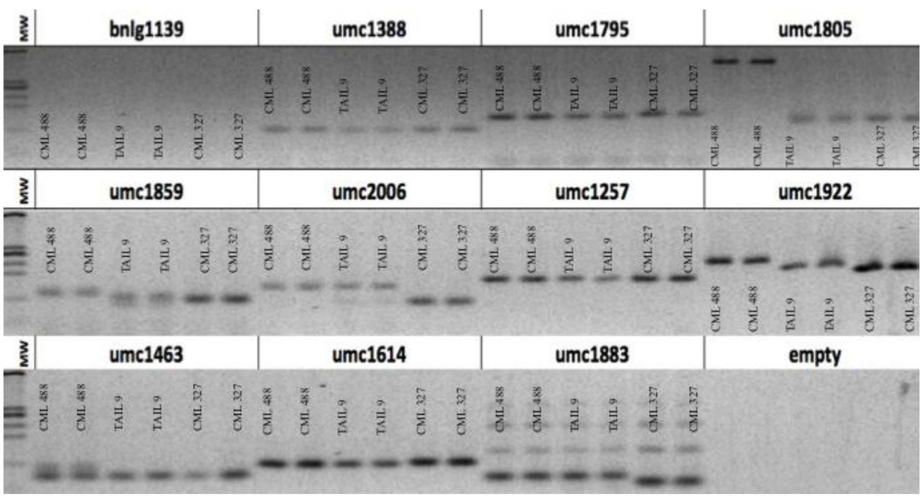


Figure 10. SSR 52 FINGERPRINTING CML 488/TAIL 9/CML 327

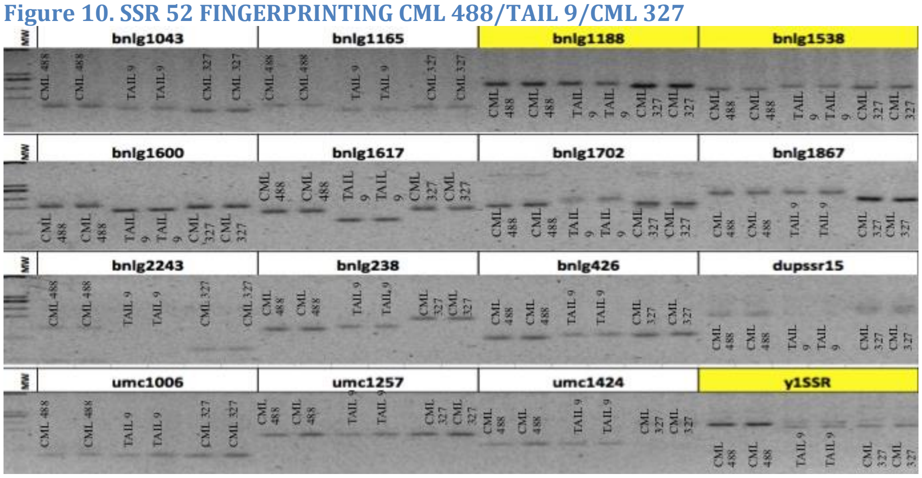


Figure 11. SSR 55 Fingerprinting CML 488/Tail 9/CML 327

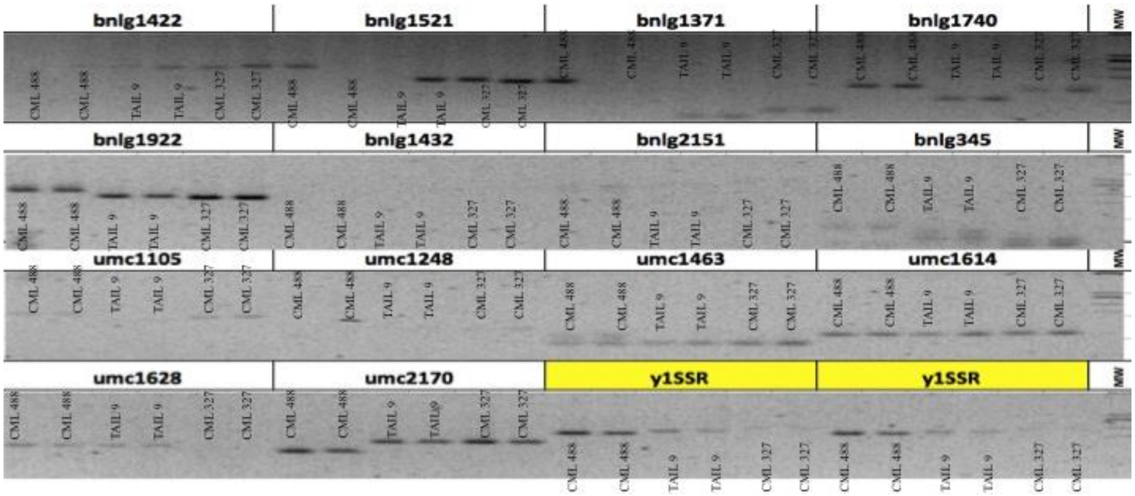


Figure 12. SSR 60 FINGERPRINTING CML 488/ TAIL 9/ CML 327

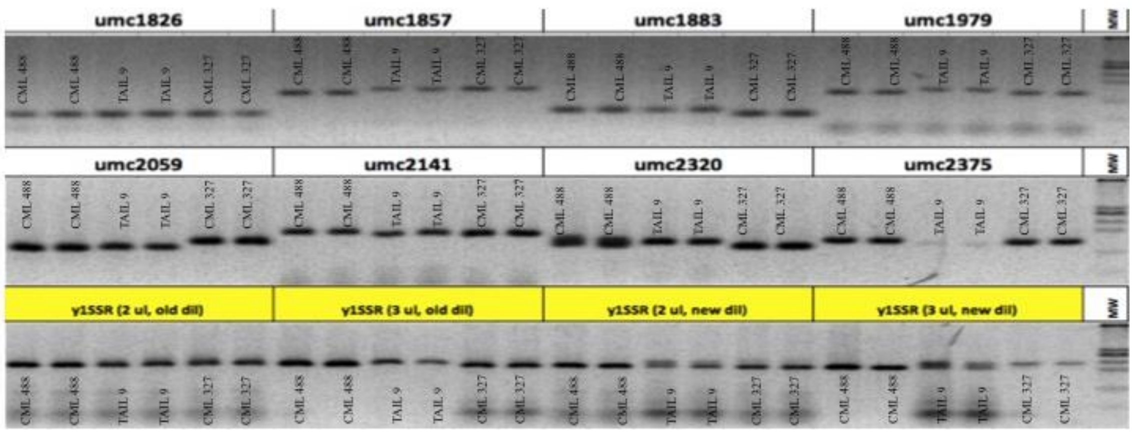


Figure 13. SSR 65 Fingerprinting CML 488/Tail 9/CML 327

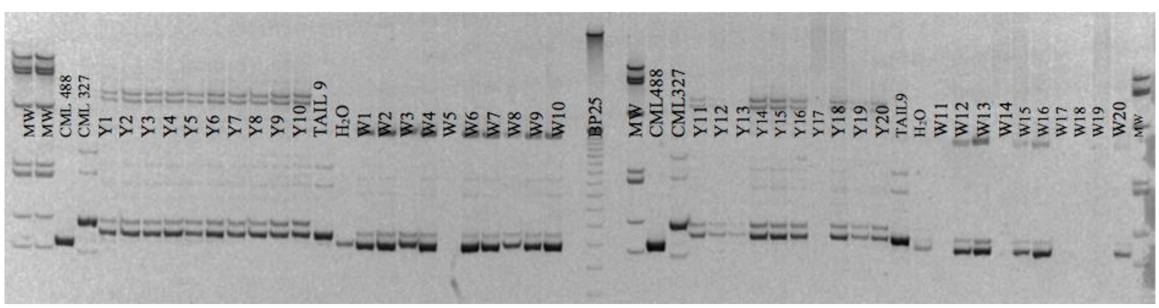


Figure 14. PAGE results with Y1SSR under SSR55

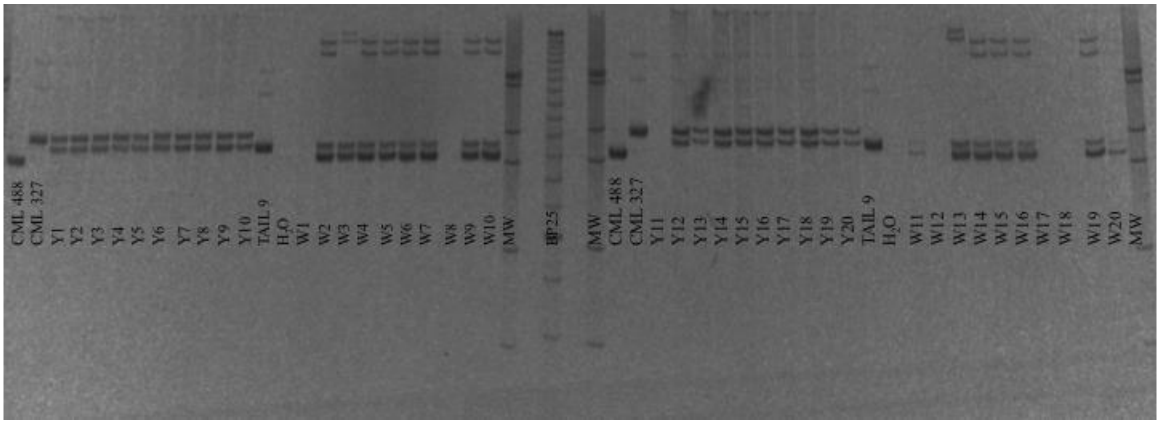


Figure 15. Optimized PAGE results with Y1SSR under SSR60

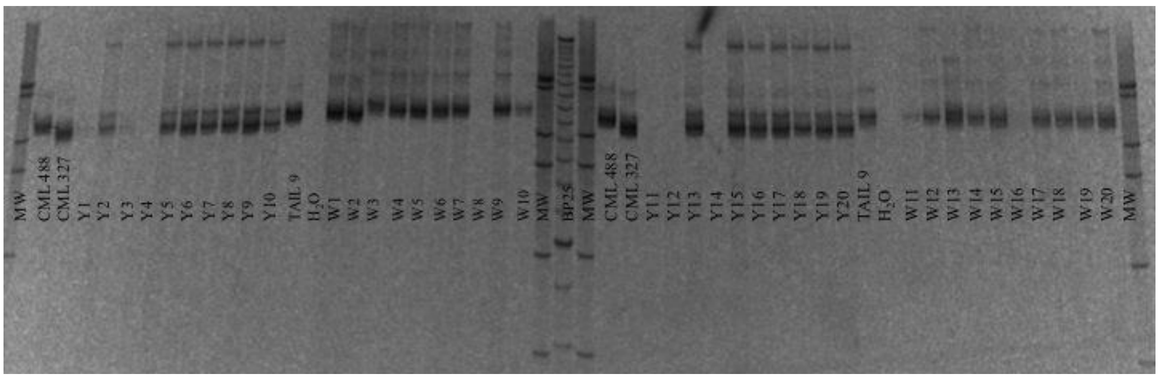


Figure 16. PAGE Results with BNLG1188 under SSR60

3.2.5 Discussion

In **Figure 15** and **Figure 16**, it can be observed that each of the samples has two bands. In both the white and the yellow samples, the bands have a bp length that is the same as Tail 9 (the inducer line). In the white population, the samples also have a band that has the same bp length as CML 488 (the white parent). While in the yellow population, the samples have a band that has the same bp length as CML 327 (the yellow parent). Hence, differentiation between alleles of the parents and the inducer line can be observed, and individual haploid kernel selection using molecular markers can be feasible. Molecular markers can help to select for haploids that contain the favorable allele because it is possible to differentiate between the unfavorable alleles of the parental populations and the inducer lines.

4 – Regional and Cultural Experiences

My experiences in Mexico were nothing short of spectacular. CIMMYT became more than just a summer internship to me. Through the Borlaug-Ruan Internship, not only was I able to experience another culture, but I was also able to learn more about my own culture and how it is different. I have learned a countless number of new things about agriculture, the Spanish language, and the Mexican culture. The experiences and the people that I have met here have defined my experience while in Mexico.

CIMMYT is a research institution comprised of an international faculty. I had the opportunity to constantly interact with people from all around the world. On my first day here, I met people with nationalities from Mexico, Belgium, Peru, Germany, the Philippines, Thailand, and South Africa. Consequently, I found myself constantly adjusting. By the end of my internship, I had adjusted to the fact that if you see someone that you know while walking, they will stop to talk and catch up with you despite their busy schedule. Most of all, I have come to know and appreciate the sense of community within CIMMYT. Everyone seemed to go out of their way to assist me in any way they see fit.

Being able to understand the Spanish language has made my experiences here all the more memorable as I was able to interact with the locals and understand the culture all-the-more. I had the opportunity to go out into the markets on the weekends and speak with vendors and other locals. The opportunity to communicate with members of the community has been a memorable experience for me. I have been able to understand the community more because of this ability. It has culminated in a wide variety of new knowledge about Mexico and the Mexican people.

More surprisingly, I was shocked to find how many people are able to speak English here. Many people inform me that it is necessary in order to write their research papers. Still, having taken four years of Spanish, I know that my language skills still have not become as fluid as some people who have only taken two years of English. I have always heard that English is the international language, and CIMMYT seems to embody this idea. With such an international faculty, I was never surprised to hear Spanish or English conversations going on among many people of all different nationalities.

Throughout my time in Mexico, I had the privilege of seeing both the modern aspects of Mexico as well as the more rural aspects. Both were entirely beautiful in their own way. The juxtaposition between the ancient, the colonial, and the modern parts of Mexico are all embodied in urban metropolis of Mexico City. Most evidently I saw how each of the different cultures built upon each other when I visited Zócalo, the main square in the Historical District of Mexico City. There, Templo Mayor, the major pyramid of the Aztecs, once stood. When the Spanish came, they used many of the stones to build the buildings such as the Palacio Nacional and the Catedral Metropolitana. Further off into the distance, the modern aspects of shopping malls and restaurants can be seen. The juxtaposition of all three times is rare to find, and it is nothing short of spectacular. This combination occurred again when I visited Cholula where there was a church built on top of an ancient pyramid. I saw the clashing of different times all throughout my visits in Mexico, and it was nothing short of spectacular.

Besides the modern aspects of Mexico, I also had the privilege of traveling to the more rural aspects of Mexico. I travelled to the state of Hidalgo where once again I was able to see the striking differences between the urbanization of the “Magic Towns” and the bucolic beauty of the landscape. The same occurred when I travelled to Agua Fría. I saw many people trying to sell fruits out of their home, barely making it by; yet, nearby, larger cities arose from the jungle. It made me contemplate that just a few kilometers away from this abject poverty there was a sprawling city. Ultimately, these experiences pushed me all the harder in my work. I often reminiscence on how one of my colleagues responded to one of my questions regarding why he went into this field. He said, “I went into this field because it really matters. People really need us to do this.” I could not agree more.

Personal Reflection

From the busy streets of Texcoco to the bucolic beauties of Agua Fría, the Borlaug-Ruan Internship at CIMMYT Mexico has been an utterly transformative experience. Being around such impassioned people has encouraged me to try, in whatever way possible, to make a difference.

During my experience here, I not only learned about what it means to be a farmer in Mexico, but I learned about the tedious agricultural processes of pollination and the careful techniques to take care of maize doubled haploid plants. I was immersed in a language in which I knew nothing about: agriculture. Agriculture does not just have to do with planting the crop, giving it water, and watching it grow; rather, agriculture is about making sure the people all around the world have sufficient food of adequate nutritional quality to ensure they can live healthy, happy lives. Through these experiences, my views on food security have also been completely altered. Agriculture is much bigger than going to the grocery store to by flour or stopping on the side of the road to by corn from a local farmer. The dedicated CIMMYT scientists have proven and taught me that agriculture has a much broader meaning than the average American consumer realizes, that I realized.

My most notable experience here occurred after I presented my findings to my mentors. I have never seen such excitement for what was to come. All of the hard work and dedication that I had put into getting the results was instantly worth it. With the help of researchers at CIMMYT, provitamin A can be and will be introduced into maize plants, which will help to combat VAD and malnutrition. I was similarly inspired by the abject poverty of some here in Mexico. Lack of food and sanitation poses a major problem for some people. Ensuring that these people get the proper materials to live healthy lives will continue to pose a problem in coming years. I am humbled as to have been part of an effort to combat issues of malnutrition and food instability while here in Mexico.

Above all, my journeys here in Mexico have forced me to realize that it is our duty, our responsibility as a global, national, and local community to ensure that the people who do not have food of adequate nutritional quality receive the help they need to live healthy, happy lives. It is our obligation as citizens of the world to fight against this universal problem of hunger and malnourishment. Consequently, it is paramount that we learn about these issues and learn how to treat them in sustainable methods. Together, we must unite to fight against the global issues of food insecurity; we cannot do it alone.

Citations:

B.M. Prasanna, Vijay Chaikam and George Mahuku (eds). 2012. Doubled Haploid Technology in Maize Breeding: Theory and Practice. Mexico, D.F.: CIMMYT.

Babu et al. 2013. Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. Theoretical and Applied Genetics: International Journal of Plant Breeding Research. 126:381-399.

"CIMMYT and Mexico." 1985. The International Maize and Wheat Improvement Center.

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Georgiev, S. 2008. Haploids in Genetic and Cytogenetical Research. *Biotechnology and Biotechnological Equipment*. 644-651.

Appendix A

Chemical treatment
38 mL Maximxl
4 L Semerin 350
4 mL Apron XL
200 g Captan
50 mL Adherente (pegamento)
tinte (rojo)
900 mL agua

Appendix B

DNA ISOLATION FROM ENDOSPERM TISSUE
(in microtubes Neptune of 1.1 ml)

Tissue collection and grinding

1. Identify the microtubes and plates. Follow the same id format to make a correct locate of the seeds.
2. Collect 20 mg of endosperm tissue in small pieces. Use a scissor to cut nails of dogs and a fitted funnel to facilitate the sampling. Avoid touching the embryo or fracturing the rest of the seed.
3. Grind to a fine powder with a ball of 5/32" (Open Diagnostics GBSS 156-500-01) per tube. Use 2-3 min for 30 1/s of frequency in the Tissuelyser (Quiagen) or 1500 rpm for 3 min in Geno/Grinder 2010 (Spex Sample Prep) machine.
4. Keep the samples hermetically capped until the extraction.

DNA isolation

1. Add 400 ul of warm CTAB-lauril sarcosyl buffer (warmed at 65^{o}C)[1]. Secure the plates with a lid fixed with rubber bands to prevent leakage. Mix by inversion.
2. Incubate the samples for 90 min at RT with continuous gentle rocking. Check the plates often to detect some leakage.
3. Give a brief spin. Add 400 ul of phenol:chloroform (1:1)[2]. Again secure the plates with the lid and the rubber bands. Mix well. Incubate the samples under stirring for 15 min at RT.
4. Centrifuge samples at 3500 rpm for 15-20 min at RT.
5. Transfer 400 ul of aqueous layer in new tubes.
6. Precipitate DNA adding 340 ul (1:1 ratio based on the amount of solution in the tubes) of cold isopropanol. Mix gentle by inversion until see the "fiber" of DNA. Incubate at -20^{o}C for 60 min (you can leave the samples in this step overnight).
7. Centrifuge at 3500 rpm for 30 min at 4^{o}C. Pour off the isopropanol.
8. Add 400 ul of 70% ethanol. Centrifuge at 3500 rpm for 15 min at 4^{o}C. Pour off the ethanol. Do this step thrice.
9. Evaporate remaining ethanol leaving the samples in a laminar hood overnight.
10. Dilute the DNA in 100 ul of TE pH 8.0 or water (Sigma W3500).

CTAB-lauril sarcosyl buffer[1] to isolate DNA using microtubes of 1.1 ml

SOL. CONC.	[FINAL]	25 rxn 10 ml	250 rxn 100 ml	500 rxn 200 ml	750 rxn 300 ml
dH20		5.6 ml	56.0 ml	112.0 ml	168.0 ml
1 M Tris-7.5	100 mM	1.0 ml	10.0 ml	20.0 ml	30.0 ml
5 M NaCl	700 mM	1.4 ml	14.0 ml	28.0 ml	42.0 ml
0.5 M EDTA-8.0	50 mM	1.0 ml	10.0 ml	20.0 ml	30.0 ml
CTAB	1 %	0.1 gr	1.0 gr	2.0 gr	3.0 gr
10% Sarcosyl[3]	1 %	1.0 ml	10.0 ml	20.0 ml	30.0 ml

[1] Use a fresh buffer every time. Before adding CTAB (Sigma M-7635) and lauril sarcosyl (Sigma L5125), heat the buffer until 65 ^{o}C. You can prepare the buffer one day before to DNA isolation, but do add neither CTAB nor sarcosyl.

[2] Take the phenol (Sigma P4557) without touching the Tris layer. Mix it vigorously with the chloroform (Baker 9180) before add to the samples. Do not use the phenol if it has a rose color.

4 Sterilize by filtration the 10% lauril sarcosyl solution before aliquote.

Appendix C

MASTERMIX USED FOR FINGERPRINTING
SIGMA water 6.4
10x 1.5
dNTPs 1 [2.5 µM]
MgCl2 0.8 [50 mM]
F + R primers 3 [1 µM]
Taq 0.3 [5 units/microliter]
DNA 2 [40 ng/ml]

Appendix D

```
                    WITHOUT DAC AS COVARIABLE       11:03 Monday, July 29, 2013

                        The GLM Procedure

                     Class Level Information
          Class        Levels    Values
          POP               2    A T
          TRIAL            10    1 2 3 4 5 6 7 8 9 10
          CHIPPED           2    C NC

             Number of Observations Read          72
             Number of Observations Used          72

                    WITHOUT DAC AS COVARIABLE       11:03 Monday, July 29, 2013
                        The GLM Procedure
Dependent Variable: GERM
                                     Sum of
        Source                DF        Squares     Mean Square    F Value    Pr > F

        Model                  1     0.00000139      0.00000139       0.00    0.9941

        Error                 70     1.77933056      0.02541901

        Corrected Total       71     1.77933194

                  R-Square     Coeff Var      Root MSE     GERM Mean
```

0.000001	18.20074	0.159433	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	1.3888889E-6	1.3888889E-6	0.00	0.9941

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	1.3888889E-6	1.3888889E-6	0.00	0.9941

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013

The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00142222	0.00142222	0.01	0.9174
Error	70	9.19912778	0.13141611		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.000155	96.03009	0.362514	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9174

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9174

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00233472	0.00233472	0.00	0.9640
Error	70	79.53155278	1.13616504		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.000029	35.57482	1.065910	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9640

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9640

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013

The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN

C	0.87583333	0.38194444	3.00194444
NC	0.87611111	0.37305556	2.99055556

The GLM Procedure

Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.27972223	0.13986111	6.44	0.0027
Error	69	1.49960971	0.02173347		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.157206	16.82962	0.147423	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00000139	0.00000139	0.00	0.9936
DAC	1	0.27972084	0.27972084	12.87	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00000139	0.00000139	0.00	0.9936
DAC	1	0.27972084	0.27972084	12.87	0.0006

The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02383952	0.01191976	0.09	0.9144
Error	69	9.17671048	0.13299580		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.002591	96.60554	0.364686	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9179
DAC	1	0.02241730	0.02241730	0.17	0.6827

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9179
DAC	1	0.02241730	0.02241730	0.17	0.6827

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.28244830	2.14122415	1.96	0.1482
Error	69	75.25143920	1.09060057		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean

0.053844	34.85418	1.044318	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9632
DAC	1	4.28011358	4.28011358	3.92	0.0516

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9632
DAC	1	4.28011358	4.28011358	3.92	0.0516

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013

The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.87583333	0.38194444	3.00194444
NC	0.87611111	0.37305556	2.99055556

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013

The GLM Procedure
Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.54717146	0.27358573	15.32	<.0001
Error	69	1.23216049	0.01785740		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.307515	15.25523	0.133632	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	30.64	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9930

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	30.64	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9930

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	7.35019785	3.67509892	137.05	<.0001
Error	69	1.85035215	0.02681670		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.798887	43.37962	0.163758	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	274.04	<.0001
CHIPPED	1	0.00142222	0.00142222	0.05	0.8185

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	274.04	<.0001
CHIPPED	1	0.00142222	0.00142222	0.05	0.8185

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	36.92395722	18.46197861	29.90	<.0001
Error	69	42.60993028	0.61753522		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.464254	26.22725	0.785834	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	59.79	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9511

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	59.79	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9511

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.88557986	0.34622569	3.08200694
NC	0.88585764	0.33733681	3.07061806

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure
Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure
Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.76776333	0.25592111	17.20	<.0001
Error	68	1.01156861	0.01487601		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.431490	13.92364	0.121967	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	36.78	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9923
DAC	1	0.22059187	0.22059187	14.83	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	0.48804110	0.48804110	32.81	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9923
DAC	1	0.22059187	0.22059187	14.83	0.0003

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013

The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7.48993972	2.49664657	99.25	<.0001
Error	68	1.71061028	0.02515603		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.814075	42.01498	0.158607	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	292.13	<.0001
CHIPPED	1	0.00142222	0.00142222	0.06	0.8128
DAC	1	0.13974188	0.13974188	5.56	0.0213

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	7.46610020	7.46610020	296.79	<.0001
CHIPPED	1	0.00142222	0.00142222	0.06	0.8128
DAC	1	0.13974188	0.13974188	5.56	0.0213

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	43.56506472	14.52168824	27.45	<.0001
Error	68	35.96882278	0.52895328		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.547755	24.27340	0.727292	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	69.80	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9472
DAC	1	6.64110750	6.64110750	12.56	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	39.28261642	39.28261642	74.26	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9472
DAC	1	6.64110750	6.64110750	12.56	0.0007

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013

The GLM Procedure

Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.88506944	0.34581944	3.08480754
NC	0.88534722	0.33693056	3.07341865

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013

The GLM Procedure

Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read 72
Number of Observations Used 72

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00000139	0.00000139	0.00	0.9941
Error	70	1.77933056	0.02541901		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.000001	18.20074	0.159433	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	1.3888889E-6	1.3888889E-6	0.00	0.9941

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	1.3888889E-6	1.3888889E-6	0.00	0.9941

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00142222	0.00142222	0.01	0.9174
Error	70	9.19912778	0.13141611		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.000155	96.03009	0.362514	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9174

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9174

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00233472	0.00233472	0.00	0.9640
Error	70	79.53155278	1.13616504		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.000029	35.57482	1.065910	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9640

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9640

WITHOUT DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN

C	0.87583333	0.38194444	3.00194444
NC	0.87611111	0.37305556	2.99055556

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure
Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.27972223	0.13986111	6.44	0.0027
Error	69	1.49960971	0.02173347		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.157206	16.82962	0.147423	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00000139	0.00000139	0.00	0.9936
DAC	1	0.27972084	0.27972084	12.87	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00000139	0.00000139	0.00	0.9936
DAC	1	0.27972084	0.27972084	12.87	0.0006

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02383952	0.01191976	0.09	0.9144
Error	69	9.17671048	0.13299580		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.002591	96.60554	0.364686	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9179
DAC	1	0.02241730	0.02241730	0.17	0.6827

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00142222	0.00142222	0.01	0.9179
DAC	1	0.02241730	0.02241730	0.17	0.6827

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.28244830	2.14122415	1.96	0.1482
Error	69	75.25143920	1.09060057		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean

0.053844	34.85418	1.044318	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9632
DAC	1	4.28011358	4.28011358	3.92	0.0516

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00233472	0.00233472	0.00	0.9632
DAC	1	4.28011358	4.28011358	3.92	0.0516

WITH DAC AS COVARIABLE 11:03 Monday, July 29, 2013
The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.87583333	0.38194444	3.00194444
NC	0.87611111	0.37305556	2.99055556

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.54717146	0.27358573	15.32	<.0001
Error	69	1.23216049	0.01785740		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.307515	15.25523	0.133632	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	30.64	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9930

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	30.64	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9930

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	7.35019785	3.67509892	137.05	<.0001
Error	69	1.85035215	0.02681670		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.798887	43.37962	0.163758	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	274.04	<.0001

CHIPPED	1	0.00142222	0.00142222	0.05	0.8185

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	274.04	<.0001
CHIPPED	1	0.00142222	0.00142222	0.05	0.8185

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	36.92395722	18.46197861	29.90	<.0001
Error	69	42.60993028	0.61753522		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.464254	26.22725	0.785834	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	59.79	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9511

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	59.79	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9511

WITHOUT DAC AS COVARIABLE + POP 11:03 Monday, July 29, 2013
The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.88557986	0.34622569	3.08200694
NC	0.88585764	0.33733681	3.07061806

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure
Class Level Information

Class	Levels	Values
POP	2	A T
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read	72
Number of Observations Used	72

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.76776333	0.25592111	17.20	<.0001
Error	68	1.01156861	0.01487601		
Corrected Total	71	1.77933194			

R-Square	Coeff Var	Root MSE	GERM Mean
0.431490	13.92364	0.121967	0.875972

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	0.54717007	0.54717007	36.78	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9923
DAC	1	0.22059187	0.22059187	14.83	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F

POP	1	0.48804110	0.48804110	32.81	<.0001
CHIPPED	1	0.00000139	0.00000139	0.00	0.9923
DAC	1	0.22059187	0.22059187	14.83	0.0003

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7.48993972	2.49664657	99.25	<.0001
Error	68	1.71061028	0.02515603		
Corrected Total	71	9.20055000			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.814075	42.01498	0.158607	0.377500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	7.34877562	7.34877562	292.13	<.0001
CHIPPED	1	0.00142222	0.00142222	0.06	0.8128
DAC	1	0.13974188	0.13974188	5.56	0.0213

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	7.46610020	7.46610020	296.79	<.0001
CHIPPED	1	0.00142222	0.00142222	0.06	0.8128
DAC	1	0.13974188	0.13974188	5.56	0.0213

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	43.56506472	14.52168824	27.45	<.0001
Error	68	35.96882278	0.52895328		
Corrected Total	71	79.53388750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.547755	24.27340	0.727292	2.996250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
POP	1	36.92162250	36.92162250	69.80	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9472
DAC	1	6.64110750	6.64110750	12.56	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
POP	1	39.28261642	39.28261642	74.26	<.0001
CHIPPED	1	0.00233472	0.00233472	0.00	0.9472
DAC	1	6.64110750	6.64110750	12.56	0.0007

WITH DAC AS COVARIABLE +POP 11:03 Monday, July 29, 2013
The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.88506944	0.34581944	3.08480754
NC	0.88534722	0.33693056	3.07341865

WITHOUT DAC AS COVARIABLE BY POP 11:03 Monday, July 29, 2013

--- POP=A ---

The GLM Procedure
Class Level Information

Class	Levels	Values
TRIAL	8	1 2 3 4 5 6 7 8

CHIPPED 2 C NC

Number of Observations Read 32
Number of Observations Used 32

--- POP=A ---

The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00340313	0.00340313	1.91	0.1766
Error	30	0.05331875	0.00177729		
Corrected Total	31	0.05672187			

R-Square	Coeff Var	Root MSE	GERM Mean
0.059997	4.330831	0.042158	0.973438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00340313	0.00340313	1.91	0.1766

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00340313	0.00340313	1.91	0.1766

--- POP=A ---

The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00002812	0.00002812	0.02	0.8959
Error	30	0.04846875	0.00161563		
Corrected Total	31	0.04849688			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.000580	197.8823	0.040195	0.020313

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00002812	0.00002812	0.02	0.8959

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00002813	0.00002813	0.02	0.8959

--- POP=A ---

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.15680000	0.15680000	0.36	0.5544
Error	30	13.15748750	0.43858292		
Corrected Total	31	13.31428750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.011777	17.44213	0.662256	3.796875

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.15680000	0.15680000	0.36	0.5544

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.15680000	0.15680000	0.36	0.5544

The GLM Procedure
Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.96312500	0.02125000	3.86687500
NC	0.98375000	0.01937500	3.72687500

The GLM Procedure
Class Level Information

Class	Levels	Values
TRIAL	10	1 2 3 4 5 6 7 8 9 10
CHIPPED	2	C NC

Number of Observations Read 40
Number of Observations Used 40

The GLM Procedure
Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00256000	0.00256000	0.08	0.7749
Error	38	1.17288000	0.03086526		
Corrected Total	39	1.17544000			

R-Square	Coeff Var	Root MSE	GERM Mean
0.002178	22.01568	0.175685	0.798000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00256000	0.00256000	0.08	0.7749

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00256000	0.00256000	0.08	0.7749

The GLM Procedure
Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00210250	0.00210250	0.04	0.8343
Error	38	1.80117500	0.04739934		
Corrected Total	39	1.80327750			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.001166	32.82531	0.217714	0.663250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00210250	0.00210250	0.04	0.8343

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00210250	0.00210250	0.04	0.8343

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.08372250	0.08372250	0.11	0.7432
Error	38	29.21425500	0.76879618		
Corrected Total	39	29.29797750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.002858	37.22000	0.876810	2.355750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.08372250	0.08372250	0.11	0.7432

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.08372250	0.08372250	0.11	0.7432

WITHOUT DAC AS COVARIABLE BY POP 11:03 Monday, July 29, 2013

-- POP=T --

The GLM Procedure

Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.80600000	0.67050000	2.31000000
NC	0.79000000	0.65600000	2.40150000

WITH DAC AS COVARIABLE BY POP 11:03 Monday, July 29, 2013

-- POP=A --

The GLM Procedure

Class Level Information

Class	Levels	Values
TRIAL	8	1 2 3 4 5 6 7 8
CHIPPED	2	C NC

Number of Observations Read 32

Number of Observations Used 32

WITH DAC AS COVARIABLE BY POP 11:03 Monday, July 29, 2013

-- POP=A --

The GLM Procedure

Dependent Variable: GERM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00392875	0.00196438	1.08	0.3532
Error	29	0.05279312	0.00182045		
Corrected Total	31	0.05672187			

R-Square	Coeff Var	Root MSE	GERM Mean
0.069263	4.383102	0.042667	0.973438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00340313	0.00340313	1.87	0.1821
DAC	1	0.00052563	0.00052563	0.29	0.5951

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00340313	0.00340313	1.87	0.1821
DAC	1	0.00052563	0.00052563	0.29	0.5951

WITH DAC AS COVARIABLE BY POP 11:03 Monday, July 29, 2013

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--------------------------------------------- POP=A --------------------------------------------

                                        The GLM Procedure
Dependent Variable: FUNGUS
                                                Sum of
        Source                      DF         Squares     Mean Square    F Value    Pr > F
        Model                        2      0.00055375      0.00027687       0.17    0.8466
        Error                       29      0.04794313      0.00165321
        Corrected Total             31      0.04849688

                        R-Square     Coeff Var      Root MSE    FUNGUS Mean

                        0.011418      200.1708      0.040660       0.020313

        Source                      DF       Type I SS     Mean Square    F Value    Pr > F
        CHIPPED                      1      0.00002812      0.00002812       0.02    0.8971
        DAC                          1      0.00052563      0.00052563       0.32    0.5772

        Source                      DF     Type III SS     Mean Square    F Value    Pr > F
        CHIPPED                      1      0.00002813      0.00002813       0.02    0.8971
        DAC                          1      0.00052563      0.00052563       0.32    0.5772

                                   WITH DAC AS COVARIABLE BY POP     11:03 Monday, July 29, 2013

--------------------------------------------- POP=A --------------------------------------------
                                         The GLM Procedure
Dependent Variable: ROOT
                                                Sum of
        Source                      DF         Squares     Mean Square    F Value    Pr > F
        Model                        2      1.92921000      0.96460500       2.46    0.1033
        Error                       29     11.38507750      0.39258888
        Corrected Total             31     13.31428750

                         R-Square     Coeff Var      Root MSE     ROOT Mean
                         0.144898      16.50223      0.626569      3.796875

        Source                      DF       Type I SS     Mean Square    F Value    Pr > F
        CHIPPED                      1      0.15680000      0.15680000       0.40    0.5324
        DAC                          1      1.77241000      1.77241000       4.51    0.0423

        Source                      DF     Type III SS     Mean Square    F Value    Pr > F
        CHIPPED                      1      0.15680000      0.15680000       0.40    0.5324
        DAC                          1      1.77241000      1.77241000       4.51    0.0423

                                   WITH DAC AS COVARIABLE BY POP     11:03 Monday, July 29, 2013
--------------------------------------------- POP=A --------------------------------------------
                                         The GLM Procedure
                                        Least Squares Means

                                                       FUNGUS
                      CHIPPED     GERM LSMEAN          LSMEAN     ROOT LSMEAN
                      C           0.96312500      0.02125000      3.86687500
                      NC          0.98375000      0.01937500      3.72687500

                                   WITH DAC AS COVARIABLE BY POP     11:03 Monday, July 29, 2013
--------------------------------------------- POP=T --------------------------------------------
                                         The GLM Procedure
                                      Class Level Information
                           Class         Levels    Values
                           TRIAL             10    1 2 3 4 5 6 7 8 9 10
                           CHIPPED            2    C NC

                              Number of Observations Read          40
                              Number of Observations Used          40

                                   WITH DAC AS COVARIABLE BY POP     11:03 Monday, July 29, 2013
--------------------------------------------- POP=T -------------------------------------------
                                         The GLM Procedure
Dependent Variable: GERM
```

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.35236125	0.17618063	7.92	0.0014
Error	37	0.82307875	0.02224537		
Corrected Total	39	1.17544000			

R-Square	Coeff Var	Root MSE	GERM Mean
0.299770	18.69033	0.149149	0.798000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00256000	0.00256000	0.12	0.7364
DAC	1	0.34980125	0.34980125	15.72	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00256000	0.00256000	0.12	0.7364
DAC	1	0.34980125	0.34980125	15.72	0.0003

WITH DAC AS COVARIABLE BY POP   11:03 Monday, July 29, 2013

-- POP=T --

The GLM Procedure

Dependent Variable: FUNGUS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.19713375	0.09856688	2.27	0.1175
Error	37	1.60614375	0.04340929		
Corrected Total	39	1.80327750			

R-Square	Coeff Var	Root MSE	FUNGUS Mean
0.109320	31.41334	0.208349	0.663250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00210250	0.00210250	0.05	0.8270
DAC	1	0.19503125	0.19503125	4.49	0.0408

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.00210250	0.00210250	0.05	0.8270
DAC	1	0.19503125	0.19503125	4.49	0.0408

WITH DAC AS COVARIABLE BY POP   11:03 Monday, July 29, 2013

-- POP=T --

The GLM Procedure

Dependent Variable: ROOT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.98917375	2.49458687	3.80	0.0316
Error	37	24.30880375	0.65699470		
Corrected Total	39	29.29797750			

R-Square	Coeff Var	Root MSE	ROOT Mean
0.170291	34.40739	0.810552	2.355750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.08372250	0.08372250	0.13	0.7231
DAC	1	4.90545125	4.90545125	7.47	0.0096

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHIPPED	1	0.08372250	0.08372250	0.13	0.7231
DAC	1	4.90545125	4.90545125	7.47	0.0096

WITH DAC AS COVARIABLE BY POP   11:03 Monday, July 29, 2013

-- POP=T --

The GLM Procedure

Least Squares Means

CHIPPED	GERM LSMEAN	FUNGUS LSMEAN	ROOT LSMEAN
C	0.80600000	0.67050000	2.31000000

NC	0.79000000	0.65600000	2.40150000