Innovating Wheat Breeding to Improve Indian Agriculture

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The World Food Prize Foundation

2017 Borlaug-Ruan International Internship

MAHYCO

Jalna, Maharashtra

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Chapter 1: Introduction

1.1 Acknowledgments

I would like to thank **Lisa Fleming**, **Ambassador Quinn**, **John Ruan, Katie Walther**, and everyone at the World Food Prize who created this program that gives young people an once-in-a-lifetime chance to explore new cultures, discover their passions, and develop their skills to join the fight against hunger. Also, special thanks to **Keegan Kautzky** for inspiring me to pursue an internship and **Kay Ellington** for organizing the Minnesota Youth Institute.

Furthermore, I would like to express my deep gratitude to **Dr. Usha Zehr**, **Dr. Chandrashekhar Chaporkar**, and **Dr. Anita Murugkar** for welcoming me into their homes with unparalleled generosity. Because of them, I always had plenty to eat, a safe place to stay, and most importantly, a home away from home.

With the fantastic mentorship of **Neelam Shaikh**, I was able to become proficient in procedures that I had not even heard of before my internship. All that I learned and accomplished in the MAHYCO labs would not have been possible without her patience and useful diagrams.

Next, I would like to express my appreciation for **Dr. Bharat Char** and **Dr. Anjanabha Bhattacharya**, who always had helpful advice and guidance. They found my research project and helped me make the most of my time in the lab.

I would like to thank **Vaishali Khanale**, **Nabeela Khan**, and **Latika Mohide** for encouraging me in the lab and teaching me Hindi during tea breaks. Most importantly, they were always smiling each morning and have become my lifelong friends.

I would like to extend my gratitude to **Leela Alamalakala**, who took me under her wing during my time in India. Because of her generosity, I was able to see more of India's agriculture and experience the culture of this beautiful country.

Special thanks to **Hannah Hornung**, **Gloria Klassen**, and **Jayme Fast**, who were fantastic editors, even when I forgot about the time change and sent them rough drafts at 4:00 A.M. Without these ladies, my propensity for spelling mistakes would have surely ruined this report.

Finally, I would like to express my deep gratitude to the people who believed in me from the beginning. Years ago, the **Mountain Lake Agriculture Teachers** ignited my passion for solving food insecurity. Throughout my entire high school career and beyond, they have continued to inspire confidence and purpose in my life. I cannot offer enough thanks for all of the opportunities and the advice that has shaped me into the person I am today. I am forever thankful for my friends and family who were extraordinarily supportive of me throughout my season in India. Special thanks to **Chad, Esther, Luke, Zach, and Caleb Klassen** who always believed in me and allowed me to grow.

1.2 The Beginning of My Journey

My name is Rebekah Klassen. In the spring of 2017, I completed my high school career at Mountain Lake Public School in Mountain Lake, Minnesota. Growing up in a refreshingly diverse small town, I have always enjoyed exploring new cultures and meeting new people.

In ninth grade, I joined my school's FFA chapter (a national youth organization) and began learning about agriculture and food insecurity. One of the components of the FFA Organization is the Public Speaking Career Development Event (CDE)—a competition among students who write and recite their own speeches pertaining to current issues in agriculture. As I browsed the list of topic ideas, "World Hunger" caught my eye. After investigating the topic further, I was captivated by this problem and the organizations who work on the cutting edge of solving food insecurity.

As my interest grew, I earnestly read books on food insecurity. To me, the thought that our world is capable of producing enough food even though thousands go starving every day was unacceptable and unignorable. As a result, with the help of my FFA chapter and advisors, I began taking action.

By communicating with the local food shelf and my school's principal, my FFA team and I were able to organize the largest food drive in our school's history. In just one week, we collected over $300 and 800 items to donate to the food shelf. Motivated by this, I continued to raise awareness of food insecurity by leading the development of the FFA Region VI Hunger Workshop, as well as several other hunger-fighting activities. In the meantime, I also traveled to five countries, observing their cultures and studying the ways that food insecurity manifests itself in different communities.

Then, my agriculture teacher, Mrs. Brockberg, introduced me to the World Food Prize and the Minnesota Youth Institute (MYI). After a year of researching and writing, I eagerly submitted my paper to the MYI. At the institute on the University of Minnesota–Twin Cities campus, I met Keegan Kautzky, who inspired me to continue to fight food insecurity, and Kay Ellington, who made it possible for me to go to the Global Youth Institute (GYI).

At the 2016 GYI, I discovered numerous world leaders, company executives, and students like me who shared the view that global food insecurity is a problem that demands attention and action. Before my time with the GYI, I had always felt as if I was on the sidelines, watching hunger rear its ugly head yet not contributing to the science that was pushing for solutions. When I learned of the Borlaug-Ruan Internship, I knew the program was exactly what I was hoping for—a chance to aid in research to decrease food insecurity.

After gathering the requried documents, I anxiously submitted my application to be an intern. This choice was difficult because since ninth grade, I had my hopes set on interviewing to be Minnesota FFA State Officer during my senior year. However, after much reflection, I concluded that the internship was more meaningful to me. When I received the email telling me I was selected as a 2017 Borlaug-Ruan Intern, I was overjoyed. My excitement grew even more when I learned I would be placed at a seed company in the beautiful country of India.

1.3 MAHYCO

Standing tall among the fields of rural Jalna, the Maharashtra Hybrid Seed Company Private Limited (MAHYCO) is situated along the Aurangabad-Jalna Highway in the state of Maharashtra. This gorgeous campus has hosted some of the biggest breakthroughs in the Indian seed industry and continues to forge solutions in this new era. Vaishali Khanale, a researcher for the company, summarized its mission by stating, "MAHYCO is always passionate to explore promising technology in the field of crop improvement for the betterment of farmers" (Khanale). While this company has reached unprecedented success, MAHYCO had a humble beginning. MAHYCO's founder Dr. Badrinarayan R. Barwale devoted his autobiography, *My Journey with Seeds and the Development of the Indian Seed Industry*, to the story of this innovative company.

In the autobiography, Barwale details how his experiences during a pivotal time in India shaped him. On August 27, 1930, Barwale was born in Hingoli, Maharashtra. In 1947, India gained its independence from Britain. India faced numerous challenges as it worked to organize a government, calm the remaining political unrest, and feed its staggering population. In this turbulent time of freedom fighting and food shortages, Barwale envisioned a bountiful future for his beloved nation and worked towards achieving his vision.

As a young and determined man, he first made headlines when he joined the Freedom Movement of Hyderabad. After months of underground work, he was finally arrested and imprisoned for over a year. Following his release, his autobiography narrates that he married a young woman named Gomati and moved to Jalna, Maharashtra to begin farming.

On his farm, Barwale dedicated himself to his crops. As his plots grew and his methods improved, the surrounding community took notice. In 1959, Barwale produced and sold his first seed—Pusa Sawani Okra. Barwale's book summarizes that success motivated him to expand his work to include hybrids and more crops. By 1962, he hired an advertising agency to sell his labeled and packaged seed.

With new colleagues and new crops, Barwale realized that he needed an official partnership to grow his business. Therefore, he founded MAHYCO on November 24, 1964. Despite a widespread drought in 1965 and 1966, MAHYCO planted wheat that came from Norman Borlaug's research, namely the Sonora-64 and Lerma Rojo, and was able to triple the company's yield. To continue its growth, Barwale established a research and development unit in 1966. His autobiography explains that Barwale hired Dr. K. R. Chapra and a number of young scientists to run the new endeavor.

As the years of profitable yields continued, MAHYCO earned international attention for its pioneering work. By 1995, Monsanto, an American giant in the seed industry, signed permission for MAHYCO to use Bt technology for its cotton crops. Eventually, this partnership grew, and Monsanto bought 26% of MAHYCO stake. In his book, Barwale recounts that he continued to collaborate with Indian farmers and companies on a smaller scale.

While improving his nation's agriculture was always Barwale's main focus, he also looked beyond seeds to benefit his community. After receiving laser treatment for glaucoma and visiting a hospital in Chennai, Barwale was inspired to build a quality eye hospital in Jalna. Even though

he faced some challenges, he established Shri Ganapati Netralaya on December 31, 1992 according to his autobiography. Invigorated by its success, the MAHYCO Research Foundation funded the Badrinarayan Barwale College with the goal of providing valuable education in microbiology, biotechnology, and computer science. In addition, Barwale established the Golden Jubilee School as a pre-primary, primary, and secondary school for the children of Jalna.

1.4 The Tissue Culture Lab

On Monday of my first week in India, Dr. Bharat Char, the Senior Lead of Biotechnology at MAHYCO, introduced me to Dr. Anjanabha Bhattacharya (everyone called him Dr. Anjan), who would be my project supervisor for the next eight weeks. At the morning meeting, Dr. Anjan introduced me to the rest of the team of Double Haploid researchers. I was then escorted to the tissue culture lab by my new mentor—Neelam Shaikh, a research associate of the Double Haploid Department.

Upstairs in the lab, Neelam attempted to explain the basics of her work and plant breeding. Having no previous lab or plant science experience, everything was overwhelming. I felt as if I had been dropped off on another planet that was much more advanced than mine, inhabited by geniuses who spoke a higher form of English and wore colorful kurtas.

I spent the entire first week of my internship learning about lab procedures and concepts. Even after I had spent hours working with lab equipment, reading about plant breeding, and diligently copying Neelam's notes and diagrams, I still had no idea how to pick a research project. As the days turned into weeks, my anxiety about the subject of my project grew. Fortunately, Neelam reassured me, "We are here for you. We want to help you."

I reached out to Dr. Anjan and Dr. Char. Together, we decided that I should experiment through the Double Haploid method. This project covered the basics of plant breeding biotechnology while giving me a structured frame to work within. Most importantly, the majority of the procedure could be completely within 2 months with only the final testing occurring 2–4 months after all of the work was completed.

1.5 Researchers on This Project

My mentor, Neelam Shaikh, had come to MAHYCO on a recommendation and had been working for the company for three years. During that short time, she had several successful projects in drought-tolerant rice, abiotic stress-tolerant cotton, synthetic biotic design, and Double Haploid breeding (Shaikh).

My supervisor, Dr. Anjanabha Bhattacharya, earned a bachelor's degree in agriculture, a master's degree in horticulture, and a PhD in plant biotechnology before joining MAHYCO to pursue his goal of becoming a scientist. At the time of my internship, he had been working for the company for over five years, during which he worked on promoter discovery, gene editing, mutagenesis, and Double Haploid breeding (Bhattacharya).

Vaishali Khanale also worked in the tissue culture lab. With a bachelor's degree in microbiology, a master's degree in botany, a PhD in progress, and 17 years of lab work at MAHYCO under her belt, she was the ultimate source of information and advice (Khanale).

Chapter 2: An Introduction to the Concepts

2.1 The Green Revolution and Wheat

In 1944, Dr. Norman Borlaug began working in Mexico as a research scientist, where he focused on breeding wheat varieties to have higher yields and be disease-resistant (Quinn). At this time, his work was especially significant because nations around the world were pinpointing hunger as an obstacle to growth and development (Spielman, Pandya-Lorch 12). Researchers estimate that by the 1950s approximately a billion people went hungry every day (Spielman, Pandya-Lorch 16). After 13 assiduous years in the fields of Mexico, Borlaug and his team finally produced wheat that was resistant to rust (Quinn). As his work expanded, he innovated new ways to rotate crops in order to capitalize on each growing season (Quinn). One of the problems Mexican farmers continued to face was the wheat plants collapsing due to the heads growing too heavy for the flimsy stalks to support (Gillis). In perhaps one of his biggest break-throughs, Borlaug and his team developed a wheat variety with a shortened and sturdier stalk that still produced the same amount of seed as taller varieties. As a result of this new "semi-dwarfed" crop, Mexico was able to produce six times more wheat in a span of only 20 years (Gillis).

Norman Borlaug's work did not stop in Mexico. In the years following the introduction of semi-dwarfed wheat, Borlaug's seed varieties and agricultural practices spread around the globe to developing nations in desperate need of a solution to food shortages, launching what is now recognized as the Green Revolution (GR). From 1960-1990, the amount of available food in developing nations rose 12-13% (Pingali 2). In only 40 years (1960-2000), wheat yields in developing countries increased by 208%, the largest increase per hectare of any GR crops (Pingali 2). Among the nations that benefitted from the GR, India was one of the first to grow Borlaug's wheat (Gillis).

In the 1960s, India faced crippling food shortages due to a skyrocketing population growth. By the middle of the decade, the relatively new nation was forced to import massive shipments of grain to supplement the inadequate crop production (Gillis). After observing Mexico's success story, scientist Dr. M.S. Swaminathan requested that the Indian government contact Borlaug for a solution to the nation's crisis (Subramanian). At their invitation, Borlaug traveled to India to assess the situation and gain insight on the subcontinent's needs (Subramanian).

Following his visit, Borlaug selected seeds from four varieties of wheat, which he hypothesized would adapt to India's climate (Subramanian). Even though some officials were hesitant to adopt Borlaug's methods, the government purchased large quantities of his wheat seed to avert famine (Gillis). As plant breeding methods and fertilizer became available to Indian agriculturalists, harvests improved, resources expanded, and food costs declined (Pingali 2). By 1968, India's wheat yields had increased from 800 pounds per acre to 6,000 pounds per acre (Subramanian).

Norman Borlaug's effort to grow rust-resistant wheat was a revolutionary for the cereal crop. In fact, recent studies show that wheat with a resistance to rust creates $600 million to $2 billion in benefits per year (Pingali 2). However, when in Norway to receive the 1970 Nobel Peace Prize, Borlaug stated, "We may be at high tide now, but ebb tide could soon set in if we become complacent and relax our efforts." (Gillis). His words ring true as the world continues to face food insecurity.

2.2 Increasing Cereal Crop Yield

As Norman Borlaug predicted, the world continues to suffer from hunger and poverty. Today, undernourishment is still a daunting problem, with almost a billion people going hungry every day (Spielman, Pandya-Lorch 17). In fact, estimates conclude that 159 million out of 667 million children under 5 are stunted, and 50 million are underweight for their height (International 2). One of the ways to rectify this massive problem is to increase cereal crop yield.

Improving agricultural production, specifically cereal production, can be extraordinarily beneficial to developing countries by increasing yield, lowering food costs, and even reducing malnutrition. As David J. Spielman and Rajul Pandya-Lorch wrote, "The interventions of the past half century have...demonstrated that agriculture can be a key driver of growth and development for many of the world's poorest countries" (17).

According to the World Bank, cereal crops can refer to wheat, rice, maize, barley, oats, rye, millet, sorghum, buckwheat, and mixed grains (Databank). Around the globe, these cereal crops are staples in most diets. Rice is the primary source of calories in developing nations, followed by wheat. Additionally, these countries' populations depend on wheat as their leading source of protein (CGIAR). Of the 7.4 billion people on Earth, 1.2 billion rely on wheat as an irreplaceable caloric source (CGIAR).

Without cereal crops, hunger and malnutrition cause a plethora of health problems. According to the Global Nutrition Report in 2016, "Malnutrition and poor diets constitute the number-one driver of the global burden of disease" (19). One of the devastating consequences of malnutrition is stunting, a condition where the child is unable to grow to full development and will lag behind international standards cognitively and physically. Without proper diets in infancy and childhood, stunted children are less likely to excel in school, more likely to suffer from other health condition, and have decreased work productivity (Programmes).

Although eating only cereal crops will lead to an unbalanced diet and deficiencies, increasing the production of cereal crops can cause food prices to go down, which can lead to better overall nutrition. Around the globe, there are numerous case studies of how higher cereal crop production can decrease food prices and increase diet diversity and health. One of the best examples of this was in a study in Bangladesh. In this example, researchers conducted a study of the relationships among underweight children, rice consumption, and rice prices. The study focused on 81,337 children from 62,959 households in rural communities. Data was collected over the course of eight years. The researchers found that rice prices fluctuated drastically over the years while rice consumption remained relatively steady. However, during the years that rice prices went down, less children were underweight, indicating a positive correlation between rice prices and underweight children. Additionally, families that were able to purchases less expensive rice were found to have more diverse diets. Therefore, the researchers concluded that as a staple crop's price declined, rural families could afford a well-rounded diet, leading to better overall health (Torlesse, Kiess, Bloem).

In conclusion, cereal crops with higher yields have the potential to greatly benefit developing nations. Due to the massive caloric dependence on cereal crops around the globe, increased

production is imperative. Higher-yield cereal crop can lead to diversified diets and improved health. If cereal crop production does not increase, hunger and malnutrition will continue to cause a profusion of health problems, most notably stunting in children. Even though increasing cereal crop yields cannot solve the problem of hunger completely, it can contribute significantly to rectifying the devastation of global food insecurity.

Chapter 3: Materials and Methods

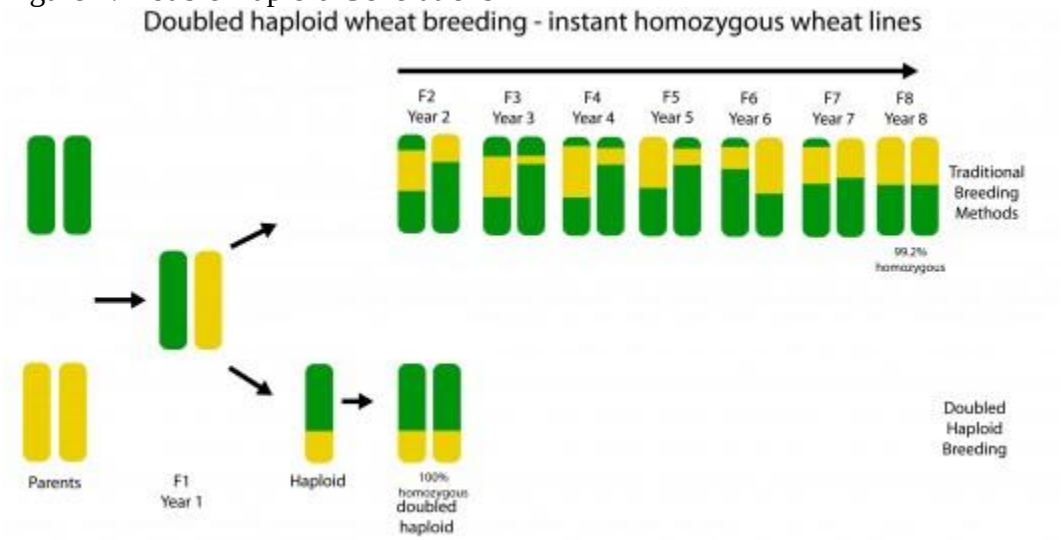
3.1 Abstract

Wheat constitutes approximately one-fifth of the world's diet, making it one of the most crucial crops grown today (CGIAR). With demand for wheat expected to rise 60% in the next 30 years, improving the quality and yield of this cereal is a decisive move in the fight against food insecurity (CGIAR). This experiment examined the timeline and effectiveness of the Double Haploid (DH) method in wheat to determine if this method can expedite the plant breeding process and produce high-yield crops. To test this, wheat samples were grown in a growth cabinet, and the samples were emasculated to prevent self-pollination. One to three days after emasculation, feathery receptive stigmas were pollinated with fresh maize pollen.To further assist the caryopsis and embryo growth, 2, 4-Dichlorophenoxyacetic acid was added 24 hours after pollination to each floret. After 14-16 days, the caryopses (which contains the embryo) were removed and sterilized; then, the embryos were set on a specialized media to grow. In order to cause the haploid chromosomes to double, the plants were placed in colchicine. The end result was several young plants that should contain Double Haploid chromosomes and traits that the plant breeders value: higher yield and increased quality. However, the wheat samples will need to grow over several months to develop a seed before they can be analyzed by the plant breeders. Unfortunately, the length of my stay at MAHYCO was not be long enough to personally confirm results. As a result, further and broader investigation needs to be done to confirm this project.

3.2 Project Objectives

The purpose of the DH method is to accelerate the plant breeding process while preserving desired traits. With the DH method, homozygous varieties can be produced in a shorter time span than traditional breeding (see Figure 1). According to the researchers at MAHYCO who work with this procedure, they are able to cut down the time needed for developing a high-yield variety from eight years to four years. This decrease in time to produce quality seed which translates into products that are suited to the current markets and better harvests for farmers with fewer inputs. In India, improvements in agriculture are especially influential because a massive population depends on it. In fact, the World Bank found that over 20% of Indians live below the international poverty line and 51% of the population is employed in agriculture (DataBank). For Indian people, improvements in agriculture can mean improvements in all other areas of life.

Figure 1: Double Haploid Generations



Source: Colorado Wheat

3.3 Preparing Samples

The samples came from wheat plants grown in a growth cabinet in the MAHYCO lab building. Since wheat is a self-pollinating plant that cradles both the ovary (female) and three anthers (male) inside of each floret, each sample needed to be emasculated to prevent self-pollination, or selfing.

To accomplish emasculation, a spike which was enclosed or partially emerged in a leaf sheath was selected. Then, the central sterile flower was removed with forceps. Next, the inner lining of each floret was opened, taking care to avoid tearing the lemma or palea, and the 3 immature, green anthers were removed with forceps. If the lemma or palea tore, the floret would no longer be viable because this thin tissue closes over the ovary after pollination, creating a space for the caryopsis (plural caryopses) to grow. Without the lemma and palea, the floret would not produce a caryopsis, the watery vesicle where the embryo develops. After emasculation, each spike was bagged and stored in the growth cabinet at 22 degrees celsius.

Wheat in Growth Chamber

Bagged Wheat

Pollination

On the next morning between 9:00 and 11:00 A.M., maize pollen was collected from a greenhouse on the MAHYCO campus. To activate the pollen, it was warmed under a heating lamp. The pollen was then dusted on stigma of emasculated flower with a brush.

After 24 hours following pollination, a drop of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) with a concentration of 100-150 parts per million (ppm) was pipetted onto each floret to aid the growth of the caryopsis (See Table 1).

3.4 Caryopsis Removal and Sterilization

After 17-18 days, the wheat stalks were cut, placed in water, and stored for 8 days at 4 degrees Celsius.

Note: Due to my limited time, I could not use the caryopses from my own samples to finish my project in time. Instead, I used samples that had been previously emasculated and pollinated for the remainder of the procedure.

If emasculation and pollination were successful, the samples developed a green caryopsis inside the floret. These healthy but delicate caryopses were removed with forceps and set on wet filter paper to prevent drying. After collecting all viable caryopses from a plant, the caryopses were placed in 2 milliliter tubes, labeled, and stored again at 4 degrees Celsius.

Caryopses Work Station

Caryopses

Sterilization

To sterilize the caryopses, the procedure outlined in Table 2.3 was followed.

After each substance was added, the samples were inverted for the allotted time. Once completed, the substance was siphoned out using a pipet before moving on to the next repetition. See Table 2.3 for specific time and repetition requirements.

When the sterilization process was complete, the caryopses were stored for 1–2 days at 4 degrees celsius in 2 ml tubes to keep them viable for embryo rescue.

3.5 Embryo Rescue and Media Preparation

Following sterilization, the caryopses were placed under a microscope. Using forceps, each caryopsis was opened along its vertical seam to locate and rescue the embryo. Each embryo was then transferred to a half-strength Murashige and Skoog (MS) formulation media.

The MS formulation is a common formula for media that supports plant growth (Smith 32). MAHYCO labs have a consistent stock of various MS components, of which the correct ingredients were selected to best support wheat embryos. The complete list of this media's ingredients is specified in Table 4.1–Table 4.5.

Each ingredient in the media played an important role in the growth of the embryos. The MS Major and MS Minor supplied the inorganic salts which are essential to ensure speed and accuracy of the experiment. Wheat plants require the salts in the MS Major formulation—Potassium, Nitrogen, Phosphorus, and Sulphur—in larger quantities than other elements. On the other hand, Manganese, Boron, Copper, Zinc, Molybdenum, and Cobalt are also required but in smaller dosages, so these are found in the MS Minor (Plant 6). These inorganic salts promote the embryos' development and are essential to proper tissue culture (Smith 32).

Potassium was added to the MS Major because this salt is utilized in osmotic regulation and enzyme activation in the plants. In this case, Ammonium Nitrate was included in the MS Major to provide nitrogen, which contributes to manufacturing amino acids and organic molecules (Plant 9). Because pure nitrate can be too alkaline, Ammonium is added to balance the pH. Phosphorus is utilized by the plant in DNA and RNA (Plant 8).

In addition to the MS Major, the MS Minor is also essential to each plant's health. Boron is instrumental in the creation of cell walls and cell membranes (Plant 9). Copper is beneficial for photosynthesis and enzyme reactions. Zinc also contributes to enzyme action, as well as protein synthesis. Molybdenum assists many enzymes and reduces Nitrogen gas. Finally, Cobalt may be partially responsible for fixation of nitrogen (Plant 10).

According to Roberta Smith's *Media Components and Preparation,* Myoinositol (or Myo-insitol) is a hexitol that is considered to be important in "cyclitol biosynthesis, storage of polyhydric compounds as reserves, germination of seeds, sugar transport, mineral nutrition, carbohydrate metabolism, membrane structure, cell wall formation, hormonal homeostasis, and stress physiology" (37).

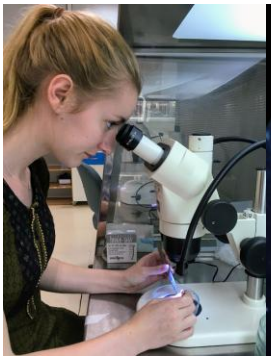
MS Iron contained iron in the form of ethylenediaminetetraacetic acid, or EDTA, which synthesizes iron sulphur proteins and haeme proteins. These proteins have vital functions in plants (Plant 10).

While plants growing in a field can synthesize key vitamins, the vitamins in the MS Vitamin formula were carefully selected for plants growing in a lab (Plant 6). For example, Thiamine, regarded as a necessary ingredient in many medias, was added to improve cellular response (Smith 36).

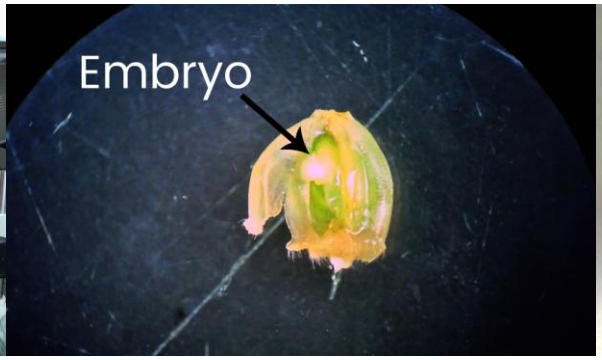
The remaining ingredients—Sucrose, Calcium Chloride, and Agarose—served a vital role in the media as well. Sucrose supplies the crucial source of carbon (Smith 36). Calcium Chloride contributes to cell and root growth (Plant 9). Finally, Agarose acted as the gelling agent which provides immobile support for the embryos and the media components (Smith 37).

Following complete mixing of the media using a magnetic stirrer, pH was adjusted to 5.8 using Sodium Hydroxide and Hydrogen Chloride. The flask was then autoclaved at 121 psi for 15 minutes. To ensure that the agarose was dissolved, the entire mixture was heated in a microwave for 3 minutes. Then, the media was occasionally stirred until it cooled before being poured into petri plates.

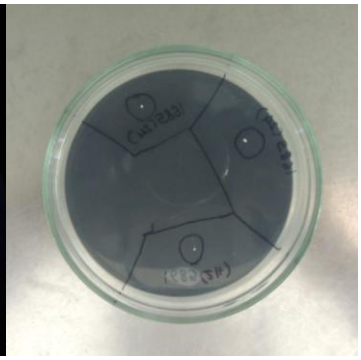
Each embryo was set on the surface of the cooled media. After being loaded with the embryos, these plates were stored in the dark at 25 degrees celsius until germination.



Rescuing Caryopses



Embryo in Caryopses



Embryos in Media

3.6 Doubling Chromosomes Using Colchicine

After 2 weeks, 1 embryo germinated and was moved to a growth chamber. The remaining embryos had not germinated yet. At this point, I had to return to the U.S., so I have written what will happen to complete the DH method.

Once roots and tillers develop, the regenerated plants will be treated with 0.1% colchicine, followed by an overnight water wash, and then transplanted in pots. The treated plants will be maintained at 22 degrees in a growth room. In the reproductive stage, the plants will be moved to a growth chamber of 25 degrees for seed set. In 3-6 months, when the seeds develop, the samples will be phenotyped and genotyped by breeders to determine if each one is genetically pure and produces a high yield or a trait of particular interest.

Chapter 4: Results

4.1 Research Results Discussion

Due to limited time at MAHYCO, conclusions on the quality of the seeds or the yield of this variety cannot be drawn. However, based on the results during the project, it can be concluded that the dosage of 2, 4 D needs to be increased, emasculation must be more precise, and a larger sampling needs to be taken to improve the efficiency of the Double Haploid method in wheat.

In the first batch of 28 caryopses from 5 samples, no embryos had developed, though 2 embryos had selfed, or self-pollinated. Sterilization was repeated with a much larger batch of 108 caryopses from 21 samples.While many caryopses had not accepted the maize pollen nor produced an embryo, 9 embryos were rescued (Table 3.1).

From a total of 136 caryopses, only 9 embryos were rescued. Interestingly, 3 embryos came from the same caryopsis. Considering only 7 caryopses developed embryos, this may point to a shortage in growth hormones. In order to test this, the experiment would need to be performed again with the amount of 2, 4-D as the variable. 2, 4-D could be administered to the plants multiple times following pollination to increase the probability of embryos developing. However, increasing the concentration of the hormone would be counterproductive because florets become sterile if exposed to 2, 4-D with a concentration over 150 ppm. Alternatively, 2, 4-D could also be added to the media (Smith 45).

In addition to very few embryos developing, a number of samples selfed. The exact number of selfed samples could not be accurately determined because they were not collected nor recorded. The prevalence of selfed seeds is due to error during the first step of the process—emasculation. To avoid this problem in the future, all anthers must be removed before bagging or pollinating.

While more haploid embryos might have developed if the amount of 2, 4-D was increased and if emasculation was exact, starting with more samples would also benefit this study and increase the number of embryos.

4.2 Significance to Food Insecurity

With the world's population continuing to rise, farmers need to produce more food on less land. In addition to land constraints, farmers also face a number of environmental challenges that can decrease yields. Seeds that are resistant to viruses, pests, or droughts are essential to successfully grow high-yield crops. While these seeds can be selected through traditional plant breeding, the timeframe for a complete experiment is often too long to produce a useful product in the current market. However, by using the Double Haploid method, improved seeds can be growing in the fields much sooner. With the seeds improving almost as quickly as problems arise, agriculturalists can make progress towards increasing yields and ending food insecurity.

4.3 How I grew as an Individual

In only a 60-day period, I changed tremendously in the way that I thought and viewed the world.

First and foremost, my internship changed the way I processed life because I established confidence in myself. By gaining new skills in the lab, I assured myself that I could grow as a learner. For example, my first weeks in the tissue culture lab were confusing and sometimes even frustrating as I struggled to grasp concepts. However, as I continued working through each procedure and asking plenty of questions, I began to understand the concepts. When my paper was completed, I was so incredibly proud of my finished product because I knew that I had worked incredibly hard to understand each part of it. Because of this trip, I know that I will be a life-long learner even after I complete my formal education.

Not only did I acquire new technical skills, I also learned more communication and interpersonal skills. Before this summer in India, I had never worked in an office before, nor had I had a full-time job. To fully experience working in a lab, I had to clearly and concisely communicate my needs and my questions. While I loved doing my own research, I relished the opportunity to witness the work of individuals who are fighting hunger in India. By working at MAHYCO for two months, I acquired valuable relational abilities that I will use in any career that I pursue.

Finally, I gained a new worldview as I lived in India. During my internship, I learned to understand and appreciate the Eastern way of thinking. Surrounded by a culture that is so different than mine, and yet so beautiful, I enjoyed discovering Indian foods, traditions, and so much more during my internship. However, India is not immaculate. Amongst the colorful tapestry of this beautiful culture, I witnessed extreme need, deep hunger etched into tired faces, and human beings straining to escape an unrelenting cycle of poverty and ruin. Outside of my comfort zone, I came face-to-face with my beliefs about the world and my place in it. As a result, I gained a new perspective and expanded my horizons.

4.4 New Clarity to My Passions

Without a doubt, this experience has grown, developed, and expanded my understanding of myself for the better. I know with absolute certainty that I want to be part of solving food insecurity. However, this may not mean that I will work in a lab or pursue a degree in microbiology. Instead, through this internship and my time with the people of India, I feel compelled to impact hunger through education or business.

As I considered career paths as a senior in high school, I wandered from one interest to another without ever feeling convinced I could find fulfillment in any of them. I considered botany; I investigated a major in linguistics. Then I pondered a career as a teacher. Tottering between different majors, I headed to India. As I studied in MAHYCO's labs, I loved working with Neelam as she taught me how to prepare samples and interpret data, yet I found myself gravitating towards my desk and my writing.

As the weeks progressed, I realized that my strengths were organizing, coordinating, planning, creating, writing, and teaching. Most importantly, I determined that my passion for hunger alleviation will probably not take me to a career behind a microscope, but rather behind a desk. This knowledge is invaluable as I head into my first year of college and decide on a major. Now that I understand where my aspirations lie, I can focus my abilities in that direction.

4.5 Looking to the Future

I will forever be grateful for my time in India; what I have been able to see, do, and learn will stay with me for the rest of my life. This internship has changed not only my summer, but also my future.

During my time in the labs of MAHYCO, I gained a deeper appreciation for the scientists and companies all over the world who work tirelessly to improve agriculture. Their research and patience have impacted billions of lives. Without my firsthand experience, I would not have been able to fully comprehend the value of seed research. Additionally, because I have worked on GMOs personally, I can bring an unique and accurate perspective to the frequent GMO discussions that cause controversy.

While this trip impacted how I view the world and the seed industry, my internship also positively affected my career path. After interacting with the children at the Golden Jubilee School, I decided to study to be an English as a second language (ESL) educator or a

businesswoman. I truly believe improving medical care, government, agriculture, and education can change the world. I am going to apply myself in the worlds of education or government.

Overall, I am so grateful for the knowledge that I gained, the friends that I made, and the experiences that changed the course of my life. My time in India challenged me to take a deeper look at how I fit into the solutions for world hunger. I realized that food insecurity will not be solved merely by money or force of will. Yes, those are pieces to the puzzle, but the real solutions will come from innovative ideas and people who persevere. As a result, I was motivated to live a purpose-driven life. On my own, I cannot change the world or solve any of the looming problems facing our world, but I can always care for the person right in front of me, hope for continual growth, and work towards a better tomorrow.

Appendix

Table 1.1: Preparation of 2, 4-D with 100 ppm Concentration

Step	Instructions
1	Mix 1 mg of 2, 4-D into 1 ml of 100% alcohol in flask
2	Heat mixture for 3 seconds in microwave
3	Add water until volume is 10 ml

Table 2.1: Preparation of 70% Alcohol

Component	Amount (milliliter)
100% Alcohol	70
Water	30

Table 2.2: Preparation of 1.5% Sodium Hypochloride

Component	Amount (milliliter)
4% Sodium Hypochloride	37.5
Water	62.5

Table 2.3: Caryopses Sterilization Procedure

Substance	Amount	Duration of Inverting	Repetition	Purpose
Autoclaved Water	1 ml	1 minute	2	Remove initial contamination
70% Alcohol	1 ml	1 minute	2	Sterilizing agent
Autoclaved Water	1 ml	1 minute	2	Wash off alcohol
1.5% Sodium Hypochloride	1 ml	5 minutes	1	Disinfectant used for cleaning plant materials in plant tissue culture
Autoclaved Water	1ml	1 minute	5	Remove all traces of alcohol or sodium hypochloride

Table 3.1: Rescued Embryos

Line Number	Caryopses	Embryos	Pollination Date*	Emasculation Date*
1685 (24)	9	3	06/25/17	06/24/17
1757 (8)	2	4	06/20/17	06/19/17
1685 (23)	9	2	06/16/17	06/15/17

*Dates written in month/day/year format

Table 4.1: Media Components

Component	Amount
Agarose	0.80 grams
MS Major	5.0 milliliters
MS Minor	0.50 milliliters
Myinositol	1.0 milliliters
MS Iron	0.50 milliliters
MS Vitamin	1.0 milliliters
Sucrose	3.0 grams
Calcium Chloride	0.50 milliliters

Note: pH was adjusted to 5.8 followed by autoclaving at 121 psi for 15 minutes.

Table 4.2: MS Major Components

Component	Amount (grams/liter)
Potassium Nitrate	1.90
Ammonium Nitrate	1.65
Potassium Dihydrophosphate	0.17
Magnesium Sulphate	0.37

Table 4.3: MS Minor Components

Component	Amount (milligrams/liter)
Boric Acid	6.20
Manganous Sulphate	16.9
Sodium Molybdate	0.250
Potassium Iodide	0.830
Copper Sulphate	0.025
Cobalt Chloride	0.025
Zinc Sulphate	8.60

Table 4.4: MS Iron

Component	Amount (milligrams/liter)
Ferrous Sulphate	27.8
Disodium Ethylenediaminetetraacetic acid	37.3

Table 4.5: MS Vitamin

Component	Amount (milligram/liter)
Thiamine Hydrochloride	0.100
Pyrodeine Hydrochloride	0.500
Nicotinic Acid	0.500
Glycine	200

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