Screening Wheat Germplasm at the Soil Borne Pathogens Program-CIMMYT-Turkey





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Personal Background

I was made aware of the World Food Prize Youth programs when a previous intern from my school, Ames High, spoke about the program to the Students Helping Eliminate Poverty and Hunger club (SHEPH). After hearing about her experience I knew the World Food Prize was something I was interested in. I wrote the initial paper required to attend the Iowa Youth Institute in April 2012. My paper was about the need for international collaboration to solve the ever-growing problem of Brucellosis in Afghanistan. Brucellosis is a pathogen of ruminants that causes chronic disease and death in humans. Brucellosis is transmission to humans from infected cattle, sheep and goats through consumption of unpasteurized milk and exposure to birth fluids. Brucellosis is common in developing countries and eradicated from most developed countries. The disease disproportionately affects women who tend sheep and goats. The focus of my paper was on the critical importance of ensuring the availability of highly nutritious and safe food as an income source to Afghan people.

After the Iowa Youth Institute I attended the Global Youth Institute in Des Moines, Iowa in October 2012. After listening to the presentations by the interns, Ambassador Quinn and others, and meeting the inspiring and passionate people attending the Institute I decided to apply for the Borlaug-Ruan international internship. The Youth Institute program made me realize that many people are actively and purposefully working to bring advanced scientific approaches to solving the largest global problem facing us and how to provide food for the millions in food insecure regions. My hope was to be given the opportunity to learn about science-based approaches to alleviating world hunger.

The Youth Institute and the World Food Prize presentations made me aware that solutions to food insecurity require numerous disciplines including animal scientists, plant scientists, sociologists and economists etc. However, based on my prior classes and general interest, I identified that I was interested in the role of genetics in food security. In particular I was interested in genomic tools that can be used to identify resistance to pathogens that decreased crop yield. During my interview for this internship I expressed this interest. Subsequently, I was offered the opportunity to pursue this interest by interning at the International Maize and Wheat Improvement Center (CIMMYT) in Turkey. Working at CIMMYT was an amazing opportunity, and I have learned more than I could have ever imagined about how research to breed for genetic resistance is one of the most valuable tools in the fight against world hunger.

BACKGROUND INFORMATION: CIMMYT

I spent the summer of 2013 in an internship at the International Maize and Wheat Improvement Center (CIMMYT)-Turkey in Eskischir Turkey. CIMMYT was founded in 1966 from a pilot program sponsored by the Mexican government and the Rockefeller Foundation with renowned wheat specialist Dr. Norman Borlaug. With headquarters in Mexico, CIMMYT has over 180 specialized research staff in over 40 countries and has 18 offices throughout the developing world. CIMMYT's goal is to carry on the Borlaug legacy of reducing poverty and food insecurity by sustainably increasing the productivity of maize and wheat cropping systems. Although enormous gains have been made in poverty and hunger alleviation, much work remains to be done, and CIMMYT continues to be part of the solution.

Much of the work conducted at CIMMYT focuses on identifying superior lines of maize and wheat to distribute to farmers in the developing world. The impact of CIMMYT has been significant. A recent estimate showed CIMMYT wheat varieties represent 75% of modern wheat varieties planted in developing nations. CIMMYT maize varieties represented 50% maize varieties planted in developing nations. (CIMMYT) Other notable accomplishments include the

joint awarding of the 2000 World Food Prize to CIMMYT researchers Dr. Evangeline Villegas and Dr. Surinder Vasal for the development of Quality Protein Maize, which has increased nutritional value.

The critical work conducted by CIMMYT researchers to increase the quality and quantity of maize and wheat production is recognized far beyond the borders of developing nations. That is why CIMMYT is part of the larger international research community. CIMMYT has partnerships with research institutes in Belgium, Denmark, France, Germany, Italy, and Portugal along with its many international centers. These international relationships allow CIMMYT to distribute its research and assistance to the numerous places that need it. For example, CIMMYT does much work breeding drought resistant strains of wheat for numerous countries in Africa. CIMMYT also works to breed for resistance to Maize lethal necrosis, which is common in Kenya and Tanzania. At CIMMYT research is carried out that directly benefits agricultural producers worldwide, from Asia to Africa to the Americas.

During my internship at CIMMYT I had the opportunity to work on a variety of projects. Consistent with the goals of CIMMYT, these projects focused on identifying lines of wheat with resistance to soil borne pathogens that cause yield loss. Genetic resistance is a vital tool in the pursuit of increased crop yield, which is essential in the battle to end world hunger. By finding lines that demonstrate tolerance to diseases and pests, and distributing those lines to farmers it is possible to protect against yield losses and reduce the need for expensive production inputs such as pesticides. While at CIMMYT I had the opportunity to work on two main projects, the foremost being one selecting for resistance against *Fusarium culmorum*.

SCREENING OF WHEAT GENOTYPES FOR THEIR RESISTANCE TO THE DRYLAND DISEASE CAUSED BY '*FUSARIUM CULMORUM*'

Abstract

The goal of this experiment is to identify wheat genotypes that show genetic resistance to F. *culmorum*. Successful identification of resistant wheat genotypes would increase wheat yields, while also reducing the need for chemicals and pesticides currently employed to combat Fusarium. The first step of the experiment is to identify the most aggressive strain of F. *culmorum* as there are many strains with different pathogenic potential. After identification of the most virulent strain through a pathogenicity test which done with strains isolated from crown tissue of wheat samples collected from wheat fields in various regions of Turkey. Each germplasm is being assessed for browning on the crown/stem at harvest and white heads at post anthesis stage which are the key symptoms of the disease. However, tolerant reaction is being estimated by evaluating the yield under both infested versus non infested plots. The desired lines are those having both resistant and tolerance reactions to crown rot.

Introduction

The dryland crown rot (foot rot, root rot) which caused by *Fusarium culmorum*, *F. pseudograminearum* (formerly *F. graminearum* group 1), *F. graminearum* (formerly *F. graminearum* group 2) are important disease of cereals around the globe and occur wherever cereal productions systems exist especially under drought conditions (Cook,1992). Crown rot is associated with reduced yields in wheat production and can be caused by numerous factors. *Fusarium culmorum* is a ubiquitous soil borne pathogen that infects cereals, especially in wheat. Fusarium has a substantial impact upon the yield of infected plants as it increases wheat susceptibility to crown rot, a disease in wheat characterized by brown discoloration of the stem

base and crown. Severe Fusarium infection can render the wheat crown dysfunctional. Crown rot also stunts plant growth and seed production. *Fusarium culmorum* is an important disease due to the worldwide prevalence of losses due to crown rot. In the United States Pacific Northwest a yield loss of 9% in 1994 was attributed to Fusarium associated with crown rot (Hogg). In Turkey crown rot has caused yield losses exceeding 50%. From 1998 to 2008 yield losses due to crown rot have increased by 9%, 3% and 1.2% in the northern, southern, and western wheat growing regions of Australia, respectively. All these countries rely heavily upon their wheat industries, and these losses have a detrimental impact upon farmers and the economy.

MATERIAL & METHODS

Under this chapter, my training course has two distinct phases. Initially, a field survey is conducted to isolate and document the Fusarium that are endemic and naturally occurring in Turkey. The second phase is an experimental study where the resistance of germplasms to Fusarium is assessed.

Isolation of Fusarium species from wheat samples:

Samples of wheat are taken to the laboratory from different field sites representing wheat growing areas in Turkey. Wheat stems are peeled, washed, sterilized, and pieces of crown samples are placed on PCNB (Penthacloronitrobenzene) agar. Crown samples placed petri dishes were incubated at room temperature with alternating of 12 hours light and 12 hours darkness for 7 days. Growth of fungi on PCNB media was observed. After fungal growth fungus from each colony were transferred to Synthetic Nutrient Agar (SNA) for sporulation for 7-14 days. SNA encourages the sporulation of Fusarium genus, however it is insufficient to isolate and identify specific Fusarium species. After incubation period (approximately 5-7 days), spore suspension of fungus was spread onto Water Agar (WA). After 24 hours one germinated spore of each sample was taken to SNA under microscope, and left for multiplication for 7-14 days at room temperature with alternating 12 hours light and 12 hours darkness. After multiplication period, samples from these cultures are transferred to sterilized 15% glycerol solution, and small agar plugs were also taken in cryo tubes for storage. These samples were stored at -80°C for long term use. The isolates of each sample is going to be molecularly identified in later stage.

Screening of wheat genotypes under growth room conditions:

In the experimental phase, to assess resistance of wheat genotypes, seeds, were germinated on humidified blotting paper in glass petri dishes that have been sterilized at 170°C for one hour. They are then left in an incubator at 20°C for 15 min for 3 successive days. Pre-germinated seeds were planted in tubes containing sterilized soil mixture and watered. Oven bags quarter filled with wheat bran were humidified and then autoclaved at 121°C for 15 min for three successive days. Following this step, sterilized distilled water was poured onto a petri dish containing two weeks old Fusarium culture and the mycelia are scraped to release spores. Then water contained mycelia is added onto the wheat bran. The bag of wheat bran was sealed with cotton at the opening to allow oxygen flow. This bran was then incubated for 2-3 weeks at room temperature to get enough spores. In order to inoculate plants wheat bran was mixed with water and then filtered through a sieve and a cheese cloth to have a solution containing Fusarium spores. These spores are then counted under a microscope and it was adjusted to 1000,000 spores/ml of water. One week after sowing wheat stem base touch with soil was inoculated with 1 ml spore suspension $(1 \times 10^6 \text{ spores/ml})$ and covered with a plastic tent to maintain temperature of $24 \pm 1^{\circ}\text{C}$ and high relative humidity of (80-90%) for 48 h in growth room. After incubation, tubes were placed in plastic trays placed in a randomized complete block design with 5 replications. Plants were grown in growth room for 8 weeks. After 8 weeks plants harvested and scored for disease symptoms. Each plant was given a rating of 1-5 (depending browning percentage on stem) based on a standardized scale for disease reaction.

Screening of wheat genotypes under greenhouse conditions:

To assess wheat genotypes under greenhouse conditions 0.5 gram fungus colonized wheat bran is added to the tubes containing soil mixtures at the same time with seed sowing stage. The experiments are conducted in wheat growing season between October and June. Plants are exposed to drought conditions to help disease improvement at spring time (April-May). Nine months after sowing, plants are harvested and scores are given by using 1-5 scale (being 1 is resistant and 5 is susceptible).

Screening of wheat genotypes under field conditions:

The trials were conducted between October and June wheat growing season. Inoculum was added to the soil at seed sowing time. Each entry was planted in 1 row of 1 m long. The experiments are arranged as lattice design and each entry is replicated 3 times. Plants are scored for typical symptoms of whitehead (WH) using a 0-5 scale (0: No WH, 1: 5-10%, 2: 10-29%, 3: 30-69% 4: 70-89% 5: 90-99%) and browning percentage on the crown (0: No WH, 1: 5-10%, 2: 10-29%, 3: 30-69%, 3: 30-69% 4: 70-89% 5: 90-99%).

I was well involved in harvesting and scoring of wheat genotypes under greenhouse and field conditions as I have arrived at wheat maturity stage in Turkey. Also I was involved in estimating yield trials to investigate the tolerant reaction of 44 wheat germplasm grown under high and low *Fusarium culmorum* inoculum. Finding new source of resistance to crown rot will assist farmers who are suffering yield losses due to the effects of this disease. The line demonstrates the greatest genetic resistance will be tested again and used by the breeding programs for incorporating into high yielding potential cultivars/lines and eventually distributed to farmers.

In addition to the *Fusarium culmorum* project, I was given the opportunity to assist on another project being undertaken at CIMMYT, the screening of wheat lines for resistance against Cereal Cyst Nematodes (CCN). CCN has been a major focus for research in CIMMYT, and CIMMYT researchers are organizers of the International Cereal Nematodes Initiative founded in 2006 and acting as a leading force in many soil borne pathogens courses/workshops globally.

CEREAL CYST NEMATODES

Introduction

Cereal Cyst Nematodes (CCN) can cause significant yield losses, however, their impact is likely underestimated, as the symptoms of CCN infestation are similar to those caused by many other factors and, therefore, often not attributed to CCN. There are two types of nematodes, aerial and root. Aerial nematodes attack components of the plant above the ground. Root nematodes feed on below the ground parts. CCN are root nematodes. However, symptoms of CCN can be seen in parts of the plant that occur above and below the ground. Aerial nematodes cause aboveground symptoms of leaf discoloration, swollen tissue growth, chlorosis, stunted growth, and thin foliage. Root nematodes cause above ground symptoms of chlorosis, stunted growth, wilting or leaf rolling, and thin foliage. Root nematodes also cause below ground symptoms of stubby roots, root cracking, deformed roots and cysts on roots.

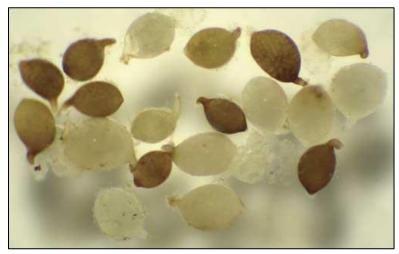


FIG 1. White cysts showing female of cereal cyst nematodes and brown cysts showing the mature cysts after females die and become a shelter to protect eggs.

These cysts are actually female nematodes, which become spheres once reaching adulthood (Figure 6) illustrates the full nematode life cycle. The objective of this project is to identify lines of wheat that are resistant to CCN. It is critical to differentiate between resistant and tolerant lines, as tolerant lines might have normal yields without having resistance. This tolerance allows for CCN multiplication. This can be problematic as a farmer may choose to plant tolerant cultivars due to its high yield, causing nematode levels in the field to increase. When a successive non-tolerant line is planted in this field dramatic yield loss can result. In order to ensure that lines are resistant and tolerant, both soil nematode levels and yield must be determined.

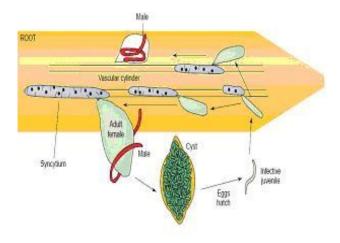


Figure 6: Life cycle of cereal cyst nematode (Heterodera spp.)

Materials and Methods

Soil samples were collected from a field in Yozgat field and *H. filipjevi* cysts were extracted according to Cobb's decanting and sieving method (Cobb, 1918). Cysts were collected and surface sterilized with 0.5% NaOCl for 10 min and rinsed several times in distilled water. The cysts were kept in the refrigerator at a temperature of 4°C. Cysts were then transferred to room temperature (10-15°C) to enhance hatching. The hatched J2 larvae were used as inoculums in screening tests.

Growth chamber experiment

Single wheat seeds were planted in standard small tubes (16 cm in high x 2.5 cm in diameter) filled with a sterilized mixture of sand, field soil, and organic matter (70:29:1 v/v). The field soil and sand were sieved and sterilized at 110 °C for two hours for two successive days, and organic matter at 70 °C for five hours. After plant emergence, three tubes were selected per genotype and were inoculated with 300 freshly hatched J2 in three holes around the stem base. In the week after nematode inoculation, plants were gently watered to increase the efficiency of nematode penetration. Plants were grown in a growth chamber with a 16 hour of artificial photoperiod and maintained at a temperature of 22 ± 3 °C with 70% relative humidity. Experimental units were arranged in a randomized complete block design with three replicates. Based on the period of time needed for cyst formation, plants were harvested nine weeks after juvenile inoculation. Soil from each tube was collected in a two liter pot filled with water for cyst extraction, while roots were washed on nested sieves with 850 µm and 250 µm mesh sizes to free cysts from the root system (Fig 7). Cysts from both root and soil extractions were collected on the 250 µm sieve and counted under a stereomicroscope.

Genotypes were divided into five groups based on the number of cysts per plant, taking into account the reaction of check varieties with known resistance to CCN. The groups were: Resistant (R) = as little or fewer cysts than in known resistant check; Moderately Resistant (MR) = slightly more cysts than in resistant check; Moderately Susceptible (MS) = significantly more cysts than in resistant check, but not as many as in susceptible check; Susceptible (S) = as many cysts as in susceptible check and number of cysts per root system considered damaging; and Highly Susceptible (HS) = more cysts than in susceptible check. Four widely-grown winter wheat check lines Katea (MR), Sonmez (MR), Kutluk (S), and Bezostaya (S) were used in this study.

Along with determining yield we determined nematode levels. Soil samples were taken using augers in the field, and directly from pots in the greenhouse. These samples were taken back to the lab where they were washed to sort out nematodes. Measuring nematodes initial population (Pi) at sowing time and nematode final population (Pf) at harvest time will enable us to study the nematodes reproduction factor by defining the Pf/Pi and give us indication of the tested lines if they are resistant or not.



Fig 2. Sieve for Extracting Cyst Nematodes



Fig 3: Pouring soil samples through the sieve system used to wash for nematodes

Results

After counting cyst number per line, the whole lines are evaluated to their resistant reaction by comparing them to the control check lines which are used in the trails. In the field, to consider the germplasm resistant the nematode initial and final populations in the field were evaluated. If the reproduction factor which is defined as the nematode final population over the nematodes initial population is less than 1 this considered resistant since nematode population did not increase. If reproduction factor is more than 1 this means the line is susceptible. Whereas, in the greenhouse or growth room, we consider resistant lines according to the known check lines; normally plants with cyst number below 5 considered resistant.

THE WHEAT QUALITY LABORATORY AT CIMMYT

While at CIMMYT I also received the opportunity to tour the quality lab in Eskisehir. Here they test the different lines of wheat for factors such as protein content, moisture absorption, sedimentation, energy level, color and seed size. This information is then sent to the breeders and they use it to select which kinds of wheat are preferable for certain tasks. While touring the quality lab I was able to see the steps for determining several of the key factors they look for in wheat. Before any quality values can be obtained the wheat must first be turned into flour. This is done in the quality lab in small batches. The seeds are first turned into whole mill, which is sufficient for most testing. However, some tests require flour, which is timelier to produce.

First I was shown the NIRS machine, which measure protein value, moisture content, and Particle size index (PSI). In order to use this machine a small glass dish is filled with whole mill (Fig. 9). This dish is then placed inside of the machine and is read for all the previously stated values. PSI is used to determine the hardness of a particular breed of wheat. A small PSI value means that the wheat is hard, and preferable for making bread, whereas soft wheat is used to make things such as cookies and biscuits. The values obtained from the NIRS machine are all compared to the Quality standard line of wheat Bezostaja, a wheat variety from Russia. Unless a line exceeds a value



FIG 4. Preparing dish with while mill in order to measure protein content

from this breed of wheat it is not considered an improvement.

I was also given the chance to learn how seeds size is determined. First a 50g sample is measured out. This sample is placed in a three-sieve system. The top sieve is 2.8mm, the middle one is 2.5mm, and the bottom is 2.2 mm. The seeds are then shaken for one minute. The sieve system is then turned on its side and the seeds fall out into their separate size categories. Each category is weighed, and this determines the average seeds size of the line.

The third process I was able to see from start to finish was identifying the sediment value of a line of wheat. First 1g whole mills are measured out. Then 8ml of Bromfenol is mixed in. After 4 minutes have passed Lactic acid solution is added. The solution is then mixed and after 21 minutes the solution has separated. The top darker portion of the mixture varies in size depending upon the sediment value. A large top portion indicates a low sediment value, ideal for cookies or biscuits. While high sediment value would be used to make bread or pasta.

Other processes run by the quality lab include the measuring of moisture absorption, which is done by a Farinograph. Another machine can measure the energy level of wheat. Low energy levels are desired for cookies and biscuits, while high for bread. Color is also determined, as consumers prefer certain colors of wheat for certain foods. For example yellow wheat is desirable for making pasta. The quality lab is an essential part of selecting for wheat lines. The information obtained is sent to the breeders and used to identify superior lines. This may mean superior in ways of nutrition, or a line that is preferred by consumers due to a feature such as color.

CULTURAL EXPERIENCE

While in Turkey I had the opportunity to completely immerse myself in another culture, as opposed to simply travelling through a country. It was this immersion that allowed a cultural exchange in which I was the primary beneficiary. The Turkish people were incredibly kind to me, and everyone at the institute made sure I was given the chance to experience all the wonders of Turkish culture. Seeing not only the differences, but also the similarities, between Turkey and America, was one of the most rewarding aspects of this internship. It is by experiencing these similarities that I was reminded that regardless of geographical, cultural, and language differences, people share the same hopes and goals, be they professional or personal. I cannot thank my mentors at CIMMYT, the Turkish people and the Bourlaug-Ruan internship enough for providing me with the opportunity to have these experiences.

During my stay at CIMMYT I was given the chance to travel to Yozgat to assist with the harvest. There I experienced the vast difference between Turkish rural and urban life. While in Yozgat I



met and got to know many of the field workers (Fig 10). Although they did not speak English they were very friendly and made me feel welcome. I learned about their lives and how they earn a living. These workers can work only during the summer. They must save their earnings to keep for the winter, when they cannot work. Seeing how difficult life can be, not only for people in different parts of the world, but for people in different parts of one country, was

FIG 5: FIELD WORKERS IN YOZGAT



an eye- opening experience.

I also experienced the everyday life of a Turkish citizen during my stay in Eskisehir. Surprisingly, urban life in Turkey is quite similar to that in America. Turkey is a relatively modern country in most regards. Some of my most enjoyable days were simply wandering around and exploring Eskisehir. Eskisehir was a beautiful city, and due to its two universities it was full of young college students and activities. Many of the leisure activities in Turkey are the same as they would be in the

FIG 6: IFTAR WITH FRIENDS

U.S. I spent time in coffee shops, going shopping, and eating at new restaurants.

The food in Turkey was one of the most enjoyable aspects of my cultural experience. Everyone made sure I had the opportunity to sample many different types of Turkish food, all worth trying. My second month in Turkey coincided with Ramadan. I truly enjoyed experiencing that part of Turkish/Muslim culture. I was given the chance to enjoy Iftar, or the breaking of the fast with some of my Turkish friends, and I could try many of the traditional Ramadan foods, such as pide.

My internship in Turkey allowed me to establish many new friendships, and meet a tremendous amount of interesting people doing important work. I cannot thank them enough for all they did to educate me, show me their country, and to make sure I felt at home. It is a testament to their hospitality that by the conclusion of my stay I did, in fact, feel at home.



FIG 7 IFTAR PARTY WITH, AMER DABABAT , GUL ERGINBAS-ORAKCI, AND ELFINESH SHIKUR

FOOD SECURITY REFLECTION

My time at CIMMYT taught me many things; the foremost being that scientific research is the main path toward alleviating poverty and hunger. Prior to this experience I would have suggested that money and donations to the needy were the most effective means of helping others. However, after seeing the critical work done at CIMMYT, and the tremendous impact that research can have on food productivity, I know that that is not the case. It is the pursuit of science, combined with CIMYYT's system of worldwide partnerships, which provides the essential tool to alleviating poverty.

Through the development of new technologies and new resistant lines of any plant, one gives farmers in developing nations the opportunity to increase their yields, and reduces the need for harmful and expensive pesticides. Finding host genetic resistance is a diverse tool that can be used to combat a wide variety of poverty inducing pathogens. By developing methods to breed for resistance one can find solutions to almost all pests, diseases and climate conditions.

While at CIMMYT I also learned the importance of international collaboration in the fight against world hunger. Through CIMMYT's wide network of centers and partnerships they can disseminate the results of their research throughout the world. International collaboration allows not only for the sharing of results, but of resources. For example, molecular identification is necessary to identify strains of *Fusarium culmorum*. The institute in Turkey does not have the equipment necessary to do this, so Elfinish Shikur will be traveling to Pullman Washington in September in order to complete this component of the experiment at Washington State University. Without international collaboration her research would have been restricted or, even impossible. During my internship I saw many of my co-workers working with other countries, such as Russia, Germany and the U.S. This constant communication with a worldwide community of scientists ensures that poverty is being addressed from all possible angles.

The Borlaug-Ruan International Internship has provided me with the opportunity to learn, and experience, more than I ever thought possible. Not only did I spend time in the laboratory expanding my knowledge of research, but also I was given the chance to travel to a rural area and see first hand the life of the workers. I met so many brilliant and passionate people who worked incredibly hard, this insight into their professional lives was more than enlightening, it was inspiring. I cannot thank them enough for all they did for me, and all that their research is doing for the world.

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