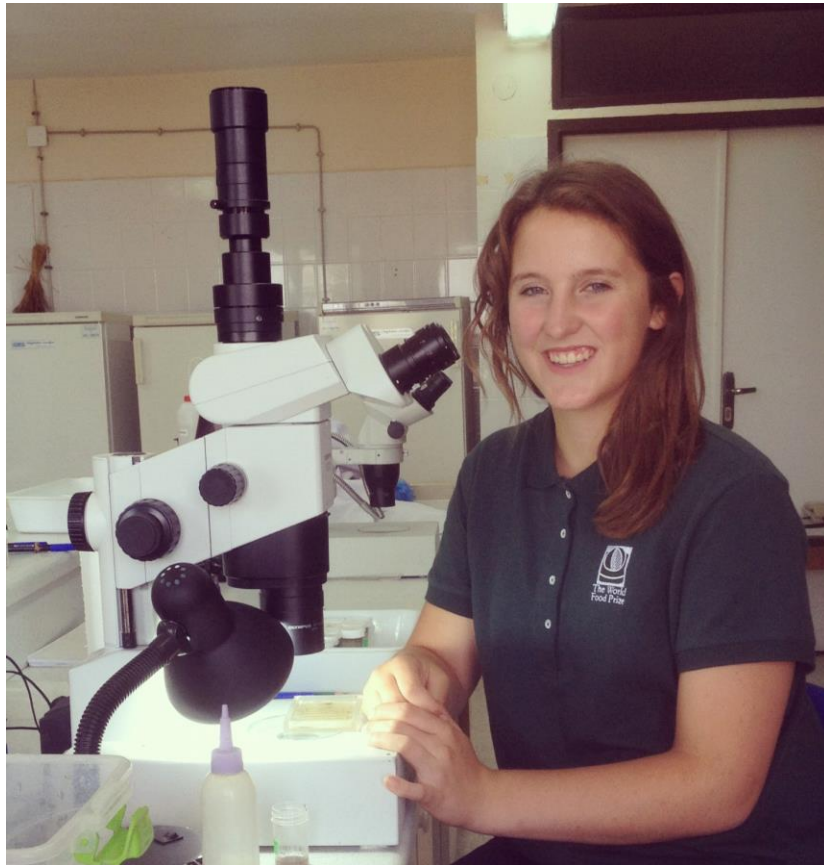


# **Summer as a Wheat Pathologist: Improving Wheat for Food Security**



**CIMMYT-Turkey**

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2012 Borlaug-Ruan International Intern

International Maize and Wheat Improvement Center (CIMMYT)

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# Introduction

My ambition to travel to another country and work on innovative science that increases food security came true when I was chosen to be a Borlaug-Ruan International Intern in Turkey. To make this dream come true, it took hard work and dedication. It was not only my hard work but also the hard work of its founders, Dr. Norman Borlaug and John Ruan, that allowed me to be apart of this program. There were also many hard working individuals who have helped me along my journey.

My journey began on my family farm in Red Wing, Minnesota where I learned the importance of responsibly growing safe food and taking care of the land. From a very young age I had a lot of responsibilities: I cared for my large garden, and my many farm animals. I was never afraid to try something new and be different; this philosophy continues in my everyday life.

I first heard about the world food prize from Addie Thompson at a genetic workshop at the University of Minnesota. With support from my family and agriculture education teacher, Christopher Sheehan, I wrote my application for the Minnesota Youth Institute. The paper I submitted with my application was regarding the need for Agricultural Education in Guatemala. At the Minnesota Youth Institute I was chosen to advance to the 2011 Global Youth Institute. After an inspiring time at the Global Youth Institute, I applied for the Borlaug-Ruan International internship. I was interested in being an intern because I believe that education and experience are the foundation of increasing food security. We can all read essays and watch documentaries regarding food insecurity, but true innovation comes when education and experience collide.

At a young age I first witnessed food insecurity when I visited Guatemala with my family. I observed erosion wiping out farmers fields and knew that this could be corrected. After this life changing experience I was inspired to dedicate my life to increasing food security. Since the population is growing rapidly, I did not want to wait until graduation. As a high school junior I started my Applied Plant Science degree at the University of Minnesota; I did not want to use the excuse of being young to limit my impact. The World Food Prize helped me live out this mission when I interned at the International Maize and Wheat Improvement Center (CIMMYT) in Turkey.

It was a true honor to work with CIMMYT, the same international program that Dr. Borlaug worked with. CIMMYT is an international organization that strives to sustainably increase global food security through increasing the productivity of maize and wheat. With CIMMYT's Soil Borne Pathogens (SBPs) program in Turkey, I focused on Cereal Cyst Nematodes caused by *Heterodera* species and Crown Rot caused by *Fusarium* species. I worked closely with Dr. Gül Erginbaş, a biologist, and Dr. Amer Dababat, a nematologist, through my entire stay and learned so much from both of them.

The SBP-CIMMYT program in Turkey mainly focuses on screening wheat germplasm for resistance and/or tolerance against Crown Rot and Cereal Cyst Nematodes under greenhouse, growth room and field conditions. The main goal of this training was to gain knowledge about the theoretical and practical screening protocols for these diseases. I was involved in a screening program run by SBP-CIMMYT staff.

# Crown Rot (*Fusarium culmorum*)

## Introduction

Crown rot caused by *F. culmorum* and *F. pseudograminarum* is one of the most economically important diseases of cereals in many rain-fed wheat cropping regions such as in Turkey, it occurs in a complex of *Fusarium* species and other pathogens. Addition of Cereal cyst nematode, CIMMYT – Turkey SBP program has also been conducting research on screening of resistant wheat germplasms for *F. culmorum* which is the predominant pathogen in cereal fields in Turkey.

An extensive survey done in 2000/2001 showed that *F. culmorum* is the dominant pathogen in Central Anatolian Plateau, Marmara, Mediterranean, South East Anatolia and Black sea of Turkey (Tunali at all., 2008). A recent survey has also showed that *F. culmorum* as the main pathogen in the three agroecological region of northern Turkey (Bentley at all., 2006). It has been found to cause yield losses of up to 43% on winter wheats (Hekimhan at all., 2004).

Whiteheads during milk dough stage and a honey-brown discoloration on the lower leaf sheaths are the typical symptoms of crown rot on cereals caused by *Fusarium*. It occurs virtually wherever wheat is grown and has a wide range of host. High populations of pathogens can be significantly reduced by a non-host crop rotation of 2-3 years (Wallwork, 2000). Although a crop rotation decreases the pathogens, this is usually not an economically viable option especially for a crop like wheat that counts for 20% of the world's caloric intake.

There are varieties with known resistance, however, many widely used varieties are susceptible (Nicol et al., 2008). Wheat varieties vary from being very susceptible to moderately resistance to *Fusarium*. All durum cultivars appear to be quite susceptible but still the use of moderately resistance cultivars to reduce grain yield losses can be an option. Wheat, barley, oats, and triticale are all equally susceptible to *Fusarium* at the end of the season as the fungi grow in the stubble debris.

Although wheat is remarkably resistant to *Fusarium*, it becomes fully susceptible under water stress (Cook and Veseth 1991). There are two winter wheat lines (ZANDER 44 and TAM 01/4/BL/AU/3/AGRT/HYS/7C/5/F/34/71/NAC) originating from Turkey-CIMMYT-ICARDA, CROC\_1/AE. SQUARROSA (224)// OPATA from CIMMYT Mexico (Nicol et al. 2008) and two spring wheat lines (GBHE 99005-084-b and GBH 01/00066/DH-45) from Australia that showed promising resistance against *Fusarium* (Erginbas et al. 2009).

To decrease the yield loss due to crown rot disease, the SBP-CIMMYT program in Turkey focuses on screening wheat germplasms for their resistance and/or tolerance against *Fusarium culmorum* under greenhouse, growth room and field conditions. The main target of the program to find high yielding, crown rot resistant germplasm under dryland conditions which will be used in breeding programs.

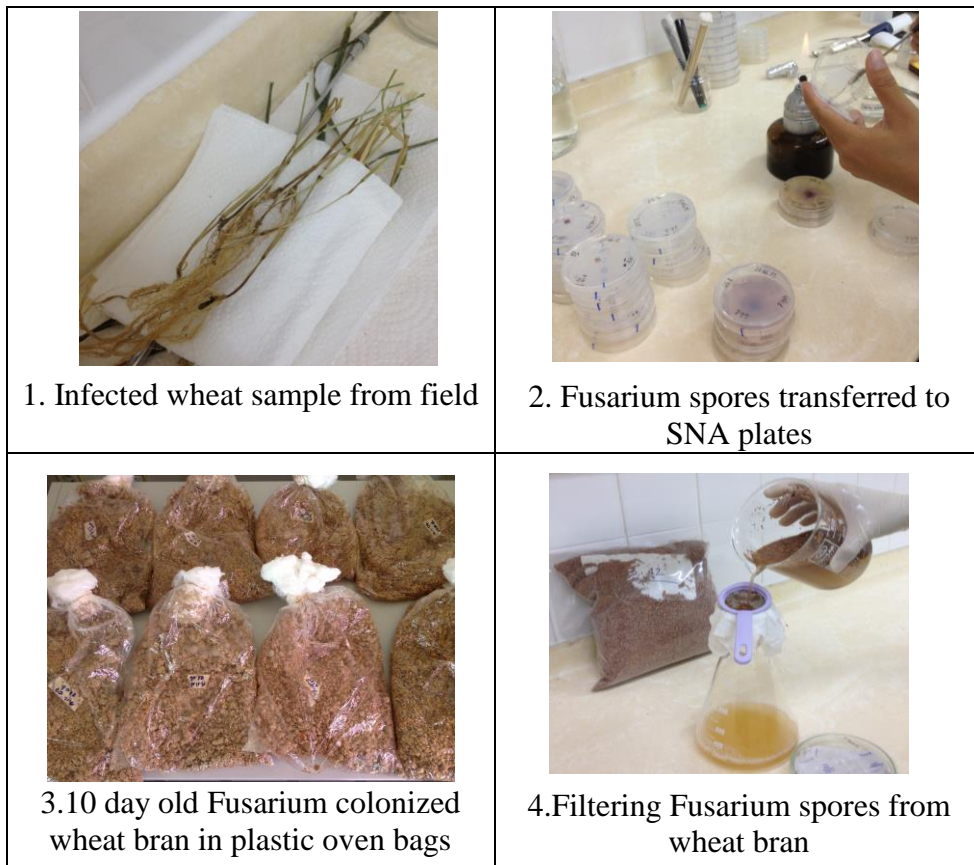
## Screening for Resistance and/or Tolerance

In my work with the SBP-CIMMYT I was unable to follow the same experiment throughout the steps, nevertheless I was able to gain knowledge and experience conducting each step. In order to have *Fusarium* inoculum to inoculate different experiments in a controlled

environment, the program has to maintain Fusarium culture. It is important for the SBP team to harvest new culture every year to ensure the inoculation of the most current evolutionized pathogen. Since Fusarium is continually changing to better fit the environment, it is important to continue to screen resistance to the pathogen.

### Material & Methods

Fusarium is isolated from infected wheat crown and/or stem (Fig. 1). Surface sterilized crown is placed on Potato Dextrose Agar (PDA) media, then left under room temperature for 4-5 days. Identification is done on water gar (WA) media then spores are transferred to SNA plates for purification and multiplication. Plates are incubated for 7-10 days for sporulation. The pure Fusarium spore suspension is added to autoclaved wheat bran under sterile conditions and grown at  $23\pm 2^{\circ}\text{C}$ , alternating under a 12 h period of light and a 12 h period of darkness. After two weeks, Fusarium colonized wheat bran is suspended in water and spores are filtered through 2 layers of cheesecloth from the wheat bran and adjusted to  $1 \times 10^6$  per ml of water.



**Fig.1: Extraction process of Fusarium Spores**

## Material & Methods (Cont)

The screening experiments for Fusarium are set up as randomized complete block design (RCBD) with 5 replicates under growth room conditions. One week after sowing, seedling stems are inoculated with 1 ml of the adjusted spore concentration (Fig. 2). Plastic tubes are covered by plastic for 48 h under humid conditions.



**Fig. 2. Fusarium Inoculation, Each seedling is inoculated with approximately  $1 \times 10^6$  spores**

Eight weeks after inoculation, plants are harvested. The roots and stem are washed and the scores are given according to the percentage of browning on the crown and stem (Fig. 3). Disease severity is recorded based on a 1-5 scale, with 1 being clean or very little diseased, and 5 being completely diseased (Fig. 4). Susceptible and resistance check lines are also included to compare and confirm the reaction of the tested germplasm.



**Fig.3. Experiment harvesting and disease scoring**



**Fig. 4. 1 to 5 scale used to score *Fusarium culmorum*.**

## Results

The table shows the mean scores of the genotypes. These newly screened germplasms are evaluated and resistant varieties, such as Sunco and Lov41//li7/le2062, are selected to breed with different high yielding varieties or evaluated again under different conditions. Screening for disease resistance and then implementing them into breeding programs, that strive to increase yields and drought resistance, is extremely necessary to double yield by 2050 to satisfy the growing population.

**Table 1. The mean of crown rot of wheat germplasms**

Wheat Genotype	Type	Mean
Kiziltan	DW	4,6
Ahmetaga	BW	4,0
Seri	BS	3,6
Cesit	DW	3,6
Konya 2002	BW	3,1
Sonmez	BW	2,9
F13011.12/Attila	BW	2,5
Bezostaya	BW	2,5
Altay	BW	2,2
2,49	BS	2,2
Sunco	BS	1,6
Lov41//li7/le2062	BW	0,7

DW: Durum winter, BW: Bread winter, BS: Bread spring



# Cereal Cyst Nematode

## Introduction

Plant-parasitic nematodes are of great economic importance. However, because they live in the soil, they represent one of the most difficult pest problems to identify, demonstrate and control (Stirling *et al.*, 1998). Their effects are commonly underestimated by farmers, agronomists and pest management consultants, however, it has been estimated that some 10 percent of world crop production is lost as a result of plant nematode damage (Whitehead, 1998).

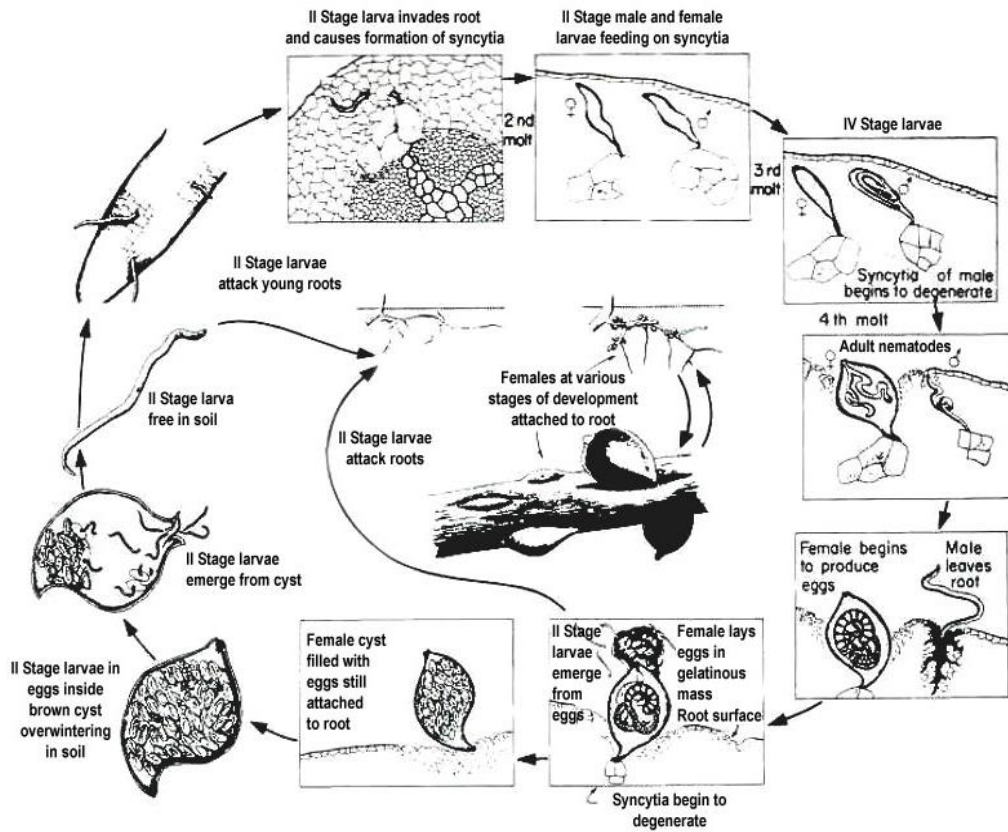
Cyst nematodes are endoparasites of plants that form specialized and complex relationships with their hosts. The female remains inactive and are developmentally committed to the feeding site. Cyst nematodes are characterized by the developing females that swell to form hardy cysts that each contain several hundred eggs, which may remain dormant in the soil for several years. If large numbers of juveniles invade, root growth is slowed and root systems are stunted making less efficient use of water and nutrients in soil and thereby reducing yields. CCN nematodes primarily occur in the Mediterranean but have also been recorded in eastern and northern Europe, the Middle and Near East, North and South Africa, and Japan. It is also known as the cereal cyst nematode and the wheat cyst nematode.

Crop rotation and nematicides have been used to decrease the effects of CCN. However, the use of nematicides to control CCN is not advisable because of health and environmental problems, as well as the economic expense. Although a crop rotation does not hinder the environment, it is not an economically viable option. Cultural methods offer some control options, but have limited effect. One of the most acceptable methods to control cereal nematodes is by the use of resistant germplasm which is environmentally friendly, cost effective, and easy to implement.



**Fig. 5. CIMMYT greenhouse, screening for resistance to Cereal Cyst Nematodes**

## Life Cycle of Cereal Cyst Nematodes



**Fig. 6. Life cycle of cereal cyst nematodes, source: South Australian Research and Development Institute-SARDI**

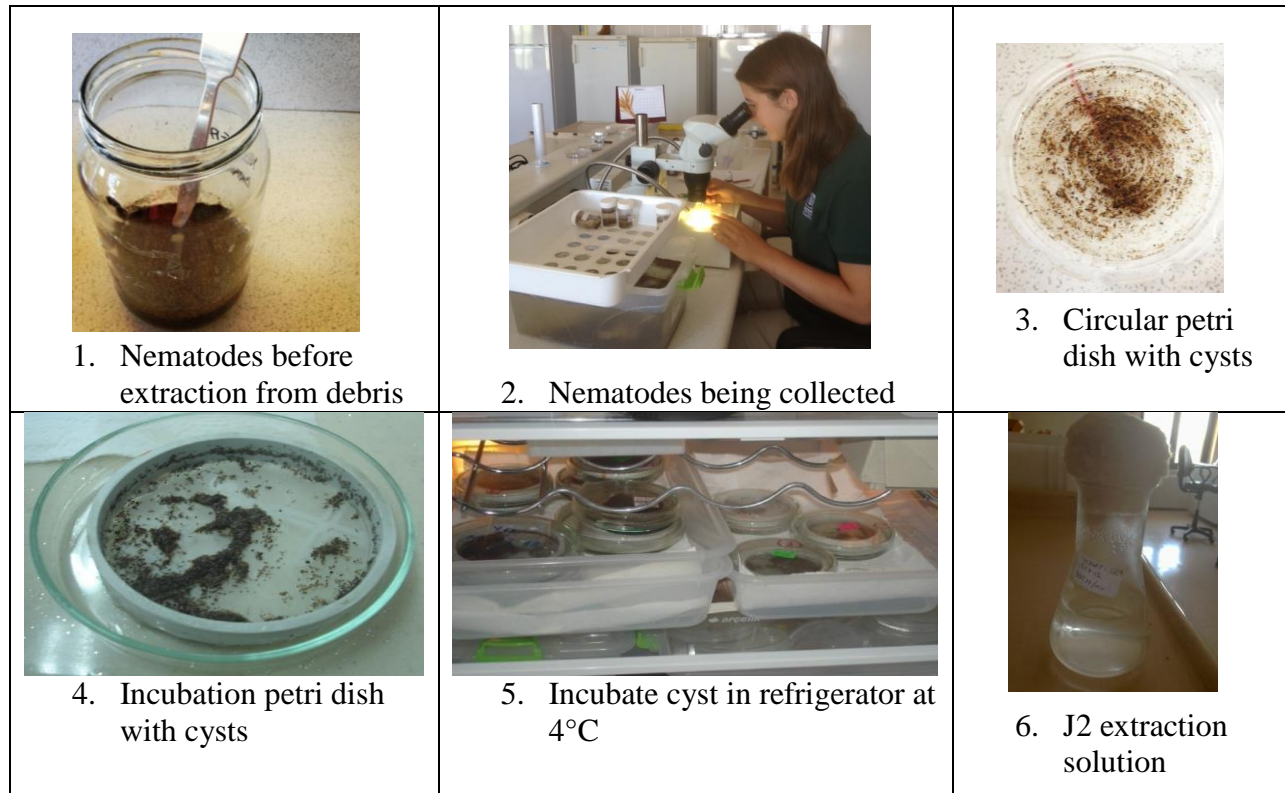
## Methodology

In order to have pathogens to inoculate different experiments in a controlled environment, the program has to maintain nematode cysts. An auger is used to collect soil samples from infested fields. The soil is then taken to the lab where the cysts are extracted from the soil by using the floatation method and then filtered through special sieves (850  $\mu\text{m}$  and 250  $\mu\text{m}$  mesh) as indicated in (Fig. 7). The floating method consists of stirring the soil mixed with water for 15 seconds, then waiting 10 seconds for the heavier debris to sink, and finally it is pored through the sieves, this process is repeated up to 5 times to ensure all cysts are extracted from the soil. The big mesh sieve is used to separate any big soil particles and/or any other undesirable materials whereas the second sieve 250  $\mu\text{m}$  is used to collect the cysts. The cysts on small sieves are sterilized in 0,5% NaOCl for 10 minutes and washed with distilled water. Sterilized cysts are transferred to petri dishes, which are labeled with field location and other information.



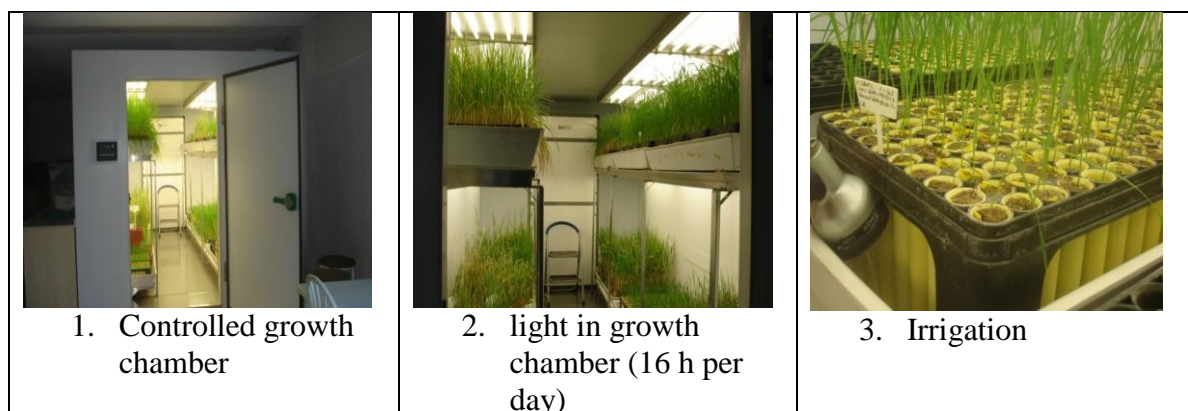
**Fig. 7. Steps for cysts extraction from soil**

The nematode cysts are then collected from the product of this filtration under a microscope with forceps. A circular dish with lines is used in order to efficiently make sure every cyst is harvested. Extracted cysts are placed in glass petri dishes, which have fine mesh gauze and a glass trough at the bottom. Distilled water is added to the petri dish to keep cyst moisturized for long term hatching encouragement. Petri dishes are kept in refrigerator at 4°C for 3-4 months to induce cysts hatching. After 3-4 months, petri dishes are taken out of refrigeration and placed under room temperature (20-23°C) for a few days. During these few days hatching is observed daily under microscope and J2s are collected in glass beakers (Fig. 8).



**Fig. 8. Collected cysts in petri dishes and hatched juveniles**

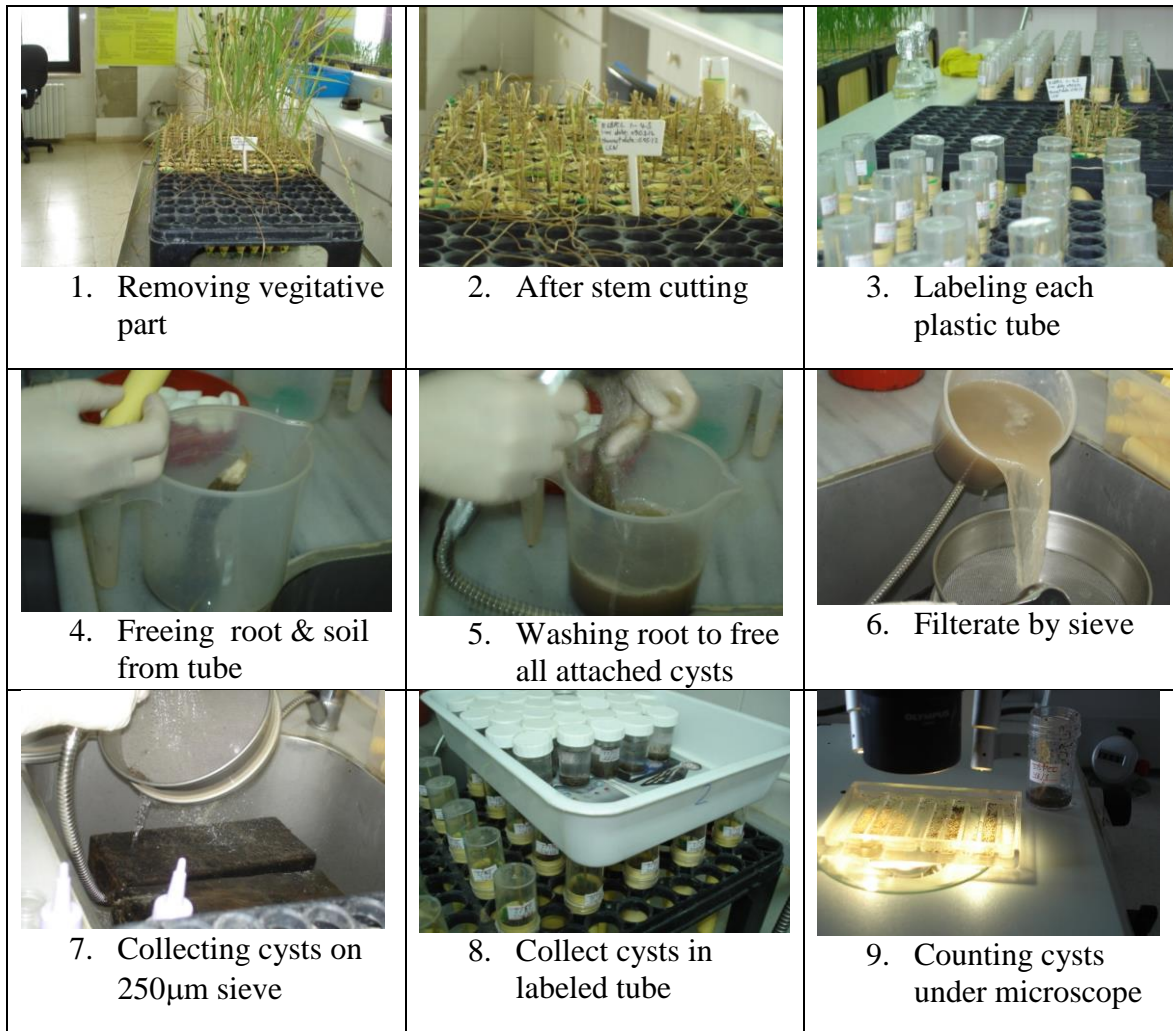
The J2 concentration was adjusted to 250 J2 per 1 ml of distilled tap water. The wheat experiments are set up in a randomized complete block design (*RCBD*) with 3 to 5 replicates. At the sowing date each tube was inoculated with 1 ml of suspension into 3 holes made around each seedling. The plants are grown in the growth room for 9 weeks, at 19 to 24 °C, 16 hours of artificial lights, and a relative humidity of 60 to 80% (Fig. 9).



**Fig. 9. Wheat plants grown under the controlled growth room conditions**

## Harvest of Cereal Cyst Nematode experiment

Nine weeks after J2 inoculated, plants were harvested. Each tube was washed by using decanting and sieving method. Collected cysts from each plant are counted under microscope (Fig.10). Germplasm performance is evaluated based on the number of cyst compared to the resistance and susceptible check lines. The 1 to 5 scale is normally followed at SBP CIMMYT Turkey Program. Score 1 is considered resistance while score 5 is considered highly susceptible.



**Fig.10. Harvesting steps for cyst extraction from screening trials**

**Resistance scale 1-5:** 1: (R) shows better reaction as good as used check line, 2: (MR) almost similar to check lines used, 3: (MS) consist of more cysts than the used check lines, 4: (S) similar to susceptible check lines and number of cysts per root system considered damaging, and 5: (HS) which is similar or highly infested compared to the used check line.

## Results

The genotypes that showed the best resistance are shown in Table 2.

**Table 2: The best performed 4 germplasms against *Cereal Cyst Nematodes***

Genotype	BW/DW	Type	OC	Genotype Reaction (1-5)
KIZILTAN	DW	WW	TK	1
YAKAR	BW	WW	TK	1
SONMEZ	BW	WW	TK	1
KATE A-1	BW	WW	TK	1

DW: Durum wheat, BW : Bread wheat, WW : Winter Wheat, Origin Country: OC, TK: Turkey

## Field Trials

SBP-CIMMYT also conducts field trails to monitor yield, drought tolerance, and disease resistance against Cereal Cyst Nematodes. SBP-CIMMYT has a variety of locations where they have field plots. I was able to travel with Dr. Amer Dababat and Dr. Gül Erginbaş to field trials in Yozgat, Adana, and of course Eskişehir. The trials compare fields with low and high nematode populations. When visiting these trials we labeled the different varieties according to a map used when planting. We would then record the average height of each variety. It is important to consider the height of wheat because height is linked to qualities, such as, straw potential and drought tolerance. If the plots were ready to be harvested we would harvest the grain and collect soil samples to be brought back to the lab.



**Fig. 11. Field Plot in Adana with Dr. Dababat, and Dr. Erginbaş**



**Fig. 12. Measuring average wheat height**

# International Winter Wheat Improvement Program and the Drought Yield Trial

When I was not working in the lab or field for my research with the SBP program, I was helping out with the International Winter Wheat Improvement Program (IWWIP) and a CIMMYT drought yield trial. I was fortunate enough to spend a week working with CIMMYT-Turkey's head wheat breeder, Dr. Alexei Morgounov, who leads IWWIP, and Beyhan Akin, who is a wheat breeder. IWWIP is an international collaboration between CIMMYT, the Turkish Ministry of Agriculture (TAGEM), and the International Center for Agriculture research in the Dry Areas (ICARDA). IWWIP strives to improve winter wheat cultivars. While working with Dr. Morgounov, I learned what it is like to be a wheat breeder.

We were selecting a nursery of more than 500 varieties for favorable traits: disease resistance, genetic uniformity, and high yields. Just by looking at a specific variety Dr. Morgounov knew if it was favorable. Although he knew right away, he took the time to explain why he selected the variety. I spray painted each variety he selected and then harvested 50 spikes from each. We then threshed the spikes to be tested at the quality lab, and/or be planted the following season. Each variety that demonstrated desirable traits will be planted the following season on a larger scale to check for genetic uniformity.

Although I was quite busy with my responsibilities with the SBP program, I was happy to assist my friend Jamala Mursalova, a PHD Student from Azerbaijan, with a CIMMYT drought yield trial. In this trial there was one nursery that was irrigated by drip irrigation, and one nursery that had no irrigation, both nurseries had the same varieties. With Jamala, I helped measure the different plots, harvest 50 spikes from each plot, thresh the spikes, and weigh the grain. This process was done every week to monitor development. The goal of this project was to find varieties that did just as good or better in the drought nurseries. I do not have this data because the data has not yet been evaluated. The experiments did however go very well and Jamala and I noticed some of the varieties to be doing better in the drought nurseries.

Additionally I was able to spend some time in the quality lab where they analyze the protein, starch, and other wheat qualities. Different wheat varieties have higher protein and therefore are better for different uses. It was very interesting to be apart of the many roles it takes to get sustainably productive seeds to farmers. It was rewarding to work on a variety of research projects.



Fig.13. Alexei Morgounov, Beyhan Akin, and Tessa Ries.



Fig. 14. Tessa Ries and Jamala Mursalova

# Cultural Experience

When I set off for my adventure to Turkey, I could have never fathomed the life-long connections, friends, and memories I would make. On top of the career-shaping experiences I had in the laboratory and field I was also immersed in the lovely Turkish culture. The whole time I was in Turkey I felt welcomed and cared for. In the laboratory I felt like a productive member of the team, and in the community of researchers I felt welcomed. In the laboratory I made life-long friends and enjoyed learning more than science but also the Turkish language and culture. Every day in the laboratory we would have Çay (tea). I became very close with the scientists I worked with and it was a pleasure to get to know them all.

While in Turkey I stayed in the institute guesthouse where I was a part of the ever-changing, always exciting community of traveling scientists. I was able to make friends with people from all over the Middle East including: Azerbaijan, Uzbekistan, Iran, Palestine, Nepal, Tunis, Russia, and, of course, Turkey. Even though there was a language barrier I was able to communicate quite sufficiently with the help of my friends Jamala and Ömer.

I learned to embrace the many different cultures. The scientists and I would come together for dinner often, and we would all share food from our homelands and talk about our cultures. I learned to respect their culture just as they respected mine. I was often showered with questions regarding America's culture and language. In the United States we are often blinded to the reality of our similarities by the exaggeration of our differences. It was an honor to be part of a culture unified with the mission of increasing food security.

The generosity of the Turkish people was overwhelming. I do not recall neither a day nor evening that I was not invited somewhere. I was able to have dinner with many different families. I even learned how to make Turkish Çay (tea), teacake, and börek, a pastry made of thin flaky dough. I felt a part of the whole community. I became friends with the teens who live in the institute and I found myself taking many walks with them learning what it is like to be Turkish. We would walk around the institute and visit their community garden and orchards.

Although I did not get to stay with a family the whole time, I was able to be a part of many of the scientists' families, and I stayed with a local farm family. The family I stayed with was very kind. Their farm was much smaller than I am used to but they grew most of their own produce and sold enough to make a small profit. I was very impressed by how many people garden in Turkey. Even people with limited yard space use all they can for a garden. Many people who live in apartments even grow vegetables in pots. The family also had many chickens that I helped them tend to. I saw the importance of agriculture research in their gardens and fields, and realized how vital it was for them to have pathogen resistant and high yielding crops.



Fig. 15. Sevil Yavuz, Jamala Mursalova, and Tessa Ries



Fig. 16. Dinner with Friends



# The Future of Food Security Reflection

Before getting on the plane that much anticipated June day, I was naïve to the world. I knew agriculture work was hard, but I never saw how hard it was with out the adequate technology. One day the wheat harvester was not available for my team to use so we harvested a field by hand. This hard work made me comprehend the work thousands of farmers in developing nations have to go through just to harvest their crops.

Throughout my work, I often stepped back and looked at the big picture. The work at times was hard and seemed tedious, but when I stepped back I realized why it was worthwhile; I realized that it was for the nearly one billion people who are food insecure. My efforts strived to help poor farmers feed their families and communities with better varieties of wheat; it was so those families could make a profit on their crops so they can send their children to school. Since I realized that my work was helping people I had a much different mentality. I was committed to magnifying my impact. In the lab I made sure to always do my best and pay attention to detail. After work and on weekends, I helped other people with their projects and learned about what they were doing.

Becoming aware of the barriers to food security was only part of my realization; my eyes were also opened to the opportunities and advances in food security. I was able to witness many passionately committed scientists who are devoting their lives to increasing food security. The process of wheat breeding is very complex and takes many hands. There are many factors to consider, such as drought tolerance, yield, pathogen resistance, and protein quality. I was able to spend time with scientists working on these different factors.

As our world becomes more and more globalized it is important to have the developed countries' expectation of a food secure nation, everywhere. Our wish for our children's food supply to be bountiful cannot come true unless the whole world's next generation is food secure. When we can be half way across the world in half a day we are truly one, therefore collaboration and cooperation is vital for feeding our population of nearly 7 billion and growing. The responsibilities and opportunities for the next generation of leaders are endless and exciting. When I walked through the CIMMYT field plots in Turkey this opportunity and responsibility came to life.

I realized how blessed I am to have the opportunities I have, and with this comes many responsibilities. After a great opportunity I wish to "pay it forward" and help others have opportunities. I am so appreciative of CIMMYT and The World Food Prize, for not only helping me learn the importance of agriculture research, but also allowing me to observe their true commitment and passion towards increasing food security. I hope to mirror their commitment and passion in my everyday life. My future endeavors are forever changed. I am now inspired to further my education in Plant Science and continue to gain research experience. After the long days in the hot Turkish sun, I am no worse for the wear. I am changed: changed for the better after seeing reality. The reality is lots of hard work ahead and, more than that, it is endless amount of hope.

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