

More Changes than Wheat

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Table of Contents

An Introduction to CIMMYT & Wheat Research	3
My Project	5
Pioneer Hi-Bred International in Mexico	10
My Personal Experience	14
Photos	18
Acknowledgements	21

The Influence of CIMMYT and Wheat & Maize Research in Fighting Hunger

In 1943, the International Maize and Wheat Improvement Center (CIMMYT) began as a pilot program, sponsored by the Rockefeller Foundation and the Government of Mexico. The pilot program became an innovative collaboration of international and Mexican scientists. CIMMYT's most renowned scientist, Dr. Norman Borlaug developed shorter, more efficient and responsive wheat varieties. These varieties helped Mexico become self-sufficient in wheat production by the late 1950s.

Having successfully accomplished the main basis for the founding of CIMMYT, CIMMYT's researchers advocated the "Mexican innovation model" in other countries that would greatly benefit from self-sufficiency in wheat production. The first country to benefit from this model was India in 1967. After the significant increase in wheat production from 1967 to 1968 in India, Pakistan began importing the Mexican wheats. By 1971, these two countries had doubled their wheat harvests. This was the beginning of the "Green Revolution."

When Norman Borlaug won the Nobel Peace Prize for his work, the world recognized the achievements of the Green Revolution. One year later, the Consultative Group on International Agricultural Research (CGIAR) was formed by development organizations, sponsors, and foundations) to spread the impact of research to more crops and nations. Even though CIMMYT has accomplished its original goal, it continues to be an innovative leader of the world in global maize and wheat improvement for the poor in developing countries. They continue conducting research to create effective livelihood systems where maize and wheat provide food security to rural households and systems in which maize and wheat lessen poverty, generate income, and encourage economic growth in all the impoverished areas of the world that depend on these two important crops.

If the world's CGIAR centers had not been created after CIMMYT, "crop yields in developing countries would have been 19.5%-23.5% lower; prices for food crops would have been 35%-66% higher; imports would be 27%-30% higher; calorie intake would have been 13.3%-14.4% lower; and 32-42 million more children would have been malnourished." (*Seeds of Innovation*)

These statistics show CIMMYT's research, and the CGIAR centers that were produced in its form, have affected billions of people in the world; yet, it keeps working for the remaining 3 billion people living in poverty around the world. Out of these 3 billion, 1.2 billion live on less than US\$ 1 a day and nearly all in South and East Asia or Sub-Saharan Africa. There are 815 million people that suffer from hunger in the world with the vast majority of hungry living in Latin America, Africa, and Asia. In developing countries, where the malnourished live, about 25% of all calories consumed are from maize and wheat that occupy 200 million hectares. It is estimated that by 2020, developing countries will need 368 million additional tons of maize and wheat, over a 50% of what is needed today (700 million tons), but nearly 6.25 million people could escape this severe poverty with only a 1% increase in agricultural yields.

The research conducted at CIMMYT focuses on creating a larger, functional, and accessible collection of maize and wheat genetic resources; promoting policies that remove constraints on the adoption of their developments in plant technologies; reducing the vulnerability of poor households to stresses like drought, infertile soils, diseases, and insects; improving nutrition of maize and wheat varieties; giving small farmers access to markets with value-added crops; promoting crop rotation practices; developing varieties conducive to resource-conserving technologies; conserving natural resources; and managing genetic diversity. Since 70% of the world's poorest people depend on agriculture for food and a livelihood, CIMMYT and the CGIAR

center's research can have drastically positive affects on the world's poverty and hunger situation.

CIMMYT. 2004. *Science and Partnerships to Reduce Poverty and Hunger: Medium-Term Plan of the International Maize and Wheat Improvement Center (CIMMYT), 2005-2007*. Mexico, D.F.: CIMMYT.

CIMMYT (International Maize and Wheat Improvement Center). 2004. *Seeds of Innovation: CIMMYT's Strategy for Helping to Reduce Poverty and Hunger by 2020*. Mexico, DF: CIMMYT.

Lantican, M.A., H.J. Dubin and M.L. Morris. 2005. *Impacts of International Wheat Breeding Research in the Developing World, 1988-2002*. Mexico, D.F.: CIMMYT.

Reynolds, M.P., S. Rajaram, and A. McNab, eds. 1996. *Increasing Yield Potential in Wheat: Breaking the Barriers*. Mexico, D.F.: CIMMYT.

Thaumatococin-Like Protein in Transgenic Wheat with Tan Spot Resistance

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Abstract

Tan Spot is a foliar blight of wheat that is becoming a more important problem in many parts of the world. It is a fungus that can survive in wheat stubble, infecting the crops year after year. In the recent past, crops have been genetically-modified for different resistance traits. At the Applied Biotechnology Center at CIMMYT, 12 related wheat lines (Bobwhite SH 98 26) known to have high transformation efficiency were modified with a barley gene for thaumatococin-like protein (TLP). TLP is a PR-5 protein, meaning it exhibits antimicrobial activity, or gives an organism increased pathogen resistance. These 12 lines and non-transformed Bobwhite SH 98 26 were evaluated after being inoculated with a Tan Spot suspension for resistance or susceptibility to Tan Spot. The results show that TLP does increase wheat's resistance to Tan Spot, also showing that the effects of a TLP wheat line usage in a Tan Spot prone area of the world could have a significantly positive effect on those areas' economies and agricultural situations.

Introduction

Tan spot caused by *Pyrenophora tritici-repentis* is one of the most important foliar blights of wheat that is becoming a more important economic problem in many areas of the world (De Viedma and Kohli 1998). The prevalence of tan spot is increasing with the use of conservation and zero tillage practices (Díaz de Ackermann and Kohli 1998; Osorio, Garcia, Lopez, and Duveiller 1998; De Viedma and Kohli 1998). Conservation tillage creates an ideal situation for tan spot to continue infecting wheat crops year after year. The fungus survives in the wheat stubble from the previous year, which guarantees perpetuation of the fungus and an increase in the inoculum load because most of the sexual spores are released during the crop's seeding time. Their release also coincides with lower temperatures and rainy periods, the perfect conditions for infection very early in the crop cycle (De Viedma and Kohli 1998).

The locations of the world where tan spot is a major constraint on wheat production are North Africa including the North African Coast, the East African Highlands, southern Brazil, the rainfed areas of South Asia, North-Central Asia, northeastern China (Lantican,

Dubin, and Morris 2005), Paraguay (De Viedma and Kohli 1998), Uruguay (Díaz de Ackermann and Kohli 1998), and Oaxaca State, Mexico (Osorio, Garcia, Lopez, and Duveiller 1998). In these areas of the world there are few to no wheat varieties that are resistant to tan spot. In Central Asia, 136 local cultivars were tested for resistance to tan spot, and no immune genotypes were found (Postnikova and Khasanov 1998). Also, both durum and bread wheat varieties are susceptible to the damage from tan spot and suffer economic losses (Nagarajan and Kumar 1998).

Plants complete a number of steps that are natural defenses when attacked by a pathogen. One of these steps in this defensive process is the induction of pathogenesis-related (PR) proteins that exhibit antimicrobial activity. The combination of steps including the induction of the PR proteins occasionally leads to formation of lesions. These lesions are caused by the death of cells around the infection sites to prevent the spread of the pathogen to healthy cells. The formation of lesions through a "programmed suicidal act of plant cells" is referred to as the hypersensitive response. Hypersensitive response is accompanied by accumulation of PR proteins in the infected tissues (Anand, Schmelz, and Muthukrishnan 2003).

Transgenic plants are often developed with the intention of making them disease resistant. Since PR proteins exhibit antimicrobial activity, genes encoding PR proteins have been used to develop genetically engineered crops with enhanced pathogen resistance (Anand, Schmelz, and Muthukrishnan 2003; Anand, Zhou, Trick, Gill, Bockus, and Muthukrishnan 2002; Selitrennikoff 2001). PR proteins are classified into 14 families; the proteins of the fifth family (PR-5 proteins) are called thaumatococin-like proteins (TLP) because of their similarity to thaumatococin, a protein from the African berry shrub *Thaumatococcus daniellii* (Breiteneder 2004; Selitrennikoff 2001). TL proteins have been isolated from *Arabidopsis thaliana*, corn, soybeans, rice, wheat, barley, and many other plants (Selitrennikoff 2001).

A past study comparing the accumulation of transcripts from genes encoding PR-1, PR-2, PR-3, PR-4, and PR-5 proteins on wheat spikes infected with *Fusarium graminearum* (FHB) found that accumulation of PR-4 and PR-5 (TLP) transcripts were greater and earlier in the genotype that was resistant to FHB than in the susceptible genotype. Other studies have also found a correlation between accumulation of PR-5 transcripts in wheat and resistance to powdery mildew and leaf rust (Pritsch, Muehlbauer, Bushnell, Somers,

and Vance 2002). This evidence supports the idea that PR-5 has a role in wheat's pathogen resistance.

After inoculation with spores from Tan Spot (*Pyrenophora tritici-repentis*), the wheat lines that are transgenic from the barley gene for thaumatin-like protein should show resistance, which is determined by a visual evaluation using a standard rating scale, and the non-transgenic variety (Bobwhite) should be susceptible to damage. If the thaumatin-like protein (TLP) gene increases wheat plants' resistance to Tan Spot, that means wheat varieties have been developed for resistance to Tan Spot. This is significant because TLP transgenic wheat varieties could possibly have resistance to other fungal pathogens, which would help areas of the world with wheat crops that are heavily stricken with fungal pathogen infection prevent severely lowered yields.

Materials and Methods

Plant material.

Twelve different transgenic wheat varieties were developed from the embryo bombardment of one of the 129 sister lines of 'Bobwhite'. 'Bobwhite' was originally obtained from the cross 'Aurora'/'Kalyan'/'Bluebird'/'Woodpecker'. The bombarded accession used for this experiment, line SH 98 26, was selected because, in a previous experiment done in the Applied Biotechnology Center at CIMMYT, researchers found that it had a high transformation efficiency (%). The 12 transgenic varieties were bombarded with a gene for thaumatin-like protein that was isolated from barley. 2 seeds of each transgenic variety and of the original, non-transgenic 'bobwhite', line SH 98 26, were planted in 15 pots. 12 pots were inoculated with *Pyrenophora tritici-repentis* (experimental group) and 3 were not (control group).

Preparation of fungal inoculum, inoculation procedures, and scoring.

Pyrenophora tritici-repentis (*Helminthosporium tritici-repentis*) isolates were collected from wheat leaves. Before collection, the leaves were washed in 2% chlorine and then passed through distilled water to remove the chlorine. Cultures from the isolates were incubated in V8 agar 30% at 22°C to 26°C for 7 days in a photoperiod of 12 h light and 12 h darkness. Spores were collected by washing the colonized agar surface of each plate with 5 ml of distilled water and straining the suspension of spores and water through four sterile cheesecloth layers. 20 µl of TWEEN 20 were added to homogenize the suspension.

Three weeks after planting, wheat seedlings from each genotype were spray inoculated with the suspension (20,000 spores per ml of water). Spraying was performed using an air brush with about 5 ml of suspension per pot. The plants were placed in a humidity room set at 25°C in a photoperiod of 12 h light and 12 h darkness for 48 h. The first 4 h the plants were in the humidity room was continuous humidity, followed by 0.5 h 90% humidity every 3 hrs for the last 44 h. As a control, plants from each genotype were not inoculated with the suspension, but similarly placed in the humidity room with the inoculated plants.

After 48 h in the humidity room, the plants were placed in the greenhouse at 25°C. The plants were sprayed with about 5 ml of distilled water twice daily for 10 days.

Then, all plants were scored by visual comparison to a standard grading scale (Figure 3).

Using a chi test, genotypes with 70% or more plants expressing a score ≤ 3 are considered as a genotype resistant to *Pyrenophora tritici-repentis*, and genotypes with 70% or more plants expressing ≥ 4 are considered as a susceptible genotype.

Figure 1: Control plants.



Figure 2: Inoculated plants.

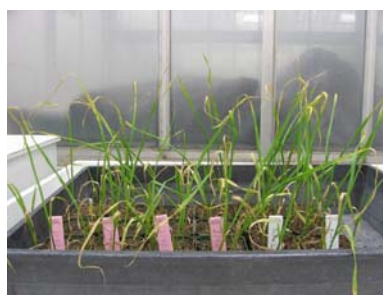
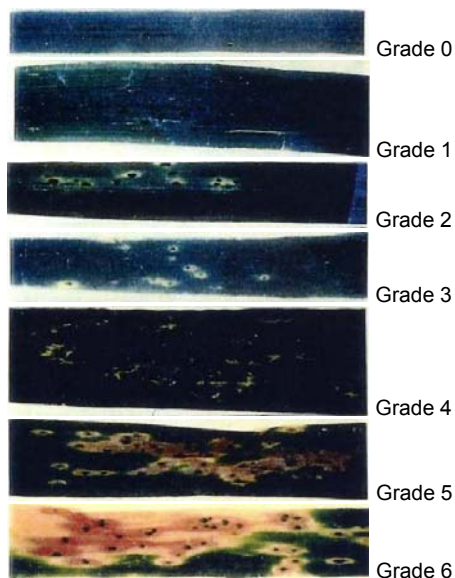


Figure 3: Grading Scale.



DNA extraction.

200 g of plant material from young leaves were collected from each row (1 pot in control group; 4 pots in experimental group) of every variety, frozen in liquid

nitrogen, and homogenized by grinding. 50 g of each homogenized sample of plant material was used for DNA extraction. DNA extraction was completed using the Nucleospin DNA Purification Kit (Clontech, Palo Alto, CA), following the manufacturer's protocol.

Molecular analyses.

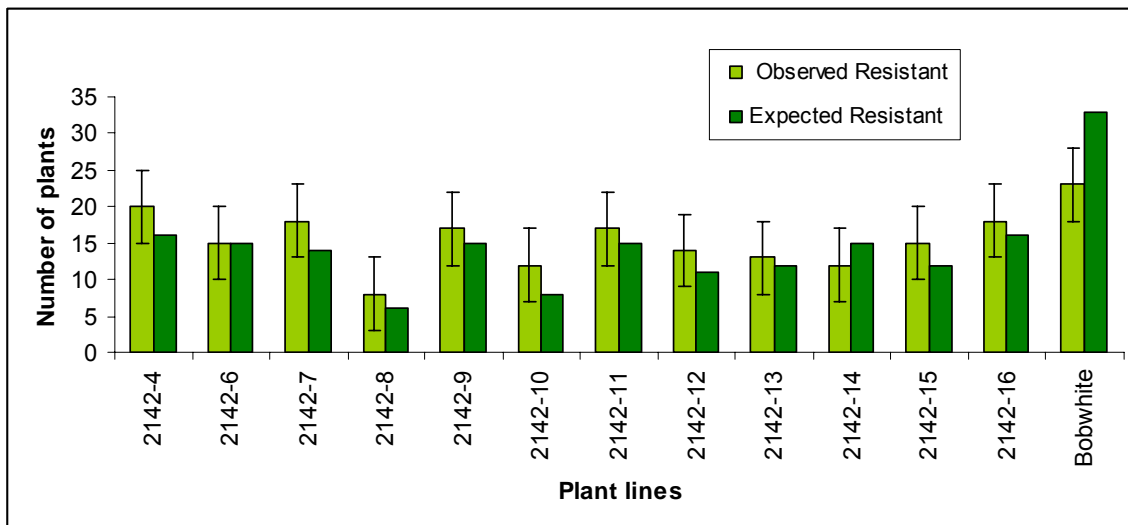
A polymerase chain reaction was performed on the plant DNA that was extracted. The PCR reactions were carried out in a total volume of 25 µl, consisting of 10 ng wheat genomic DNA, 1x Taq Buffer, 2 mM MgCl₂, 15% Glycerol, 50 µM dNTP, 0.5 U Taq DNA polymerase, and 0.2 µM of each (forward and reverse) primer. For PCR analysis of the *bar* gene, DNA was denatured at 94°C for 1 min, followed by 25 amplification cycles of 94°C for 1 min, 56°C for 2 min, and 72°C for 2 min, and 1 cycle of 72°C for 1 min. 5 µl 5x SGB to each sample; the DNA samples were fractionated in a 3% agarose gel at 40 mA in 1X TBE buffer for 3 hrs, stained in 1 µl/ml ethidium bromide for 15 min, destained in dH₂O for 15 min, and photographed with UV light.

Results

Scoring and statistical analysis.

A standardized grading scale (Figure 3) was used to determine each individual plant's resistance or susceptibility to Tan Spot. If 70% or more of one line's total inoculated plants have ratings ≤ 3, then a line is "resistant" to damage from Tan Spot. If more than 30% of one line's total inoculated plants have ratings ≥ 4, then a line is "susceptible" to damage. Lines 2142-4, -6, -7, -8, -9, -10, -11, -12, -13, -15, and -16 are resistant; line 2142-14 is susceptible, along with the control line Bobwhite. In this situation using a chi test including the 12 transgenic lines, the Null hypothesis states that the observed resistant and expected resistant will be equal. From the data collected, the value of Chi squared is 7.84, meaning that the data is insignificant (that the difference of the expected and observed is small), so the Null is accepted. The Chi test verifies that the 11 of the 12 transgenic lines are resistant to Tan Spot and that one transgenic line and Bobwhite are susceptible to Tan Spot.

Figure 4: If the observed resistant bar (left bar) is taller than the expected resistant bar (right bar), the line is resistant; if the bar of the observed is shorter than the expected, the line is susceptible. The Chi test confirms that the data collected is consistent.



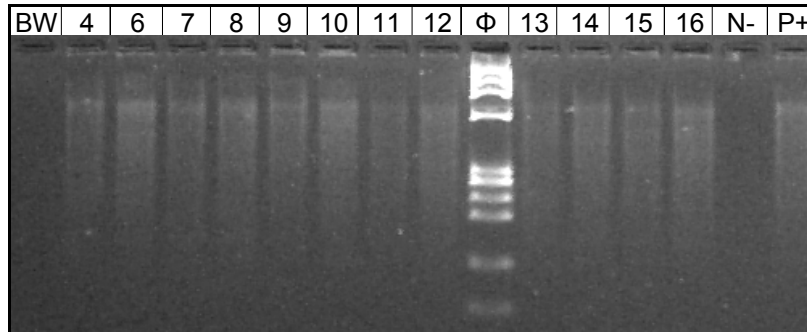
Line	Total	Susceptible	Observed	Expected
2142-4	23	3	20	16
2142-6	22	7	15	15
2142-7	20	2	18	14
2142-8	8	0	8	6
2142-9	22	5	17	15
2142-10	12	0	12	8
2142-11	22	5	17	15
2142-12	15	1	14	11
2142-13	17	4	13	12
2142-14	21	9	12	15
2142-15	17	2	15	12
2142-16	23	5	18	16
Bobwhite	47	24	23	33

Figure 5: The first column is simply the total number of inoculated plants of each variety (dependent on number of seeds that germinated after planting). The second column is the number of plants in that line with scores ≥ 4. The third is the number of plants in that line with scores ≤ 3 (Scores are according to scale in Figure 3). The fourth column is the number of plants equivalent to 70% of a line's total number of plants.

Genomic analysis.

DNA samples from the transgenic plants that were screened with PCR to verify the presence of barley TLP gene in the plant genome. Results indicated (Figure 6) that all 12 SH 98 26 lines contained the bar gene because the amplified DNA fragments from the transgenic plants were identical in size to the positive control.

Figure 6: N- is the negative control, P+ is the positive control for the barley TLP gene, Φ is the standardized molecular marker. All transgenic lines of SH 98 26 share the same band at the same horizontal location as the P+ marker for the barley TLP gene. BW (Bobwhite) SH 98 26, which is not transgenic, does not contain the gene because it is lacking a band at the P+ band's location.



Discussion

The goal of this experiment was to determine if the addition of a gene for thaumatin-like protein to wheat line SH 98 26 increases the wheat's resistance to the foliar blight, Tan Spot. The 12 plant lines that were transformed with a barley TLP gene showed a 91.7% effectiveness at increasing Tan Spot resistance. Past research has indicated that TLP increases pathogen resistance; the findings of this project support the past research's results. Since 11 out of 12 transgenic lines were resistant lines to Tan Spot, while only 1 of 12 transgenic lines was susceptible, and all transgenic lines contained the gene for TLP, the 91.7% effectiveness shows that TLP probably increases Tan Spot resistance. If this is true, any developing area of the world that is afflicted with serious Tan Spot damage could greatly benefit from using a TLP wheat line. The most important thing about TLP increasing Tan Spot resistance is that TLP probably also increases resistance to other damaging pathogens. If a transgenic wheat variety that has significantly increased pathogen resistance abilities is developed, moderately to heavily pathogen stricken areas of the world could prevent significantly lowered yields from one season to the next, providing small-scale farmers with more limited fluctuations in profit from season to season also. A crop that helps small-scale farmers, as opposed to just wealthy farmers, could be an important tool for aiding small farmers, which would reduce international poverty and hunger.

Acknowledgements

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References

- Anand A, Schmelz EA, Muthukrishnan S.** 2003. Development of a lesion-mimic phenotype in a transgenic wheat line overexpressing genes for pathogenesis-related (PR) proteins is dependent on salicylic acid concentration. *Molecular Plant-Microbe Interactions* **16**, 916-925.
- Anand A, Zhou T, Trick HN, Gill BS, Bockus WW, Muthukrishnan S.** 2003. Greenhouse and field testing of transgenic wheat plants stably expressing genes for thaumatin-like protein, chitinase and glucanase against *Fusarium graminearum*. *Journal of Experimental Botany* **54**, 1101-1111.
- Breiteneder H.** 2004. Thaumatin-like proteins – a new family of pollen and fruit allergens. *Allergy* **59**, 479-481.
- Díaz de Ackermann M, Kohli MM.** 1998. Research on *Pyrenophora tritici-repentis* Tan Spot of Wheat in Uruguay. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*. Mexico, D.F.: CIMMYT.
- De Viedma LQ, Kohli MM.** 1998. Spot Blotch and Tan Spot of Wheat in Paraguay. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998.

Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Mexico, D.F.: CIMMYT.

Francl L. 1998. Components of the Tan Spot Disease Cycle. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*. Mexico, D.F.: CIMMYT.

Hammond-Kosack KE, Jones JDG. 1996. Resistance gene-dependent plant defense responses. *The Plant Cell* **8**, 1773-1791.

Lantican MA, Dubin HJ, Morris ML. 2005. *Impacts of International Wheat Breeding Research in the Developing World, 1988-2002*. Mexico, D.F.: CIMMYT.

Nagarajan S, Kumar J. 1998. Foliar Blights of Wheat in India: Germplasm Improvement and Future Challenges for Sustainable, High Yielding Wheat Production. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*. Mexico, D.F.: CIMMYT.

Osorio LA, Garcia IA, Lopez FF, Duveiller E. 1998. Improving Control of Tan Spot Caused by *Pyrenophora tritici-repentis* in the Mixteca Alta of Oaxaca, Mexico. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998. *Helminthosporium Blights of*

Wheat: Spot Blotch and Tan Spot. Mexico, D.F.: CIMMYT.

Pellegrineschi A, McLean S, Salgado M, Velazquez L, Hernandez R, Brito RM, Noguera M, Medhurst A, Hoisington D. 2001. Transgenic wheat plants: a powerful breeding source. *Euphytica* **119**, 133-136.

Pellegrineschi A, Noguera LM, Skovmand B, Brito RM, Velazquez L, Salgado MM, Hernandez R, Warburton M, Hoisington D. 2002. Identification of highly transformable wheat genotypes for mass production of fertile transgenic plants. *Genome* **45**, 421-430.

Postnikova EN, Khasanov BA. 1998. Tan Spot in Central Asia. Published in: **Duveiller E, Dubin HJ, Reeves J, McNab A**, eds. 1998. *Helminthosporium*

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP. 2002. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Molecular Plant-Microbe Interactions* **13**, 159-169.

Selitrennikoff CP. 2001. Antifungal proteins. *Applied and Environmental Microbiology* **67**, 2883-2894.

Pioneer Hi-Bred International's Operations in the High Valleys of Central Mexico

Any area of central Mexico with an elevation above 2000 meters is considered to be part of "High Valleys." Pioneer Hi-Bred International is currently trying to develop a maize hybrid for commercial sales in High Valleys. On July 8, 2005, I was taken to two of Pioneer's commercial plots at different elevations in the High Valleys by two Pioneer employees in Mexico, Silvia Rojas and Jesus Gonzalez. Silvia and Jesus spent the day explaining the company's problems with marketing to the indigenous farmers, hybrid development, legalization of transgenic crops, and the environmental difficulties of maize production in High Valleys to me.

High Valleys is actually not one uniform area; it is many small valleys in a vast range of elevations (2000 meters above sea level) with varying characteristics such as water composition. The first commercial plot was in the area of Zumpango, 2200 meters above sea level. Zumpango is an area plagued with countless agricultural obstacles. The valleys in High Valleys that are similar to the Zumpango demonstrative plot have many characteristics that need to be taken into consideration with hybrid and possibly in the future, genetically-modified, crop development. Currently, transgenic crops are outlawed for commercial production in Mexico. If it were not for the ban, Pioneer would like to introduce Bt corn varieties because one serious problem in this area is borer damage, and Bt corn has been genetically-modified with the Bt gene for corn borer resistance. Not only does damage from corn borers, insects that tunnel through corn stalks, lower yields, but this damage creates a situation for the maize plants that is conducive to infection from other diseases, especially ear rot. Ear rot prevention is extremely important since heavy ear rot damage can reduce yields up to 30%.

At the present time, while genetically modified crops are illegal in Mexico, Pioneer must focus their resources in this area on creating new hybrids. The company's biggest difficulty with the creation of a new hybrid is that the High Valley area's broad range of characteristics from valley to valley. Pioneer must also be cautious about their introduction of a new line to any valley. Their focus is on finding one or more very adaptable varieties. Many of the indigenous farmers are still using native varieties that were in Mexico before the Spaniards arrived. Most of these farmers also feel an almost religious bond or spiritual connection to the native varieties because, one: they have been using them their entire lives, their fathers used them, and their grandfathers used them, and two: the native varieties are very pretty and come in many colors like yellow, red, and blue. So aside from the difficulty of convincing farmers to abandon these varieties for a new, less visually appealing hybrid from a company, Pioneer needs to keep situations similar to this in mind before selling a new variety to the local farmers:

Pioneer sells a new hybrid to a farmer that they know works very well in this specific valley. This farmer has great yields, so he gives some to his brother that lives 100 km. away. His brother still lives in High Valleys, but this variety does not work well in the different area for one reason or another. Because of his bad experience with the Pioneer hybrid, the brother shares his view about the quality of Pioneer hybrids, which is obviously poor, with all the farmers in his area. So even if Pioneer develops a different variety that works well in the brother's area of High Valleys, the farmers in that area already have a preconceived notion that Pioneer Hi-Bred International's seed will not be productive in their area.

Situations such as the above example are why one extremely adaptable variety is so important. If a new hybrid is not capable of producing high yields and quality grain in almost any area of High Valleys, Pioneer will never be able to use the area for financial gain because of the social interactions of the native Mexican farmers.

Fields completely "choked" with weeds are common in the Zumpango region. An area of valleys

with approximately 20,000 hectares total will be irrigated by a single source. If the rainy season begins late, like the summer of 2005, this causes a significant shortage of water that is needed for an effective elimination of weeds with the herbicides, which are 24D Amine and residual Atrazine and do not damage the corn. However the problem with them is that they require moist soil before application. The farmers are only given the opportunity to irrigate once every couple of months. Since they are not able to irrigate more than once within a few days, these farmers are unable to effectively apply the Amine and Atrazine combination to their entire fields.

Mexico's soil has a sandy composition that only retains moisture for a very short period of time, and the Mexican farmers do not have access to a technology or process that can apply herbicide quickly enough. The soil's sandy composition also prevents herbicide application with modern farm equipment (tractors with sprayers) because the equipment can not be driven through the wet, sandy soil. The farmers also can not have the herbicides applied by hand because there is a shortage of field workers caused by the large numbers of citizens from the small, rural communities that are moving into the cities to look for better job opportunities. Farmers are also unable to eliminate the weeds by tilling with machinery while the soil is dry. The reason for this is that these weeds have a unique root system. As opposed to destroying the root systems, tilling actually cuts them up and spreads more weeds throughout the fields.

A possible solution I see for the fields completely overrun with weeds is a new irrigation idea that has been adopted by small farmers in India and Bangladesh. It is called the treadle pump, which only costs \$25 to install. It is a simple human – powered pump that is operated by a person walking in place on a pair of treadles made of a locally, pumping water from a shallow groundwater source. The pump has been very successful in these areas where small farmers have actually increased their profits by several times the cost of the pump in just



Weed problem in Zumpango



Irrigation system in Zumpango

wall, releasing naturally-occurring bacteria in the stomach into the borer's bloodstream and killing it. The borers tunnel through the stalks and ears of the corn plants. This tunneling causes ear rotting, which in turn causes poor quality grain.

Aside from the fact that transgenic crops are still illegal in Mexico, Pioneer would find it very difficult to sell Bt corn to the local farmers of Hidalgo. This is a region where use of transgenic crops have been discouraged by the media. Like Zumpango, many of these farmers are also partial to their native, "beautiful," corn varieties. The media has portrayed GMOs as the cause of a possible annihilation of their native corns. Even though these farmers are not open to the idea of GMO usage, many are open to buying hybrids with a shorter growing season. Since the temperature is suitable for corn production the entire year in Mexico's temperate climate and this area has access to enough irrigation, the farmers try to get two complete crops seasons in each year. Pioneer has proven that they have a hybrid, suited to this area, that flowers one week earlier than the competitor's (Monsanto) variety. One week may not seem like much time, but it is actually very helpful for the farmers who are on a very pressed schedule get one crop harvested in time to plant the other.

The area has extremely polluted water, making it very inexpensive for the farmers' usage. The contents of the water's pollution are actually a help, not the expected hindrance, for growing corn. The pollution's "nutrients" are working as a highly effective fertilizer, nearly eliminating the necessity of buying fertilizers, in an area (High Valleys) that has soil lacking the nutrients needed for the sustenance of corn. The downside of this polluted water is the attraction of insects, particularly the corn borers that are drastically lowering kernel quality. Mexico, by not legalizing usage, and its native corn producers, by lack of acceptance, are preventing the greatest possible agricultural production improvement available in this area. It takes large amounts of insecticide to keep the insect population under control. Farmers would actually benefit more from 12,000 kilos per hectare (2.5 acres) of a Bt corn variety than with 18,000 kilos of a standard hybrid variety because the grain quality would be drastically improved.

These two areas show the extreme differences from one area of High Valleys to the next and the significant amount of difficulty there would be in trying to create a hybrid that is adapted to all areas of High Valleys. I personally see the treadle pump, or a similar cheap irrigation system, and the legalization of GMOs in Mexico as the two most valuable potential possibilities used to increase maize production yields in High Valleys, Mexico.



Mezquital Valley

Polak, Paul. "The Big Potential of Small Farms." *Scientific American* (Sept. 2005), 84-91.

Del Arenal, Rodolfo. "Carta Hidrogeologica del Area de Actopan-Ixmiquilpan, Estado de Hidalgo." *Universidad Nacional Autónoma de México, Instituto de Geología Revista* (1978), Vol. 2: 98-103.

From Corn to Wheat: My Personal Experience

Not every high school student is willing to do a research project that is not required for a class, and most high school students do not have any desire to see a corn field in any way other than from outside their car's window. So not every high school student would actively pursue the completion of science fair project that requires hours working in Iowa's afternoon August heat...in a corn field.

You could probably say that I was not "every high school student." When most of my classmates at Glidden-Ralston Community School still really had little to no idea what they wanted to do with their lives, I was struggling to decide what ONE field to pursue. My problem, particularly with selecting a major, was that I had too many interests. I strongly considered majoring in political science and law. Then the next day I was going to be an astronaut. A week later, I would be reconsidering psychology or wondering how cool it would be to be a paleontologist. I was as clueless as my fellow classmates, just in a different sense of the word. During my junior year, I decided that I was going to be a civil engineer; my cluelessness came from my inability to see that the exact career I wanted had been right in front of me all along.

My college major and career selection coincided with my active years in the National FFA Organization, an outstanding program for high school students that intertwines practical contests like public speaking, individually-pursued agricultural projects, leadership positions, service activities with agricultural education in high school and college. I was devoted to many of the FFA's missions. The contest, Prepared Public Speaking, was a part of my FFA experience as a sophomore. Speaking on the benefits and detriments of conventional agricultural production techniques like genetic modification, the addition of unnecessary antibiotics to livestock feed, and the uses of chemicals versus organic farming practices which use none of the techniques previously listed. As I was writing that speech, I found myself doing more and more research. I could not seem to get enough because I was so intrigued by the subject. The piles of information I read about the two topics seemed to stir up new theories in my mind. These stirrings became two things: 1. That science fair project from the beginning, The Effects of Genetic Modification with the Bt Gene on Corn's Ability to Resist Disease, and 2. My near obsession with corn.

I grew up in an extremely small town; Glidden only has about 1000 citizens. It never ceases to amaze people I meet outside of Iowa that there are towns that actually educate all of their kindergarteners to seniors in one single building. I have found, over my 18 short years, that many negative stereotypes are given to kids that grow up in rural farming communities. While I was in the FFA, I made it my goal to prove that those stereotypes, that also often apply to FFA members, are false. I literally told myself that I would not pursue an agriculturally related career. This goal kept me from realizing how interesting, modern, and important current agriculture is. In turn, it also kept me from realizing how much I enjoyed working in agricultural research, particularly with plant (well, actually corn) genetics.

I had made myself a list of the best civil engineering programs in the United States by the winter of my junior year. That same winter, I was also putting the finishing touches on that genetically-modified corn research project for competitive science fairs. Completely oblivious to the fact that my theory (genetic modification for one trait may affect the plant's overall ability to resist natural enemies by disrupting the evolution of the plant and its predators, like fungal pathogens, viruses, insects, etc.) was a novel idea to the scientists that judged our projects and the conclusions to this scientific inquiry could show a significant effect on the environment, food supply, and economy, I was caught completely off guard when I won the biological sciences category at the South-Central Iowa Science and Engineering Fair, advancing my project to the 2004 Intel International Science and Engineering Fair in Portland, Oregon.

In Portland, I met Rachael Collier, a 2004 Ahmanson Intern Award winner, and Megan Srinivas, the 2004 John Chrystal Award winner; both girls had been World Food Prize Borlaug-Ruan International Interns the previous summer. Before meeting Megan and Rachael, I was oblivious to the existence of Borlaug-Ruan International Internships and the World Food Prize and to the fact that this program would be one of the most meaningful experiences of my life. Megan and Rachael's willingness to share their experiences with me was probably one of the most influential factors to alter my career path's direction into the biological sciences. It was not that I had not realized I am fascinated with plant genetics; it was that I had not realized I wanted to work with plant genetics.

At Intel ISEF, I started thinking about new directions a biology career could take me. A longtime goal of mine has been to become a "world traveler." As someone who is interested in the world around herself, it is not surprising to think there are so many places and cultures that I want to experience first hand. When Rachael mentioned that she had studied potatoes in Peru and Megan made it clear that there are all kinds of different centers to intern at, especially in plant research, I finally made the connection; there is a perfect career for me: agricultural research in developing countries.

Once I made this important connection, I decided to pursue a future in international agriculture and plant genetics, beginning with the World Food Prize Youth Institute and an application for a Borlaug-Ruan International Internship at the International Maize and Wheat Improvement Center (CIMMYT). Fortunately, applying to CIMMYT where I might be able to continue working with corn, and choosing this career path also coincides with one of my other life pursuits: to make a difference in the world. As idealistic as it sounds, I believe that every person can make a positive difference on the world. Mohandas Gandhi perfectly stated his famous quote, "Be the change you want to see in the world," to remind us that it is necessary to be proactive if you want to make a difference or see a change. I am one who personally believes that there is a lot of luck and chance involved with how a person's life plays out. What I mean by this is, in my life specifically, I have been given many opportunities just from things that are out of my control, like being born in America. I do not believe that an African mother who has fallen victim to AIDS or a hungry family in India has necessarily done something that warrants the life they are living anymore than anything I have done to warrant the happiness I have been given with my life. I believe that every person deserves the opportunity to be well-fed, educated, and free from illnesses that are preventable. The international inequality (of opportunities) at birth is something that I believe needs to be changed. Where one is born should not be the main determining factor to the quality of life one will live. Equality at birth, giving people the right to guide their own futures, or at least a significantly narrower gap of overall quality of life between citizens of developing nations and citizens of developed nations is the change that I would like to see in the world, and it is the change that I think I need to "be."

How will we narrow the gap? How can I "be a change?" World hunger, poverty, and inequality are complicated, intertwined issues. My belief is world hunger needs to be abolished before poverty can be eliminated, and equality can not exist until severe poverty is eliminated. I chose to pursue a career in the biological sciences researching world hunger solutions through plant genetics because it is a way that I think I can have a positive impact on the long, complicated process of ending hunger, poverty, and inequality before I left for Mexico. Even though I had already chosen this path, my internship at the International Maize and Wheat Improvement Center in Mexico still had an enormous effect on my future; I even branched away from my researching standby, corn, to wheat because of the projects available to me, the wheat research was closer to my interests: plant and pathogen interactions. However, I am pursuing the same career, but I am not pursuing it in the exact same fashion. Now through the WFP Youth Institute and my Borlaug-Ruan internship, I understand the complexity of world hunger and know that the approach to solving it is not simply to produce higher yields. Any developments in this field that I may find will be looked at in a different light; I understand, now, that I should not be trying to discover a transgenic maize variety that will increase yields but trying to see the immediate and long-term effects of that transgenic corn on an area's economy, environment, and different

classes. Now I know that even if a new crop has higher yields and causes no environmental degradation is expensive, than, in turn, it could actually have a negative overall effect. Only the wealthy could benefit from using it, extending the gap between the upper class and lower class, who probably would not be able to afford to buy it from markets or to raise it. There are many things that need to be taken into consideration when a new crop is developed or agricultural practice is created; that situation is only one example.

Before I arrived in Mexico, not only did I think I understood what causes world hunger, but I also had an image in my mind of what Mexico would be like. I imagined that all thirteen of the Borlaug-Ruan International interns would face similar situations just with different races and cultures; I imagined that we would all be facing immense amounts of poverty and starvation. I had heard Mexico City was extremely dangerous and Mexico was very dirty. I had visions of unpaved roads, people begging on every corner, and trash everywhere. Basically I imagined a situation that would make me extremely appreciative of the life I have in the United States. Except what I really found was a situation that not only made me appreciate the amenities of living in a developed country but also see what an outrageously incorrect vision I had of Mexico and some things that our society is missing.

Aside from realizing that roads really are paved in Mexico, the first thing I realized about Mexico is the kindness of the Mexicans. Even though my Spanish speaking skills are extremely limited, they all treated me with kindness and with a certain amount of understanding that I do not think I would have been given in America as a non-English speaker. Over the two months I spent in Mexico, I encountered all kinds of situations where I did not know enough Spanish (or hand signals) to get a point across to someone else; I greatly appreciated that the Mexicans would not become impatient. Instead, trying to help, they would ask us more things in Spanish, a kind gesture, but often ineffective. I could see their patience- something that I would now like to see more in myself and other Americans.

Currently, I am studying at Indiana University-Bloomington, and one thing I have noticed on campus is, even though we have many international students, they tend to gravitate towards other students of their own ethnicity, just like the American students also gravitate toward other Americans. Watching this lack of interaction, makes me appreciate the experience I had this summer that was nearly one-of-a-kind. My one-of-a-kind experience was living, working, and socializing with people from all over the world all the time. CIMMYT had such an extremely diverse staff, including their visiting scientists and interns, but the best part was that everyone worked with one another. CIMMYT lacks the "cliques" of nationalities I see around my university's campus; there are not many places in the world where all the races and religions mix together during times of work and play. It was incredibly interesting to witness and experience the interactions of multiple cultures at the same time and everyday. My favorite experience with this was a 4th of July barbeque that an American threw to celebrate, so, considering the demographics of the attendees (only 4 Americans with Canadians, French, Mexicans, Italian, and Dutch), the 4th of July was basically just an excuse to throw a barbeque. There was also a group of us who would play volleyball after work, our regulars were Australian, American, Mexican, Argentinean, Dutch, Italian, French, Austrian, and Belgian. Meghan and Suzy, the Iowa State University Borlaug interns (fellow lowans), and I would go on trips into Mexico City and to Teotihuacan (the pyramids) with three visitors from Argentina, Malawi, and Turkey on the weekends. I learned that diversity is only important when there are real interactions between the people of different backgrounds. Without the interactions, one misses the most important part of learning about other cultures; they were, quite possibly, my favorite thing about working at CIMMYT. Since everyone was from all over the world, not only did I get an understanding of Mexican culture, but also the opportunity to gain knowledge of numerous other countries.

It is probably slightly ironic that I spent my summer thousands of miles from Iowa and with interesting people from all over the globe, but the most meaningful moment, to me, was not actually a moment with an international person, but time spent with a fellow lowan, Dr. Norman Borlaug. When he is not busy traveling around the world, he frequently lives in an apartment

there. I was fortunate enough to get to eat dinner with him a few times in my two short months. When my friend, Meghan, and I got to eat with him, there was the possibility of many different topics popping up, but one night was especially meaningful. In all honesty, I can not remember what lead up to this specific conversation, but at some point Dr. Borlaug started to talk about why he decided to do the research he did (develop shorter wheat varieties that are attributed to saving millions of lives around the world). He said that during the Great Depression, he left Iowa to go to college in the Twin Cities; it was the first time that he had been exposed to hunger. Yes, people were poor in Iowa during the Depression, but they were not hungry. That was where he realized what hunger looked like. He elaborated on it much more than I have, but it was so humbling to have an amazing man like him just sit down and talk to me, an average American teenager, like an equal about why he did what he did for mankind. Since I learned about Dr. Borlaug in my freshman agriculture class, I have always had immense amounts of respect for him. Meeting him reminded me that I should never be ashamed that I am from Iowa and an agricultural background because of all the people I met it was an Iowan; not an Italian, or an Australian, or a Mexican that was the most inspiring; that was the most inspiring and interesting of all.

Many things changed about how I see the world. Not only do I now have an equal affection for wheat as to corn, but I see that other cultures are better in certain ways than the American way and that some American ways are can be even more interesting than some foreign ways. I see that its more important for different cultures to mix together, as opposed to simply spending time physically in another culture. I understand the true roots of hunger in our days around the world. Now I see and understand important, and often necessary, changes the world needs because of the ways that my Borlaug-Ruan World Food Prize International Internship changed me.

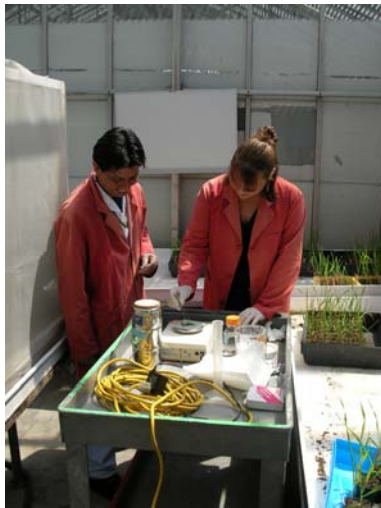
Photos



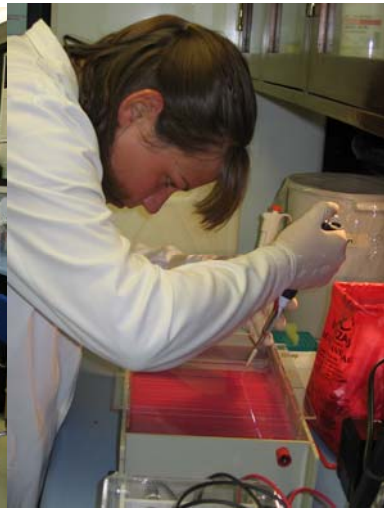
Collecting spores for suspension



Inoculating wheat with tan spot suspension



Collecting & grinding plant samples



Loading gel with PCR samples



Scoring plants for resistance/susceptibility



Preparing DNA samples for PCR

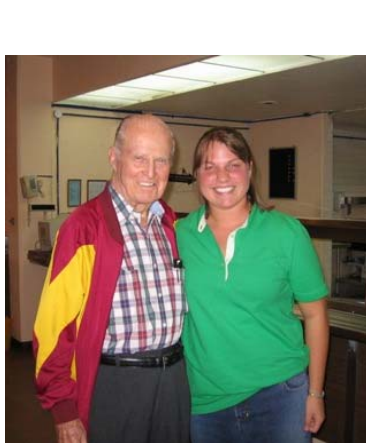


Teotihuacan (Pyramids of the Sun & Moon)



Volcanoes National Park

Xochimilco



Dr. Borlaug



Palacio de Bellas Artes



Castle of Chapultepec

My Favorite Images of Mexico



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