# Shireen Bhatia AVRDC-The World Vegetable Center Shanhua, Taiwan



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### **Introduction:**

In my 16 years on this Earth, I can now say I've seen a lot. In truth, one could say that I have been exposed to many worldly issues since my early childhood. My parents had decided to first take me to India when I was about 8 months old and ever since that time, we kept coming back to visit my family every few years. Aside from the fond memories I have of my family, there is one thing I am certain that the experience had opened me up to. India showed me what it means to face injustice. The young children who were clad in ripped clothing walking about the streets of any market I would come to were noticeably malnourished while I walked among them, healthy and well clothed. Since then, I've been told that I am lucky but it wasn't until recently that I was able to focus on the injustice side of the story.

Though I had truly never held a focused interest in agriculture, it wasn't until this year that I began to see its relevance. At the end of my sophomore year of high school at Laurel School in Shaker Heights, Ohio, I became aware of an opportunity to become engaged with food insecurity issues through the World Food Prize. After hearing one of our own students speak about her experience in writing her paper and getting the chance to meet so many specialists on hunger in Iowa, I was intrigued. I began to think of the hunger problems I had seen in India much earlier in my life and wondered if perhaps, I could make even a small impact on the problem by writing a research paper.

Later during the fall of my junior year, I had finally finished my paper for the Ohio Youth Institute to be held that September. I had decided that my topic of discussion would be on the food insecurity in Ethiopia. Due to the frequent droughts, many farmers in Ethiopia have not been able to plant crops or maintain livestock, which causes great detriment to the health of its people as well as the economy of Ethiopia. My proposed solution in this paper was entomophagy, the consumption of insects that are safe for humans. Because this solution is energy efficient and cost efficient, I proposed that Ethiopian farmers could build self sustaining farms so that they could raise the insects themselves. Because insects are extremely beneficial to the human diet, farmers could easily use the insects the raise to supply their own families and sell them to the general public for consumption.

While at the Ohio Youth Institute, I had the opportunity to present my paper to a group of peers as well as two adults in the room. After everyone's presentations, I finally received word that I had been asked for an interview. In a small conference room, I was asked questions about my project as well as my life inside and outside of school. After a day's time, I was informed that I had been selected to represent the state of Ohio along in a group of 6 delegates at the Iowa Global Youth Institute. Here, I would spend four days meeting a variety of specialists and professionals in different fields all connected to agriculture. Not only did I get the opportunity to network, but I also learned about the truth of hunger around the world. By the end of the conference, I was moved by all that I had seen and heard. The most intriguing to me were the

presentations of the 2012 Borlaug Ruan Interns who had interned at various countries to learn about food insecurity and what they could do against it. As I left the conference, I knew that if given the chance, I would take the internship in a heartbeat. With courage and support from my family and teachers, I applied. Months later, in March of 2013, I received an email informing me that I was to spend two months at AVRDC in Shanhua, Taiwan to conduct research on pepper plant accessions and screen for resistance.

## **AVRDC-The World Vegetable Center**

AVRDC-The World Vegetable Center is an international nonprofit and non-governmental research center founded on May 22 1971 by the Asian Development bank. Its mission is "The alleviation of poverty and malnutrition in the developing world through the increased production and consumption of nutritious and health-promoting vegetables". Though the center began only in tropical Asian countries, their work has expanded into other regions such as Sub-Saharan Africa, Central Asia, and Southern Asia.

The headquarters of AVRDC is in Shanhua, Taiwan, while there are regional centers and offices are located in Thailand, Tanzania, India, Dubai, Uzbekistan, Korea, Cameron, Indonesia, and Bangladesh.

The annual budget for AVRDC of about \$18 million US Dollars includes funding from national governments, and private foundations. Donors include the Asia & Pacific Seed Association, Asian Development Bank, Australian Centre for International Agricultural Research, Germany's GIZ/BMZ, Republic of China, United Kingdom's Department for International Development, United States Agency for International Development, UK Department for International Development.

The center strives to achieve its goal of producing better varieties of vegetables through studying the genetics of the plants and working together with other departments to enhance the quality of the crops. This is achieved primarily through the collection of germplasm that AVRDC has and through a multitude of breeding projects. The major breeding projects include development of multiple disease resistance in tomatoes, peppers, cucurbits, onions, and other plants. (AVRDC website)



Figure 1: AVRDC-The World Vegetable Center Headquarters in Shanhua, Taiwan

### Resistance in chili pepper accessions to two potyvirus species

#### Abstract-

This study concerns the plant potyviruses *Pepper veinal mottle virus* (PVMV) and *Pepper mottle virus* (PepMoV). The main objective of this study is to assess twelve chili pepper (*Capsicum annuum*) accessions for resistance to one or both viruses by mechanical inoculation. All the pepper accessions were from AVRDC's germplasm collection and previously had been found to be resistant to another potyvirus, *Chilli veinal mottle virus* (ChiVMV). Plants of each accession were inoculated twice with either PVMV or PepMoV and observed periodically thereafter. After three observation sessions, the symptomless plants were tested for virus infection using enzyme-linked immunosorbent assays (ELISA). The results showed that three of the lines carried resistance to PVMV, but none carried resistance to PepMoV.

#### Introduction-

Among the wide and diverse germplasm of AVRDC, *Capsicum peppers* are one the main six crops that the research center works on. After conducting a study in 1983 to 1984 in which 15 crops were studied to determine which would be the most useful to farmers, the results indicated that sweet and hot peppers were some of the best options. In 1986, AVRDC added peppers to its

main crops list. After conducting trials to evaluate the crop, many of the features of peppers were found and the conclusion was reached that hot pepper were best suited to hot climates (Fletcher, 1993). Since peppers were introduced to AVRDC, the center has continued to work with this crop to find more details about the problems it undergoes during tropical weather. One of the more significant constrants is virus diseases. Worldwide, at least 40 different virus species can infect peppers, and nine of these are potyviruses (Genus *Potyvirus*, Family *Potyviridae*). Some of these viruses have been found to cause major losses in crop production, such as *Chilli veinal mottle virus* (ChiVMV) causing a reduction in pepper yields up to 50% (Tsai, 2008). Fortunately, pepper accessions resistant to certain viruses have been found. Within AVRDC's germplasm collection, there are peppers known to be resistant to ChiVMV. Even so, research continues to discover resistance to different pepper viruses such as *Pepper mottle virus* (PepMoV) and *Pepper veinal mottle virus* (PVMV).

*Pepper veinal mottle virus* (PVMV) was discovered in Taiwan in May 2006 when a study of samples from tomato plants was conducted. Double antibody sandwich-ELISA testing showed that three of the eight samples with virus disease symptoms were infected with ChiVMV. Reverse-transcription Polymerase Chain reaction (RT-PCR) with universal potyvirus primers showed that the other samples were also infected with a potyvirus. Sequencing the RT-PCR products and homology searching the sequences showed that the virus infecting the tomato plants was PVMV (Cheng, 2009). *Pepper mottle virus* (PepMoV) was also first detected in Taiwan in 2006. Again sequencing of RT-PCR products, in this case from bell peppers from southern Taiwan, showed the presence of mixed infections of PepMoV with PVMV or ChiVMV (Cheng, 2011).

The purpose of this study was to identify if pepper lines resistant to ChiVMV were also resistant to PVMV and/or PepMoV. Because the starting materials were all resistant to ChiVMV, the idea was to challenge inoculate separate sets with PepMoV and PVMV and observe if they became infected or not. Initially, the plants were observed for symptoms of virus, and after three weeks samples were assayed by ELISA to confirm that plants without symptoms did not have symptomless infection and were really resistant. The long-term objective would be to use pepper accessions with resistance to different potyvirus species to develop improved pepper lines with multiple virus resistance

#### Methods and Materials-

#### Hot pepper accessions

Prior to my arrival at AVRDC, twelve accessions of hot pepper had been selected from the AVRDC genebank; 11 of these had resistance to ChiVMV (= less than 20% infection rate) and the other accession (VC27a) served as a virus susceptible control for the experiment. See Table 1 for accession codes. The experiment had two treatments (inoculation with PepMoV and inoculation with PVMV), and for each treatment 12 plants of each of the 12 accessions were grown individually in 3" pots in the virology glasshouse (=244 plants total).

#### Virus sources and mechanical inoculation

Two potyviruses, PVMV (isolate S00005-2) and PepMoV (isolate APB6) were used. Both virus isolates were multiplied in the *Nicotiana glutinosa* in order to prepare the inoculum. Virus inoculum for the experiment was prepared by blending infected leaves of N. glutinosa in inoculation buffer. Each test plant was inoculated at the 4-5 leaf stage with one of the two viruses. For the mechanical inoculation, carborundum-grit was first dusted onto the test plant leaves. Then, the plant sap inoculum solution was rubbed onto the leaf surface using a cotton wool pad. After 3-5 minutes, the inoculated leaves were washed with distilled water to remove excess carborundum and inoculum. Each plant was inoculated a second time with the same virus isolate four days later. The inoculated plants were placed in a net house and were checked for development of symptoms at 4, 11, 17, and 18 days after the final inoculation.

#### **ELISA testing**

Seventeen days after the final inoculation, samples were collected from each plant for testing by enzyme-linked immunosorbent assay (ELISA). PVMV was detected by using double-antibody sandwich ELISA (DAS-ELISA) and PepMoV was detected by indirect ELISA testing.

#### DAS-ELISA- (PVMV)

For DAS-ELISA, each well of a96-well microtiter plate was filled with 100 micro liters of purified immunoglobulin G (IgG) diluted in coating buffer at a dilution of 1:1000 for anti-

PVMV-IgG. The plates were then incubated at 37°C for four hours to allow the IgG to adhere to the surface of the wells.

While the plates were incubating, the leaf samples were ground in a mortar with a pestle and diluted with extraction buffer containing PBS-T (Phospathe-buffered saline-Tween 20) of 2% PVP-10 (polyvinylpyrrolidone MW 10,000) at a ratio of 1:5 (weight:volume) to create samples that would be used for ELISA testing. After incubation, the plates were washed with a washing buffer that contained PBC-Tween with a concentration of 0.5 ml Tween 20 per liter of PBS. The wells were then filled with 100 micro liters of the leaf sample solution. The first column of every plate was left empty as a buffer control. Each plate contained samples with virus and healthy samples as controls. These plates were incubated at 4°C overnight to allow for any virus present in the samples to bind to the IgG coated within the plates. After incubation, the plates were washed with washing buffer, and then each well was filled with 100 micro liters of enzyme conjugated IgG diluted 1:1000 in conjugate buffer. This buffer contained PBS-T, 2% PVP-10, and 0.2% ovalbumin. The plates were incubated again at 37°C for 4 hours to allow the IgG-alkaline phosphate conjugate to bind to the virus. Following incubation, these plates were washed with the washing buffer.

In the final step, the wells were filled with 200 microliters of 4-nitorphenyl phosphate disodium dissolved in substrate buffer, containing 97 ml diethanolamine diluted in 800 ml dH<sub>2</sub>O, at a dilution of 1 mg per ml of buffer. The plates were then incubated at room temperature until the yellow coloration became noticeable, showing that reaction was taking place. The degree of the coloration revealed the titre of the virus in the sample; the darker the yellow the more virus was present. To quantify this, the plates were then read at a wavelength of 405 nm using a Thermo scientific Multiskan EX ELISA plate reader.

#### Indirect ELISA- (PepMoV)

Wells of the microtiter plates were filled with 100 micro liters of plant sap diluted in extraction buffer containing PBS-T of 2% PVP-10 (polyvinylpyrrolidone) with a concentration of 1:5. These plates were incubated at 37°C for 3.5 hours. After incubation, these plates were washed by washing solution that contained 1000ml of PBS and 0.05% Tween 20. The plates were then filled with 200 microliters of blocking solution made with 100ml of PBS and 2% non-fat milk. This was followed by incubation overnight at 4°C. After incubation, the plates were washed and filled with 100 microliters of anti-PepMoV-IgG diluted in antibody buffer, made with 1000ml of PBS-T, 0.2% eggalbumine and 2% PVP, at a concentration of 1:800 and incubated 3 hours at 37°C. Following incubation, the plates were washed by washing buffer containing 1000ml of PBS and 0.05% Tween.

100 micro liters of alkaline phosphate conjugated anti-rabbit IgG diluted in conjugate buffer at 1:30000 ratio were then added to the plates, which were then incubated at  $37^{\circ}$ C for 3 hours. After incubation, the plates were washed by washing buffer, filled with a substrate solution (1 mg per ml 4-nitorphenyl phosphate disodium) containing 97ml of Diethanolamine, 0.2g of NaN<sub>3</sub>, 800 ml of distilled H<sub>2</sub>O at a pH of 9.8. Until the color developed, the plates were read using an ELISA reader as mentioned in the DAS-ELISA method.

#### **Results and discussion**

Tables 1 and 2 show the number of pepper plants of each accession that had been infected by PVMV and PepMoV respectively over the course of 4 symptom observation times. The plants infected with PVMV developed a mild mottle symptom (Fig. 3) whereas the PepMoV-infected plants developed mosaic symptoms (Fig. 2). In addition to these four observations, the results of the ELISA tests are listed (Tables 1 and 2).

In this study, 12 accessions of pepper were inoculated with each virus. From Table 1 it is apparent that only one of the accessions, C00266, may carry some resistance to PepMoV since five of the 12 inoculated plants did not develop virus symptoms and tested negative for presence of virus by ELIZA 18 days after inoculation. All 12 plants of VC27a (the susceptible control) became infected with PVMV, though only presented clear symptoms 19 days after inoculation (Table 2). Of the 12 accessions, three (VC241, PBC370 and PBC518) were probably immune to PVMV since none of the plants of these lines developed symptoms or tested positive for virus by ELIZA within 18 days of infection. A further four accessions (VC160a, C00266, VC35a and PBC371) presented only low frequency of infected plants. At least 8 out of 12 plants of each of the other four accessions developed virus symptoms by 19 days after inoculation and so are

regarded as relatively susceptible to PVMV. In general, when PepMoV infected a plant it caused severe mosaic symptoms (Figure 2), whereas PVMV tended only to cause mottling of infected plants, and these symptoms generally only developed at least two weeks after inoculation (Figure 3).

The 11 test accessions of pepper had previously been shown to be resistant to ChiVMV by the Virology group at AVRDC. Since many of the accessions were shown to probably carry some resistance to PVMV in this study, it seems likely that some of the resistance to ChiVMV is also effective against PVMV. However, the experiment reported here should be repeated at least once to confirm the resistance of the accessions before inheritance studies are made with the resistant accessions.

If the resistance to PVMV is confirmed, then these accessions could potentially be used as parents to breed commercial pepper varieties with multiple virus resistance. The problem of crop loss and infection of plants that continues to harm the productivity of farmers, mentioned in Tsai's report, will be reduced should the breeding be successful in producing resistant varieties.

Variety		Symptoma	<b>ELISA</b> positive		
	(4 DAI)	(11 DAI)	(17 DAI)	(19 DAI)	plants / total
PBC569	4/12	12/12 Mt	12/12 Ms	12/12 Ms	12/12
VC160a	11/12 VC	12/12 Ms	12/12 Ms	12/12 Ms	12/12
VC241	0/12	12/12 Mt V	'C 12/12 Ms	12/12 Ms	12/12
PBC370	0/12	12/12 Ms	12/12 Ms	11/12 Ms	11/12
VC255	0/12	12/12 Mt	12/12 Ms	12/12 Ms	12/12
VC247	6/12	12/12 Mt	12/12 Ms	12/12 Ms	12/12
COO266	0/12	8/12 Mt	10/12 Ms	7/12 Ms	7/12
PBC518	2/12 VC	12/12 Mt	12/12 Ms	12/12 Ms	12/12
VC35a	12/12 Ms	12/12 Ms	12/12 Ms	12/12 Ms	12/12
PBC371	0/12	12/12 Mt	12/12 Ms	12/12 Ms	12/12
VC244:	10/12 Mt	12/12 Ms	12/12 Ms	12/12 Ms	11/11
VC27a	12/12 Ms	11/11 Ms	11/11 Ms	11/11 Ms	12/12

Table 1. Development of symptoms and detection of PepMoV in 12 accessions of hot pepper following mechanical inoculation with PepMoV.

Table 2: Development of symptoms and detection of virus in plnts of 12 hot pepper accessions following inoculation with PVMV

Variety	Symtomatic plant/total inoculated				Elisa positive	
	(4 DAI)	(11 DAI)	(17 DAI)	(19 DAI)	plats/total	
PBC569	0/12	0/12	9/12	9/12 Mt	9/12	
VC160a	0/12	0/12	0/11	3/11	3/12	
VC241	0/12	0/12	0/12	0/12 R	0/12	
PBC370	0/12	0/12	0/12	0/12 R	0/12	
VC255	0/12	0/12	2/12	10/12 Mt	10/12	
VC247	0/12	0/12	0/12	10/12 Mt	10/12	
COO266	0/12	0/12	3/12	6/12 Mt	6/12	
PBC518	0/12	0/12	0/12	0/12 R	0/12	
VC35a	0/12	0/12	0/12	4/12	4/12	
PBC371	0/12	0/12	0/12	1/12	1/12	
VC244	0/12	0/12	0/12	8/12	8/12	
VC27a	0/12	0/12 Ms	0/12 Ms	12/12 Ms	12/12	

\*Note: For both tables, the key is as follows: VC: Vein Clear Ms: Mosaic Mt: Mottle R: Resistant



Fig. 2 Plant infected with PepMoV. (VC35a) (Mosaic and leaf distortion)



Figure 3: Plant infected with PVMV. (VC27a) (mottling)

## Learning around the Center

While I worked on my main project, I would have opportunities to explore the Virology unit and other units within AVRDC to learn about the different research conducted in these departments.

In Virology, I learned how to conduct a gel electrophoresis potyvirus detection by Reverse transcription polymerase chain reaction (RT-PCR). The procedure involved the total RNA extraction from leaf tissue of 5 samples by Plant Total RNA Miniprep purification Kit. This was done by following the manufacturer's protocol. Viral cDNA was synthesized using Oligo(dT) as downstream primer and the protocol for Moloney murine leukemia virus reverse transcriptase (M-MLV-RT, invitrogene) was followed. PCR amplification was then performed by using primer pairs Sprimer1/Oligo(dT) (Tsai et al, 2009). The PCR product was checked by gel electrophoresis to analyze the results.

#### GRSU

The first of these opportunities was my tour at GRSU, the Gene bank of AVRDC. During this tour, I had the opportunity to see the facilities as well as learn about the seed storage work done within this department.

AVRDC's gene bank has world's largest public germplasm collection with more than 59,507 accessions from 156 countries. In the gene bank, some of the main goals in the work conducted there include the conservation and distribution of vegetable germplasm, development of DNA markers for marker assisted selection, and training for proper germplasm management. (AVRDC-The World Vegetable Center: Managing Germplasm)



Figure 4: Work in GRSU

**Global Technology Dissemination** 

I also got the opportunity to go on two field trips with the Global Technology Dissemination group. On these trips, we visited commercial grafting nurseries and composting plants. The purpose behind these trips was to observe the different methods these farmers used for conducting their work and to hear their feedback on AVRDC's training Global Technology Dissemination group's main responsibility is to provide training to poor farmers and introduce different methods to help enhance the production of crops. In addition to training, the GTD group is also responsible for helping with agriculture during disaster relief efforts for short term development. (AVRDC-The World Vegetable Center: On-Farm)





Figure 5 and 6: Commercial nurseries that use grafting.

#### **Pepper Breeding Unit**

The next opportunity I received was to take a tour of the fields of AVRDC with the pepper breeding unit. During this tour, I had the chance to learn about the work of the Pepper Breeding Unit. Not only did I learn about the facilities under which the work was conducted, but I also became acquainted with the different varieties of peppers grown in the greenhouse and the fields. One of the main goals of AVRDC is to breed and produce more nutritious crops to help families and farmers alike. The pepper breeding unit works to enhance this goal through trying to produce a more nutritious and beneficial pepper.



Figure 7: Pepper plants in the greenhouse.

#### **Bacteriology and Mycology**

I also assisted with work in both the Bacteriology and Mycology unit. In these two units, I undertook a project that took lasted through the month of July. During this project, I took part in the propagation, inoculation and evaluation of different plants. Through this, I was able to observe the different methods and preparations of inoculations compared to those of virology. After preparation and inoculation, I received the chance to come back and observe the symptom post inoculation



Figure 8: Mycology greenhouse.

#### Biotechnology

I also had the chance to work with the Biotechnology unit. Because biotechnology is one of my major scientific interests, this opportunity was especially intriguing to me. Here, I learned about different kinds of genetic markers, Polymerase Chain Reaction (PCR), and gel electrophoresis.



Figure 9: Working on gel electrophoresis.

# **Exploring Taiwan**

As I left for Taiwan, I never thought that I would end up doing so much exploring or traveling. However, this journey for me has been incredibly educational. Not just within the lab, but also outside in the real world. Nearly every weekend, I got the chance to travel and explore different parts of Taiwan. Thanks to my professors and friends, I was able to see nearly all that Taiwan had to offer.

Near AVRDC is a small town called Shanhua. During my time at the research center, my friends and I would often go out to explore the town. Sometimes, would take trips to the local night market and others we would simply enjoy an evening a café. Although Shanhua is quite small, the town's atmosphere did provide me with a sense for the culture in Taiwan. From seeing my first Daoist temple, to simply going out to dinner with a group of friends, I learned that the Taiwanese people are extremely friendly and quite kind.



Figure 10 and 11: Daoist temple in Shanhua.

Coming from Ohio, a landlocked state with only Lake Erie nearby, I had never been to a real beach before until my second weekend in Taiwan. My advisor, Dr. Lawrence Kenyon, and his family were so kind in choosing to take me with them to Kenting, an incredible beach in Taiwan. On the way to Kenting, we stopped in a small old town called Hengchun. This permitted me to see the old architecture of Taiwan.





Figure 12 and 13: View of Hengchun and the waves on the beach in Kenting.

Another experience I thoroughly enjoyed was going to the Mango Festival held annually in Taiwan. A few people who worked in the Global Technology Dissemination group decided to get together and explore the Mango Festival. Not only did I see the plethora of fruits grown in Taiwan, but I also got to try some amazing activities, including archery.





Figure 14 and 15: Archery and the mango festival.

Perhaps one of my favorite trips to make on the weekends was to Taiwan. I was fortunate enough to be living extremely close to a railway station that was just outside of campus that I could take to Tainan, a slightly larger city in Taiwan. Tainan is unique in that it was the first city of Taiwan, providing an excellent historical experience to those who visit there. One of the major places to visit within Tainan was Anping district, a major historical site filled with old forts, and plenty of attractions. In addition to this, I also had the chance to visit Chikan tower, another historical sight in the main part of Tainan. I also had numerous shopping trips while in the city. The urbanized lifestyle of modern Tainan reminded me very much of walking through the streets of a safer New York or Chicago.



Figure 16: Chihkan tower in Tainan city.

Another city I had visited called Taichung was also quite an enjoyable experience. This city was extremely modern and also full of art exhibits for the public to enjoy. Spending a day in Taichung felt like walking through an enormous modern art exhibit.



Figure 17: Art exhibit in Taichung.



Figure 18: Chihkan tower.



Figure 19: Eternal Golden Fort in Tainan



Figure 20: Fort Zeelandia in Anping district, Tainan

Overall, experiencing Taiwan's culture and society has been extremely enjoyable. Although there were times when I experienced difficulties with the language barriers, I had friends who were more than willing to help me in any situation. Along the way, I even learned a few words in Mandarian Chinese and helped some of the Taiwanese Undergraduate students practice English, Spanish, or Hindi. The very exchange of language I experienced with other students is what helped me to overcome my fears and grow more comfortable in my environment. I learned to embrace different ways of communication and be patient with learning about my surroundings.

# Conclusion

Prime Minister of India Jawaharlal Nehru once said, "We live in a wonderful world that is full of beauty, charm and adventure. There is no end to the adventures we can have if only we seek them with our eyes open."

Looking back on my two months in Taiwan, I realize that spending my summer in a country completely different from my own opened my eyes to the world around me. Although I was separated from my family and dearest friends back in the United States, I still embarked on this journey with hope and with eyes wide open.

Because I kept my eyes open, I was able to make the friends that I have and share moments that I will cherish for the rest of my life. I met professors and researchers alike who welcomed me with open arms. They strived to teach me all that they knew in their departments. From the moments in the labs, to the hours in the greenhouses or the fields, I knew that the knowledge I gained here would stay with me for the duration of my lifetime.

While in Taiwan, I discovered a whole new world. One filled with rich history, beautiful temples, views of the mist covered mountains and beaches with waves crashing against their shores. The memories I've made here are truly priceless and ones that will always bring profound emotions whenever I look back on them.

The day I went running for the first time in the fields of Shanhua was the day I knew that I had found adventure. I remember running alongside two of my friends, as we laughed feeling the very freedom that those fields openly gave us. The sun had been shining for a little while through the dark gray clouds as we ran. Soon enough, I could hear thunder rumbling above me as I ran through those fields feeling as though there was nothing else in this world. Only what I saw before me. When the rain started to finally come down upon all three of us, I remember feeling as though I had found the true meaning of what it meant to have an adventure. The power and the beauty of rain gracing the fields made me feel as though I was witnessing some sort of miracle. From that moment I knew that my eyes were, and now always will be, wide open.



Figure 21: Looking out from Chihkan tower.

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