From Moths, to Molecular Markers, to Memories in Taiwan

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INTRODUCTION

PERSONAL REMARKS

Throughout my years in high school, I have taken an active interest in gaining knowledge about the issues of global poverty, hunger, and malnutrition. From my participation in debate I have researched and delivered speeches expressing my own solutions to these intertwined and far-reaching problems. In Model United Nations, I have worked, as a delegate to the Economic and Social Committee from Nigeria and Brazil, with other students representing countries of the United Nations and consequently experienced comparable difficulties the current global community faces in trying to create resolutions and strategies for unified action to alleviate poverty and increase food production and security.

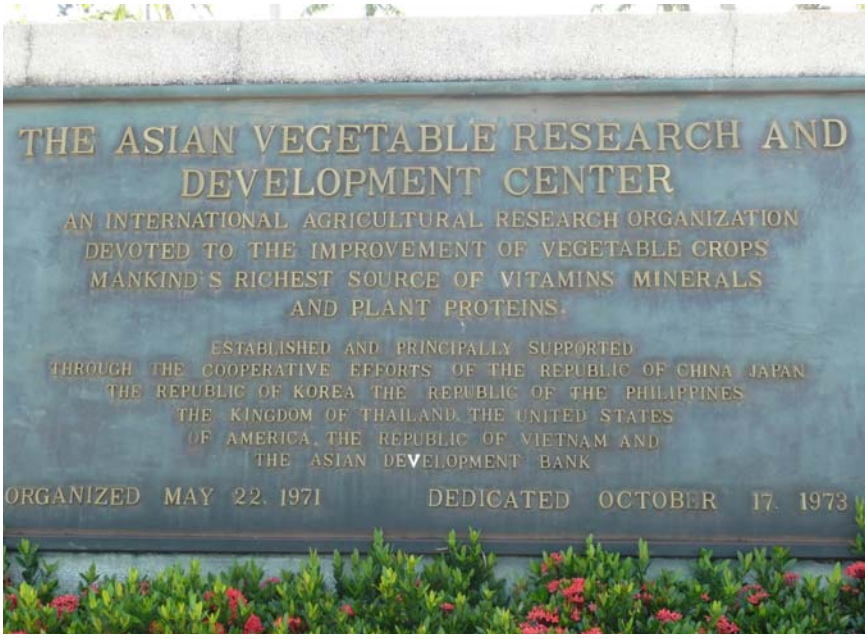
However, information alone does not interest me. As Rutherford D. Rogers of Yale University once insightfully stated, "We are drowning in information and starving for knowledge." As with the grave issues of world hunger and malnutrition, sole information frustrates me while fueling a motivation to take individual action—experience through which one gains true knowledge. Well-aware of the frightening statistics and accounts of the millions of ailing and impoverished found in all parts of the world, I experience difficulties presenting such information in speeches and debates when I cannot possibly understand the life situations of whom I speak from mere memorized statistics. The descriptions of people's lives cannot be reduced to sole numbers. As a young adult, though my assessments of problems prevalent in the world may be considered naïve compared to those of my elders, I too have thoughts of solutions and future actions. Information gained from reading, listening, and talking about these concerns is not enough for me—I want to take action to gain knowledge.

This deep longing, this need for a way to make a change and contribute my own efforts to improving the lives of others around the world was answered when I learned about the World Food Prize Foundation's Youth Institute. The opportunity to immerse among individuals dedicated to improving lives around the world prompted me to immediately contact my teacher about my interest in attending the Symposium in 2006, and soon I began to develop a research paper on the essential role that the education of family farmers plays in creating sustainable paths to food and nutrition security in East Africa.

At the Symposium I was captivated by the speakers, as well as the individuals I spoke with at mealtimes, who passionately presented information on their contributions to gaining research and developing technologies for creating agricultural sustainability in the future. On Saturday after I spoke about my paper to dignitaries in the small group setting and presented my group's findings to all the attendees in the auditorium, I listened to the 2006 Borlaug-Ruan interns' presentations on their experiences. Having read about the International Internship Program and spoken with previous interns before attending the Symposium, I knew that I would apply and already had begun to imagine the experiences I could have if I were selected.

After the long application and selection process, I was ecstatic upon opening a letter from the World Food Prize Foundation to find that I had been selected as the 2007 intern to AVRDC–The World Vegetable Center. Thus, my journey to the World Vegetable Center began long before this summer, and I have learned and experienced more than I could possibly have imagined.

AVRDC—THE WORLD VEGETABLE CENTER



Founded in 1971 as the Asian Vegetable Research and Development Center, the World Vegetable Center was renamed to more accurately describe the extent of its contributions in alleviating malnutrition and poverty in developing countries through improved vegetable crop production and consumption (AVRDC). The World Vegetable Center collaborates with national agricultural research centers and public and private organizations in Asian and African countries and other regions of the world to increase food and nutrition security with a dedication to vegetable research and development.

According to the report "Better Health through Horticulture– AVRDC's Approach to Improved Nutrition of the Poor," AVRDC improves human nutrition in four ways: 1) "increased vegetable productivity and availability through improved varieties with disease resistance and tolerance to environmental stresses, and crop management practices to enable year-round and safe vegetable production; 2) enrichment of the nutrient/phytochemical content of vegetables through the collection and promotion of nutrient-rich but under-utilized vegetables such as indigenous vegetables; 3) enhancing nutrient bioavailability through optimum food preparation and recipe design, and studies

of a whole food approach to investigate the bioavailability of phenolics; 4) assessing the benefits from the consumption of vegetables high in nutrients/bioactive compounds on health and overall economic development."

AVRDC is a non-profit research center that receives roughly US$18 million from national governments and several private foundations based in countries throughout the world. Research concentrations at the World Vegetable Center are divided into different unit divisions, including Bacteriology, Biotechnology, Molecular Breeding, and Plant Physiology, Crop and Ecosystem Management, Entomology, Genetic Resources and Seed, International Cooperation, Mycology, Nutrition, and Virology.

Though I spent the summer working in the two units of Entomology and Biotechnology, Molecular Breeding, and Plant Physiology, I received the uncommon opportunity to work on two projects directly relating the work of both. I also received the opportunity to visit and learn from scientists in the Biotechnology, Bacteriology, Genetic Resources and Seed, Nutrition, and Pepper Units about the importance of their ongoing research in establishing food security for the future.

IMPACT OF BT TRANSGENIC PLANT RESEARCH

The diamondback moth, or *Plutella xylostella*, caterpillars are major pests of glucosinolate-rich crucifers found in over eighty countries around the world (CIMBAA 2005). In fact, *P. xylostella* is known to cause losses of 30-80% of annual cabbage and cauliflower yields in developing countries, which amount to a global cost of over US$1 billion (Talekar and Shelton 1993). The significant negative impact of insect pests on crucifer production reduces the availability of *Brassica* species and subsequently increases their costs. Since cabbage is a vital cash crop that provides vitamins and fiber for peoples living in rural and urban areas, this truth presents several concerns for farmers, and especially those of developing countries of Asia and Africa with limited land and resources. (CIMBAA 2005).

Adding to the concern of pest prevalence is the truth that diamondback moths have developed resistance to almost all types of insecticides around the world. Unfortunately resource-poor farmers who are unable to bear the costs of seeds of Bt transgenic plants continue to use the conventional method of spraying pesticides on plants (Kumar 2004). As farmers spray more and more pesticides, the insects gradually develop resistance over time until the use of insecticides becomes futile. Indian farmers who are the world's largest cauliflower and second-largest cabbage growers spend approximately 38% of total costs of production to buy and apply insecticides to crops, a cost that is greater than US$168 million for 6,000 tons of insecticides for diamondback control (CIMBAA 2005). Thus, the continued spraying of insecticides is a costly, unfeasible solution for farmers in addressing the problems that pest prevalence imposes.

The development of genetically engineered, insect-resistant plants with the Bt gene(s) presents a feasible long-term solution to the problems posed by insect pests. In fact, adoption rates of the insect-resistant plants producing active δ-endotoxins (crystal protein toxins) of the soil bacterium *Bacillus thuringiensis* are "among the highest for a new agricultural technology" (Zhang et al. 2006). The development of transgenic plants as a method for management of insects has been facilitated by the improvement in recombinant DNA technology, and research has shown that the Bt plants are effective in controlling the population of *Lepidoptera* species (Liu et al. 2005).

Since the initial commercialization of Bt transgenic plants in 1996, the development of engineered plants with genes encoding Bt toxin proteins has greatly expanded (Zhao et al. 2003), with growth of Bt plants on 23 million hectares globally (CIMBAA 2005). Many cruciferous hosts of *Plutella xylostella* such as cabbage, broccoli, and oilseed rape have been engineered with Bt genes (Shuler et al. 2004). Bioengineered plants that function in the control of pest populations have the potential to "promote agricultural growth, improve environmental safety, and alleviate hunger and poverty in Africa and Asia" (CIMBAA 2005). The reduction in pesticide use can only have positive impacts on the environment and human health, and because the ingestion of their crystal toxin proteins has been largely successful in killing pests, Bt plants offer a promising solution for insect management that can prevent the devastating loss of crop yields.

Bioengineered Bt plants offer an even more promising solution for delaying pest resistance to the δ-endotoxins with the technique of gene pyramiding, the expression of two or more insecticidal proteins in a single plant. According to the research published by Zhao et al. (2003), "Theoretical models suggest that varieties pyramiding two dissimilar insect toxin genes in the same plant have the potential to delay the development of resistance much more effectively than single-toxin plants." Thus, if a pest develops resistance to one of the protein toxins in the Bt plant over time, the likelihood that the pest will develop resistance to all the different toxins expressed in the plant is significantly reduced.

As with any new agricultural technology, scientists must conduct risk assessment studies of Bt transgenic plants on flora and fauna of their surrounding environments. Risk assessments of the effects of bioengineered, insect-resistant plants on target and non-target insects are especially important. While many studies have been conducted to assess the impact of the Bt plants on target insects such as *Plutella xylostella*, with generally positive results in terms of success in pest control, more studies are needed to assess the impact of the plants on non-target insects, or parasitoids of the pests.

Some risk assessment studies found "no apparent negative effects of transgenic plants on parasitoids, while some reported lower parasitoid survival rates due to premature host death, lower parasitoid emergence rates, increased parasitoid larval development times, or reduced longevity and fewer female ova" (Liu et al. 2005) The inconclusive findings on the impact of Bt toxins on non-target insects necessitate further studies. Furthermore,

there are only a few reports on the effects of transgenic plants expressing two insecticidal proteins on parasitoids (Ren et al., 2004).

I studied the impact of the Bt toxin proteins on *Plutella xylostella*, a target pest of cabbage, and *Diadromus collaris* (non-target insect), an endoparasitoid of *Plutella xylostella*. Due to specific national and international restrictions on the release of genetically modified organisms into the environment, I was unable to use bioengineered cabbage plants with the Bt gene in my experiments. I used a commercial Xentari solution instead to imitate the role that multiple Bt genes would play in a plant. The Xentari solution contains crystal protein toxins, including Cry1A(a), Cry1A(b), Cry1C, and Cry1D (and more) *Bacillus thuringiensis* toxins. The integrated action of multiple Bt crystal toxin proteins in the Xentari solution are representative of gene pyramiding. Thus, experimental results using the Xentari solution provide a way to predict the impact that a bioengineered plant with most likely more potent toxin proteins would have on target and non-target insects.

The risk assessment study I performed at AVRDC is part of the work the Center is conducting for the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA), a public/private sector project that will directly address the UN millennium development goal of eradicating poverty and hunger by providing farmers of the developing world access to seeds of insect-resistant cabbage and cauliflower plants with two different Bt proteins. Thus, the expanded scope of research and development of Bt transgenic plants in the future provides hope for the establishment of a long-term solution for effective pest management.

SIGNIFICANCE OF MOLECULAR MARKER USE IN PLANT BREEDING

The genetically and morphologically diverse *Brassica* species have global economic importance because they have been cultivated to provide edible roots, stems, leaves, buds, flowers, and seeds and have also been used as forage, sources of oil, and as ornamentals (Ren et al. 1995). The three major crop/species groups include *Brassica rapa* (A genome; turnip, swede, and Chinese cabbage), *Brassica nigra* (B genome; mustard), and *Brassica oleracea* (C genome; cabbage, brussel sprout, kale) (Lowe et al. 2004). Greater knowledge of *Brassica* genetic diversity allows for genetic improvement, more effective germplasm collection and conservation, and the scope for finding and developing accessions with beneficial traits such as disease resistance and heat tolerance.

The World Vegetable Center (AVRDC), as of July 31, 2007, has 1,805 *Brassica* accessions in its Genetic Resources and Seed Unit. The value of large germplasm collections is based in the genetic diversity of its accessions, and more knowledge is needed on the amount of variation present in the *Brassica* collection. While knowledge of accession variation can be determined by morphological studies, study of genotypes provides a more standardized and accurate approach for classification and evaluation.

Using information on genetic diversity, scientists can use the accession variations to establish successful breeding programs; this information can be obtained using DNA-based molecular markers.

Molecular markers provide an effective method for evaluation of genetic diversity and relationships among species. Information on the extent and distribution of genetic diversity among species provides a way for the effective conservation and use of plant genetic resources by improving identification and management (Hodgkin et al. 2001). Use of molecular markers leads to the identification of quantitative trait loci (QTLs) that are stretches of DNA found close to desirable traits that allow scientists to use specific genes located on a genetic map in crop improvement strategies known as marker-assisted breeding (Piquemal et al. 2005).

Although use of RFLP (Restriction Fragment Length Polymorphism) markers has been instrumental in developing an understanding of genome structure and evolution in the *Brassicas*, this type of marker lacks high throughput potential, ease of data interpretation, and application of simple and transferable technology, making it a deficient tool for breeding programs (Lowe et al. 2004). Over the past few years new PCR-based markers have emerged such as RAPDs (Random Amplification of Polymorphic DNA), AFLPs (Amplified Fragment Length Polymorphism), and microsatellites for plant genetic research. Of these, microsatellites or simple sequence repeats (SSRs) have a great deal of potential (Lowe et al. 2004). SSR markers are usually di- or tri-nucleotide motifs randomly interspersed within eukaryotic genomes and are highly variable with regard to repeat number, mostly co-dominant and highly efficient in the pedigree analysis of most crops (Piquemal et al. 2005).

In my experiment, I used SSR markers to assess the genetic relationships among 32 *Brassica* accessions. Knowledge of genetic relationships will be used in future experimental crosses between various accessions at AVRDC to determine outcrossing potential and cross-compatibility of *B. oleracea* subspecies. This assessment of cross-compatibility is part of the risk assessment of Bt-transgenic vegetable *Brassica* because determining the potential for hybridization between transgenic crops and their relatives is a major component of risk assessment. The international community is wary of the possible negative consequences resulting from the release of transgenic plants into the environment, which has motivated researchers to seek information on the potential for transgene escape into the environment through hybridization (FitzJohn et al. 2007). Knowing which species are reproductively compatible and incompatible can help define future risk assessment studies.

RISK ASSESSMENT OF Bt TRANSGENIC VEGETABLE BRASSICA: EFFECT ON TARGET AND NON-TARGET INSECTS

I. Materials and Methodology:

I completely dissolved 10 mg of Xentari solution in 87.5 mL of distilled water. In the first trial, I obtained young cabbage leaves and cut them into one hundred circular disks. I dipped fifty of the cabbage leaf discs into the Xentari solution to which I had added 30 microliters of a "Sticky Solution" (blend of polyoxyethylene alkylaryl ether and sodium salt of dialkyl sulfosuccinate) to ensure the absorption of the crystal toxin proteins by the cabbage. For my control, I dipped the other fifty cabbage discs into a solution consisting of distilled water and "Sticky Solution."

I placed one cabbage leaf disc in each small plastic cup covered with a thin tissue paper (to absorb excess moisture in the cup) and plastic lid. I added two fourth- instar *Plutella xylostella* larvae (200 total–100 for Xentari-treated cabbage and 100 for control) to each cup to feed on either the control or the cabbage soaked in Xentari solution for two days. This ensured that the larvae had enough time to digest the Bt crystal proteins.

After two days, I removed the dead diamondback moth larvae from all the cups and collected the living diamondback moth larvae on one cabbage leaf that I placed in a hollow plastic cylinder with netting covering the ends. I exposed the surviving larvae to adult *Diadromus collaris* flies for parasitization. I removed the *Diadromus collaris* parasitoids after two days, allowing enough time for the diamondback moth larvae to pupate so that the female *Diadromus collaris* flies could lay their eggs inside the pupae. I then waited until the *Diadromus collaris* parasitoids emerged from the pupae.

In my second trial, I did not use leaf discs; I soaked two young, whole cabbage leaves in Xentari and "Sticky Solution" and added 100 fourth-instar diamondback larvae. I obtained two other young, whole cabbage leaves which I dipped only in "Sticky Solution" and distilled water (control) and added 100 fourth-instar diamondback moth larvae. I placed the leaves of the two experiments in two hollow plastic cylinders with netting covering the ends. I allowed the larvae to feed for two and a half days.

In the second trial, after allowing the larvae to feed on the cabbage leaves, I counted the number of larvae dead on the Xentari-treated cabbage leaves. I picked the same number of surviving larvae from the control as there were on the Xentari-treated cabbage for parasitization. I collected the surviving larvae from the Xentari-treated cabbage on one leaf and the same number of surviving larvae from the control onto another leaf and set up two different net-covered cylinders for *Diadromus collaris* parasitization. I removed the *Diadromus collaris* adults after one day and then waited until the next generation of *Diadromus collaris* parasitoids emerged from the diamondback moth pupae.

NOTE: Refer to Appendix A for a photograph of Plutella xylostella pupae and Diadromus collaris adults.

II. Results:

Percent Parasitism of Diamondback Moth Host Larvae:

Trial One:

In the experiment with Xentari-treated cabbage, 55 diamondback moth larvae (out of 100) survived after two days of feeding. 12 pairs of the *Diadromus collaris* flies were released for parasitization of the larvae that fed on the Xentari-treated cabbage leaves. In the control experiment, 83 diamondback moth larvae (out of 100) survived after two days of feeding. 16 pairs of the *Diadromus collaris* were released on the larvae that fed on the normal cabbage leaves. After the parasitoid adults were removed and I waited for the development of the eggs, 3 parasitoids emerged from the larvae that fed on the Xentari-treated cabbage (5.45% parasitoid emergence) while 6 parasitoids emerged from the larvae in the control (7.23% parasitoid emergence).

Trial Two:

In the experiment with Xentari-treated cabbage, 40 diamondback moth larvae (out of 100) survived after two and a half days of feeding. I collected the same number (40) of diamondback moth larvae from the control. I exposed the larvae in each experiment to ten pairs of *Diadromus collaris* adult flies for parasitization. After the parasitoid adults were removed and I waited for the development of the eggs, only 1 parasitoid emerged from the larvae that fed on the Xentari-treated cabbage (2.5% parasitoid emergence) while 10 parasitoids emerged from the larvae in the control (25% parasitoid emergence).

Taking data from the two trials, an average of 2 parasitoids emerged from the larvae that fed on Xentari-treated cabbage and 8 parasitoids emerged from the control experiment. There was an average of 3.98% parasitoid emergence from the larvae that fed on the Xentari-treated cabbage and an average of 16.12% parasitoid emergence from the larvae that fed on the normal cabbage. Thus, the use of the Bt crystal toxin proteins reduced the parasitoid emergence to roughly one-fourth of that in the control. A possible explanation of this result is that the larvae that survived after feeding on the Xentari-treated cabbage became weakened and could not serve as effective pupal hosts for the development of the *Diadromus collaris* eggs that were laid inside the pupae. Thus, fewer eggs could develop to maturity in the diamondback pupae in the Xentari-treated cabbage experiment.

Development of *Diadromus collaris* Parasitoids:

In both trials, the parasitoids emerged thirteen days after the removal of the *Diadromus collaris* adults after parasitization of the diamondback moth pupae. Thus, my experiment did not find necessarily a positive or negative impact on the time required for the development of the parasitoids that some studies have found.

Sex Ratio of *Diadromus collaris* Parasitoids:

Xentari-Treated Cabbage Experiment:

Of the 3 parasitoids that emerged from the pupae in the Xentari-treated cabbage experiment in the first trial, 2 were male and 1 was female. The one parasitoid that emerged in the second trial was male. Thus, overall there were more male *Diadromus collaris* adults that emerged in the Xentari-treated cabbage experiments.

Control Experiment:

Of the 6 parasitoids that emerged from the pupae in the control experiment in the first trial, all of them were male. In the control experiment of the second trial, 9 out of the 10 emerged parasitoids were male. Overall there were more male *Diadromus collaris* adults that emerged in the control experiments.

Number of Dead Diamondback Moth Pupae:

In the first trial of the experiment, there were 12 dead pupae that had fed on the Xentari-treated cabbage, while there were 30 dead pupae that had fed on the control. Exposure to the Bt crystal toxin proteins caused more of the diamondback moth larvae to die while in the larval stage, whereas in the control more of the larvae that had fed on the normal cabbage were able to continue their development and to pupate after completing the larval stage. The results of the second trial of the experiment confirmed the findings of the first trial: 6 dead pupae remained on the Bt-treated cabbage while 21 dead pupae remained in the control. This general trend demonstrates the effectiveness of the Bt crystal toxin proteins, whose ingestion truncated the development of the larvae and prevented most from reaching the pupal stage. Only a few of the larvae that fed on the Bt-treated cabbage survived to pupate, and they served as potential hosts for the parasitoid *Diadromus collaris*. As a result, the parasitoid can be affected by the presence of the Bt crystal toxin proteins in the food ingested by the host larvae, demonstrating the need for evaluation of the potential effects on non-target insects associated with the use of transgenic plants.

RISK ASSESSMENT OF Bt TRANSGENIC VEGETABLE BRASSICA: USE OF MOLECULAR MARKERS TO ASSESS GENETIC RELATIONSHIPS IN ASSESSMENT OF CROSS-COMPATIBILITY AND OUTCROSSING POTENTIAL

A. Materials and Methodology:

Acquisition of Plant Materials:

I initially planted 118 accessions of *Brassica* subspecies: 35 accessions of kailan (Chinese kale), 25 accessions of cabbage, 25 accessions of cauliflower, 29 accessions of broccoli, 2 accessions of mustard, 2 accessions of other kales. The seeds for the plant materials were obtained from the AVRDC Genetic Resources and Seed Unit, which maintains a database for the classification of the accessions which were used in this experiment.

Ten seeds per accession were sown in peat moss in 3-in. diameter pots in the greenhouse on June 21st and 26th. The greenhouse temperature was an average of 31° Celsius daytime and 25° Celsius during nighttime.

On July 10th (collection of tissue from first set of planted samples) and July 16th (collection of tissue from the second set of planted samples), after I harvested ten to twelve of the youngest, healthy leaves from each of the accessions, I folded and stored them in 6 mL Eppendorf tubes. The 36 accessions whose leaves I collected for DNA extraction represent a diverse array of *Brassica* subspecies that have economic importance to peoples in eleven different countries, encompass a wide range of phenotypic diversity, and have varied end use.

The plant tissue was immediately stored after collection in a –80° freezer until use. The tissue was then freeze-dried overnight to prepare for DNA extraction the following morning.

NOTE: Refer to Appendix B for information on which samples were collected in DNA Sets 1 and 2, with classification of the samples into major subspecies groups. Refer to Appendix C for more detailed information (cultivar name, accession number, country of origin, if applicable) on the Brassica subspecies planted.

Genomic DNA Extraction:

MiniBeadbeater

After freeze-drying the plant tissue overnight, I added seven to ten (3 mm) glass beads to each Eppendorf tube containing the leaf tissue, and then I placed the tubes in the MINI Beadbeater. The tissue samples were ground twice, for twenty to thirty seconds each time, depending on the amount of plant tissue collected. Immediately after the tissue grinding, I added 2.52 mL of extraction buffer (50 mM EDTA, 10 mM Tris-HCl, 500 mM NaCl, 10 mM sodium metabisulfite, and 100 µg/mL Proteinase K) to each Eppendorf tube. I had prepared the 50 mM EDTA (Ethylenediaminetetracetic Acid), 10 mM Tris-HCl, and 500 mM NaCl the previous day from the stock solutions, ensured that both the EDTA and HCL had a pH of 8.00, and sent these solutions to be autoclaved to ensure purity.

I vortexed the Eppendorf tubes for two to three seconds until all the leaf powder was mixed with the extraction buffer. After the mixing, I added 0.25 mL of 20% SDS (sodium dodecyl sulfate) solution to each tube and mixed gently by hand to incorporate it with the plant material and extraction buffer. The tubes were then incubated in a 65°C water bath for 15 minutes. I evaluated the color of the leaf tissue in the tubes after two, five, ten, and fifteen minutes for evidence of browning (the color of the leaf tissue changed from dark green to brown throughout time) of the plant material. At those same time intervals, I gently mixed the tubes by hand and returned them to the water bath.

After removing the Eppendorf tubes from the water bath, I added 1.25 mL of 5 M Potassium Acetate solution (500µL per 1 mL of extraction buffer). I shook the tubes by hand and stored them in the 4.0 °C freezer for twenty minutes. After removing the tubes from the freezer, I centrifuged the samples for twenty minutes at 4500 rpm.

The centrifuging process separated the DNA from other compounds in the leaf tissue (the other compounds settled to the bottom of the tubes as a brown mass), and the supernatant was the resulting solution in the tube containing the DNA. I was careful in transferring the supernatants by pipetting them to new 6 mL Eppendorf tubes by avoiding the area close to the brown mass at the bottom of the tube. This mass contained salts and other compounds that would have reduced the purity of the supernatant if also pipetted.

After transferring the supernatants, I added 3.5 mL of cold isopropanol and mixed the tubes gently by hand to avoid breaking the DNA. I allowed the samples to cool in the –20° C freezer for about two hours, after which the DNA became visible as a milky white cloud of "fibers" in the solution.

I centrifuged the samples for five minutes at 2500 rpm and then washed the DNA pellet by pouring off the supernatant and filling enough 70% ethanol to the 5 mL mark on the Eppendorf tube. I gently shook the samples by hand afterward. I centrifuged the samples again for three minutes at 4500 rpm, poured off the supernatant, and added enough 70% ethanol to the 4 mL mark on the Eppendorf tube. I centrifuged the samples for the last time for six minutes at 4500 rpm and poured off

the supernatant. This "washing" with ethanol process ensures the purity of the DNA sample pellet.

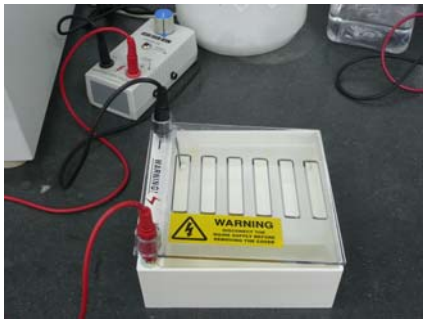
I allowed the samples to dry overnight by placing them in the freeze-dryer. The freeze-dryer had one shortcoming in that the DNA pellet from one of the samples fell into a tube containing the DNA pellet of another sample. Thus, I eliminated both of the samples due to the DNA contamination. By the end of the DNA extraction procedure, I had 34 samples for further experimentation.

DNA Quantification Analysis:

To the DNA Set 1 pellets I added 450 µL of a solution containing 9 mL of 1xTE buffer and 180 µL of RNAse. To the DNA Set 2 pellets I added 400 µL of a solution containing 8 mL of 1xTE buffer and 160 µL of RNAse. I incubated the Eppendorf tubes in a water bath at 37°C until the pellet was dissolved and the buffer was clear in each tube. I then centrifuged the tubes at 4500 rpm for ten minutes and transferred the liquid to new tubes to discard other contaminants. I stored the DNA samples at 4°C until use.

I used an agarose gel electrophoresis system for the DNA quantification analysis. The DNA quantification process was necessary because varying amounts of DNA were extracted from the different plant samples. I used λ DNA for comparing the DNA concentration in the bands on the 0.9% agarose gels. I added 4 µL of the 25 ng/µL λ DNA in one well to have a DNA concentration of 100 ng/µL, 3 µL of the λ DNA into the following well to have a DNA concentration of 75 ng/µL, and so on to have DNA concentrations of 50 ng/µL and 25 ng/µL. These λ DNA bands served as standards for comparison of the intensity of the bands of the other DNA samples. After finding the DNA concentration of the original plant samples, I was able to dilute all the DNA samples to 10 ng/µL working solutions for PCR amplification.

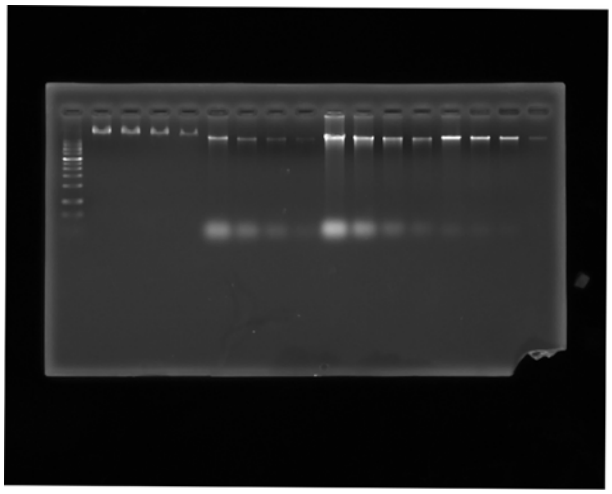
To prepare the 0.9% agarose gel electrophoresis system, I first added 48.5 mL of 0.5xTBE buffer to a flask in which I dissolved 0.45 g of agarose powder. I heated the resulting solution for two minutes in the microwave and allowed the solution to cool slightly before pouring into the balanced gel system. I then added the comb for the formation of wells and allowed the gel to solidify for thirty minutes before adding the DNA samples to the wells.



I conducted a pre-trial quantification analysis with three DNA samples to gain an idea of how much the other DNA solutions would have to be diluted to obtain a concentration in the range 25 ng/µL to 110 ng/ µL (which would be needed for PCR amplification later on). I diluted samples TB00520, TB00707, and TB00709 five, ten, twenty, and forty times and added the proportionate amount of 6x loading dye to each. The loading dye increases the molecular weight of the DNA sample and allows

it to settle into the gel well instead of diffusing entirely into the buffer. I added 4 μL of each diluted DNA sample to the wells in the agarose gel, and ran the electrophoresis machine at 50 V for eighty minutes.

After allowing the gel to run, I stained the gel in Ethidium bromide for about ten minutes. Ethidium bromide fluoresces with a red-orange color when exposed to UV Light, and thus it is effective for detecting nucleic acids in a gel. After removing the agarose gel from the staining box, I rinsed off the extra Ethidium bromide on its surface with water and placed the gel on top of the UV light. I then adjusted the camera to obtain a photograph. The picture below is a photograph of the first gel I ran as the pre-trial for the DNA Set 1 samples.



Order of Wells (numbered from left to right):
DNA Ladder (well 1)
TB00520: 5x, 10x, 20x, and 40x (wells 2-5, respectively)
TB00707: 5x, 10x, 20x, and 40x (wells 6-9, respectively)
TB00709: 5x, 10x, 20x, and 40x (wells 10-13, respectively)
λ DNA: 100 ng, 75 ng, 50 ng, and 25 ng (wells 14-17)

After obtaining a photograph of the agarose gel, I used the UVIband and UVIsoft programs on the computer, which compare the intensity of other DNA bands with the intensity of the λ DNA bands (which function as a standard) to determine the concentration (in ng) of DNA. I was able to determine that the DNA concentration of the 20x dilutions of each of the samples and the 40x dilution of two of the samples was in the range required for further analysis.

Thus, after the pre-trial, I diluted all the 18 DNA Set 1 samples (samples 1-18) by 20x and 30x for quantification analysis. The quantification analysis was a long process because I ultimately had to run different agarose gels with some or all samples diluted 10x, 5x, 15x, 20x, and 30x multiple times to accurately determine the concentration. In one gel, a particular dilution of a specific sample might be in range, but the same dilution of another DNA sample may not, requiring another agarose gel run with different dilutions of that sample to determine its concentration. I did a pre-trial analysis with the 16 DNA Set 2 samples (samples 19-34), and followed a similar process of dilution to determine their concentrations.

NOTE: Refer to Appendix E for photographs of DNA Set 1 quantification analysis agarose gels. Refer to Appendix F for photographs of DNA Set 2 quantification

analysis agarose gels. Refer to Appendix G for information on the original concentrations of the DNA samples.

Using the calculations of the original concentration of the DNA samples, I was able to create working solutions of the DNA with a concentration of 10 ng/µL for PCR amplification using the equation $M_1V_1=M_2V_2$ (which shows the relationship between concentration and volume). I prepared a total of 250 µL of each working DNA solution.

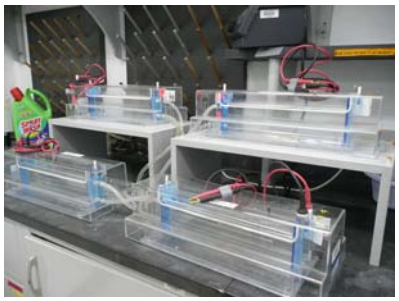
NOTE: Refer to Appendix H for information on the amount of gDNA (genomic DNA) and MQ water added to create working solutions of each of the samples.

PCR Amplification:

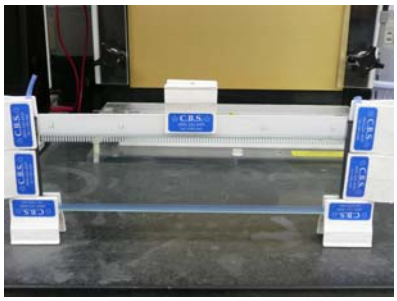
The next stage in the process was amplification of the DNA samples using PCR, polymerase chain reaction. I first prepared the PCR cocktail containing proportionate amounts of MQ water (doubly distilled and filtered), 10x buffer, 2.5 mM dNTP, 20 µM forward and reverse primers, and Taq polymerase. I prepared the PCR cocktail by keeping all components on ice so that degradation would not occur. I obtained the primers from the –20°C freezer immediately before adding them to the cocktail to ensure that they were in the best condition. I divided each 96-well plate into three sections of 32 wells, each section containing a different primer.

I screened 20 SSR primers for the experiment using the SSR50 program in the PCR machine. Using information obtained from the Electronic Supplementary Material on SSR loci, I chose those primers which would produce a PCR product of expected size within the *Brassica* subspecies used in my experiment. Each run of the PCR machine took little more than two hours, during which I prepared the acrylamide gels I would use to run the PCR products.

I first prepared the 6% acrylamide gel solution. I added 99 mL of a solution containing DDI water (740 mL), 5x TBE buffer (100 mL), and 40% acrylamide (150 mL). To this I added 70 µL of TEMED and 0.08 g of ammonium persulfate completely dissolved in 1 mL of DDI water. I allowed the resulting 100 mL solution to stir on the lowest setting for one minute. After this, I immediately poured the solution into the two acrylamide gel systems I had set up before, inserted the 96-well comb, and allowed the gel to solidify for roughly forty minutes.



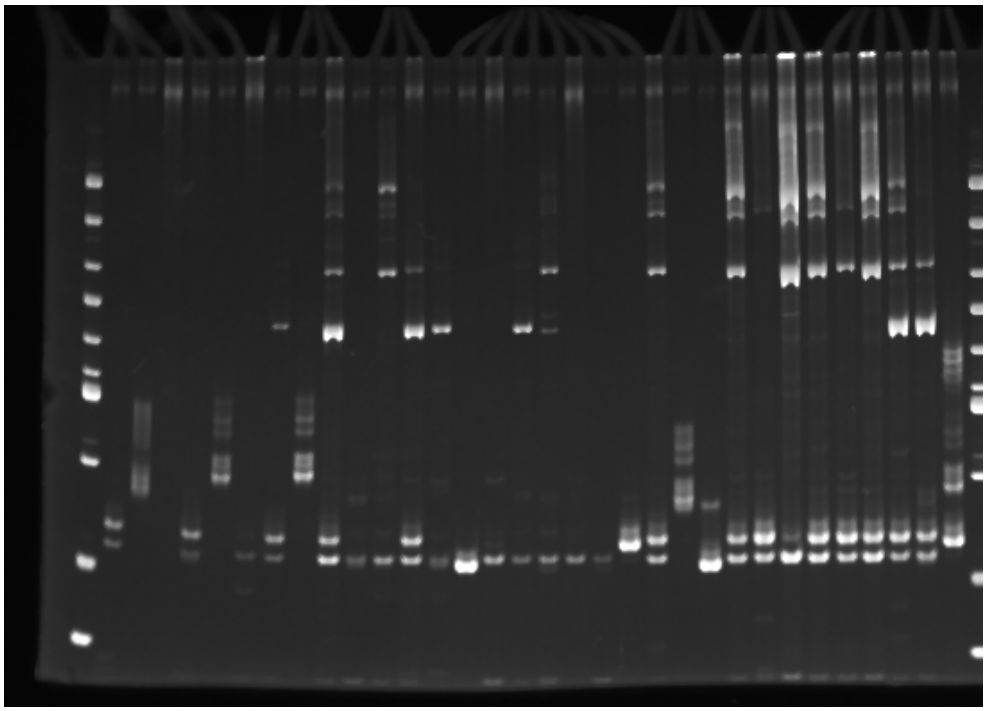
This is the electrophoresis machine used to run the acrylamide gels.



This is an acrylamide gel system with the 96-well comb inserted into the gel.

After the gel finished solidifying, I loaded the wells with the PCR products (with the addition of 6x dye) and DNA ladders (which served as standards for the authentication of good acrylamide gel electrophoresis results). I loaded 7 µL of PCR product (plus dye) into each well and ran the machine for 40 minutes at 180 V. At first I loaded the DNA solutions individually, and it took about an hour to load the 96 wells with the ladder and solutions. However, I mastered the use of the 8-channel pipette which significantly reduced the time required for loading to less than twenty minutes. The shorter amount of time for loading the DNA also ensured better results in the electrophoresis process because the DNA solutions from the wells did not diffuse as much as they did in the hour's time. The addition of the ladder in the gel served as a tool for assessing the accuracy of the results. If the distinct bands of the DNA ladder did not show clearly in the final photographed gel, this occurrence would indicate the inaccurate nature of the electrophoresis trial.

After allowing the electrophoresis machine to run, I removed the acrylamide gel and stained it in Ethidium bromide for ten minutes. I rinsed off the gel with water before photographing it above the UV light. Below is a picture of one of the gels with the screening of SSR primer 50 with the 32 DNA samples:

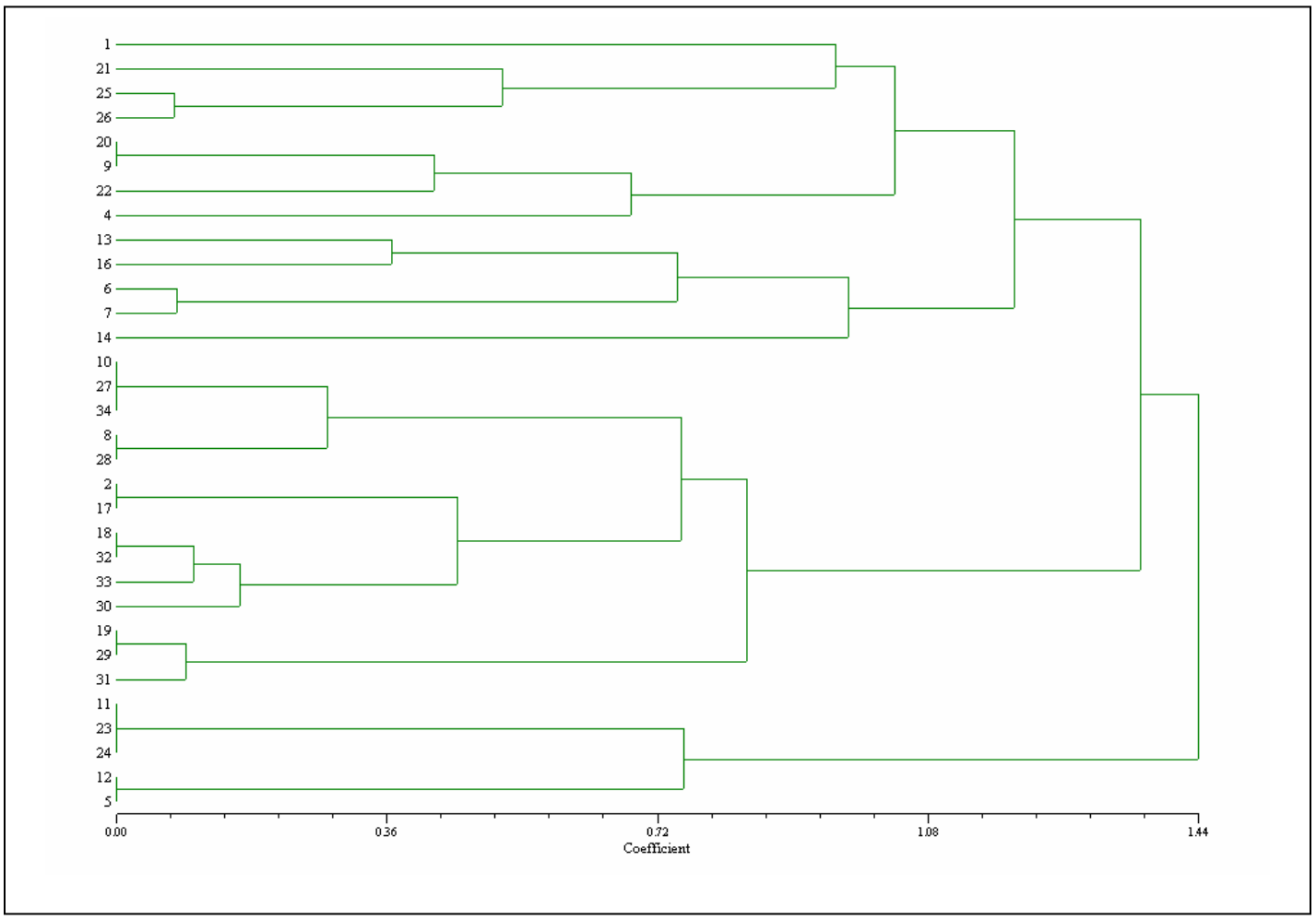


From this photograph, one can see the distinct banding patterns indicating polymorphisms of the DNA samples. The bands of the DNA ladder in the first well (to the far left) served as reference marks for the scoring of the primer. After screening twenty primers, I scored eleven of the gel photographs and inserted the data into an Excel spreadsheet. Though I screen twenty primers, eight of the primers amplified fewer than 18 (out of 32) DNA samples, and thus I eliminated those primers from the scoring to maximize the accuracy of the scoring results. I chose to score the eleven primers that produced the greatest number of amplified DNA samples. I inserted a "1" if the band was present in a DNA sample, a "0" if the band was absent, and a "9" if the band presence was questionable (perhaps a small amount of DNA from the surrounding wells were responsible for a very faint band in a certain location).

NOTE: Refer to Appendix I for photographs of the scored acrylamide gels used to run the PCR products. Refer to Appendix J for the Excel spreadsheet used in the scoring of the primers. Refer to Appendix K for summary information on the specific SSR primers screened and scored.

Results—Creation of Dendrogram:

Dr. de la Peña helped me insert the Excel spreadsheet data indicating band presence, absence, and questionable presence (indicated by 1's, 0's, and 9's, respectively) into the program NTSYS which uses mathematical formulas and matrices (with information on samples' similarity and dissimilarity) to ultimately formulate a dendrogram (shown below) with clusters displaying the genetic relationships among the *Brassica* species.



The greater the coefficient at which the species are connected corresponds to the more distant genetic relationship. Thus, one can see the distinct clusters of species more closely related to one another compared to others. My results demonstrate the significant variance among accessions, even within particular subspecies. For example, though samples 20 through 34 are all cauliflower accessions, some are more related to other *Brassica* subspecies. While samples 19, 29, and 31 are closely related cauliflower accessions, the cauliflower accessions 27, 28 and 34 are more closely related to samples 8 and 10, which are both Chinese kales. The crossing of accessions from the three primary *Brassica* species groups throughout time has created a large number of varieties. It is this knowledge of genetic diversity and relationships among species that can aid in studies of cross-compatibility and outcrossing potential.

PERSONAL REFLECTION

Whether I was cheering for the AVRDC scientists' team in the dragon boat race of the Dragon Boat Festival in Tainan, praying in the Julian Temple of Hsinchu, strolling around the night market in Taichung, bargaining for jewelry in the Tainan jade market, sampling preserved fruits sold in the narrow market street of Amping, singing and dancing at KTV, or visiting the Confucius Temple in Kaoshiung, I experienced numerous cultural aspects of Taiwan and developed a love for the country and its people.

I will never forget my trip to the mountains in the northeast with the Entomology Unit. Ms. Lin, Ms. Liang, Franzi, and I went to Wu Ling Farms for three days to collect diamondback moth larvae and pupae for our experiments because many of the specimens in Shanhua were dying due to the heat and the rainfall. I stared out of the window throughout the seven-hour car ride to Wu Ling Farms, admiring the greenery of the flatlands and the breathtaking mountains and repeatedly whispering *"Hao piaoliang"* ("How beautiful"). Fully equipped with white rain boots, wooden hat, gloves and sleeves, and tweezers and collection box, I worked with them for nine hours in the field to collect the elusive larvae and pupae from decaying cabbage plants. My favorite memory from the trip was when I asked Ms. Lin to sing her favorite Taiwanese songs for us, and we listened as the mist settled on the mountains surrounding the field that cool, breezy evening.

I also have many fond memories from the weekends I spent volunteering at the Shanhua church. The children may have thought my (most likely mispronounced) sentences of single Chinese words and hand gestures silly, but in any other sense I could not feel any language barrier. We drew pictures, played Chinese checkers, and I succeeded in conversing with them in the little English they were not shy to speak. Ms. Jin, one of the researchers at AVRDC, conducts a horticultural therapy class for children who have disabilities, and even though her program was finished by the time I reached

Taiwan, she conducted the same program with the children at the church so I would get a chance to experience it. So, Annie, Wendy, Ruth, Linda, Mark, and I made flower pots from a mixture of ingredients including cement and sand. I enjoy volunteering in the hospital and the library of my local community, but the experience I had while in Taiwan was truly unique.

One of the best aspects of the internship experience was the opportunity I had to meet people from many different countries and learn about their work. I really enjoyed talking with many of the researchers about the state of world insecurity and the impact of AVRDC's work around the world. From those discussions I learned that research and development are only the first two stages for establishing agricultural sustainability. Though the subsequent stages of extension, utilization, and repetition are equally important, I learned that the international community still needs to invest in extension means so that the resource-poor farmer can access the information and technology discovered and developed at research centers. My favorite quote from the discussion I had with Ms. Clavero is, "Output without impact is meaningless." Most scientific knowledge currently resides within the academic community of developed countries, and ensuring that the rural farmer has access to disease or drought-resistant crop or simply knowledge of improved farming techniques will be the greatest challenge in the coming decades.

These discussions further defined my own thoughts on what steps individual countries, independent organizations, and the international community as a whole can take to address the state of food and nutrient insecurity around the world. In a conversation I expressed my desire to work abroad in the future in rural communities, one that was once based solely on knowledge obtained from reading alarming statistics and information on the state of world hunger and malnutrition before this internship but confirmed through my firsthand experiences this summer. I have always believed that education is a means of empowerment. Educating farmers and empowering them with the knowledge and the tools for producing healthier and plentiful food is an effective long-term solution for establishing food and nutrition security, one that can allow farmers to engender economic and social developments to improve their societies. From the discussions I had with the researchers, however, I learned that empowerment needs an added component to education. One cannot go to a rural community and only teach the farmers; one must provide the people a binding vision that motivates each individual to work not only to improve his/her life, but to also improve the community as a whole.

A corollary can be drawn for the international community as well in trying to find a solution for establishing agricultural sustainability in the future. There are so many barriers to improving food and nutrition security, but the wise investment of resources in research, development of technology, and extension means for educating farmers and increasing their access to advances can transform the futures of millions of individuals in developing nations—an undertaking that will require the focus and unification of all countries in improving the world as a whole.

I was deeply inspired by a presentation of one scientist, Mr. Plewa, who had established a vegetable seed collection and distribution program in Laos. After he traveled in all areas of the country and collected seeds for a variety of vegetable crops, he screened the seeds that would be able to withstand the most intense selection and then mass-produced the hardiest seeds and distributed them throughout Laos. This research took an unparalleled integrative approach, for not only did the scientists discover the best strains of vegetable crops, but they also distributed them to farmers and marketed the seeds throughout the country. While people today are quick to mention inadequate infrastructure and limited monetary supply for the difficulty in spreading agricultural knowledge and technologies to farmers, especially those who are impoverished and/or live in rural areas, scientists such as Mr. Plewa are using their knowledge to assimilate research, production, and distribution, and education of farmers to improve and increase agricultural production and ultimately provide opportunities for a better way of life.

Centers like AVRDC are truly making significant strides in improving the livelihoods of resource-poor farmers, and from my experiences this summer I gained first-hand knowledge of the complexities related to world hunger and nutrition. My experiences at AVRDC have shown me the close relationship between my passions for scientific research and for helping others. Until this internship, I had thought the two were mutually exclusive–one involves conducting experiments in a laboratory and the other involves interacting with people to improve their lives. As I saw at the World Vegetable Center, the search and application of science transcend the walls enclosing the laboratory and provide the opportunity, and the means, to help others, especially in the field of agriculture. I have now experienced the connection between the two and can attest to their increasingly important union.

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APPENDIX A: *PLUTELLA XYLOSTELLA* AND *DIADROMUS COLLARIS* PHOTOGRAPH

APPENDIX B: *BRASSICA* ACCESSIONS

*Key for Chart (below):
Blue Highlight: First set of DNA samples collected on July 10th
Yellow Highlight: Second set of DNA samples collected on July 16th
Pink Highlight: Collected sample, but contaminated DNA pellets were eliminated
No Highlight: Planted, but not collected because did not grow in time

Cabbage	Cauliflower	Broccoli	Other Kales	Mustard	Kailan
BB006	CF03	BR001	TB00760	TB00709	CR046
BB008	CF10	BR002	TB00761	TB00707	CR059
BB018	CF16	BR005			CR060
BB019	CF17	BR048			CR061
BB020	CF28	BR069			CR098
BB021	CF31	BR071			Ba08
BB022	CF32	BR072			Ba09
BB023	CF33	BR075			Ba13
BB110	CF34	BR076			Ba14
BB112	CF35	BR086			Ba17
BB113	CF36	BR093			Ba18
BB148	CF37	BR100			Ba19
BB149	CF38	BR117			Ba20
BB214	CF39	BR151			Ba28
BB236	CF40	BR161			Ba32
BB268	CF41	BR169			Ba33
BB280	CF42	BR190			Ba34
BB289	CF43	BR195			Ba35
BB331	CF44	BR334			Ba39
BB342	CF45	BR393			Ba41
BB343	CF46	BR394			Ba44
TB00299	CF47	BR395			Ba45
TB00571	CF48	BR406			Ba46
TB00823	CF49	BR407			Ba47
TB00824	TB00342	BR483			TB00216
		BR515			MC02
		BR516			MC26
		TB00349			MC27
		TB00350			B00639
					TB00520
					TB00603
					TB00604
					TB00799
					TB00800
					TB00815

APPENDIX C: DETAILED INFORMATION ON COLLECTED ACCESSIONS

Accession Number	Pedigree/Cultivar Name	Source
BB006	Shia Fong No.1 (F_1)	Nong Sheng Seeds / Japan
BB113	Golden Cross 40 (F_1)	Takii Seeds / Japan
TB00571		Indonesia
TB00823		Taiwan
TB00824		Taiwan
CF10	Snow Jade 65 (F_1)	Ray Long Seeds / Taiwan
CF16	Kabayan (F_1)	East-West Seeds / Philippines
CF17	Montblance (F_1)	East-West Seeds / Philippines
CF28	S-65 (F_1)	Ching Nong Seeds / Taiwan
CF34	SF 06236 (F_1)	Sing-Flow Seeds / Taiwan
CF35	HD-02	Huadi Seeds / China
CF36	HD-04	Huadi Seeds / China
CF37	HD-05	Huadi Seeds / China
CF38	HD-06	Huadi Seeds / China
CF39	HD-07	Huadi Seeds / China
CF40	HD-14	Huadi Seeds / China
CF41	ST 50A	Huadi Seeds / China
CF42	ST 50B	Huadi Seeds / China
CF43	ST 50C	Huadi Seeds / China
CF44	ST 70	Huadi Seeds / China
CF45	CJ 80	Choonjin Seeds / China
CF47	SF 1306 (F_1)	Sing-Flow Seeds / Taiwan
CF48	SF 1806 (F_1)	Sing-Flow Seeds / Taiwan
CF49	HT King 45	Shenliang Seeds / China
TB00342		
TB00349	Medium	Japan
TB00350	De Cicco	Japan
TB00761	Large Leaf Kailan	Thailand
TB00707	Cai Be Xanh	Vietnam
TB00709	Cai Be Xanh	Vietnam
MC27	Fukuokasai (F1)	Shou Chih Seeds / China
TB00520	Ka-Na	Thailand
TB00799	Mbeya Green A	Tanzania
TB00800	Mbeya Green B	Tanzania
TB00815	Chinese Kale	Taiwan
TB00603	Chayam	Phillippines

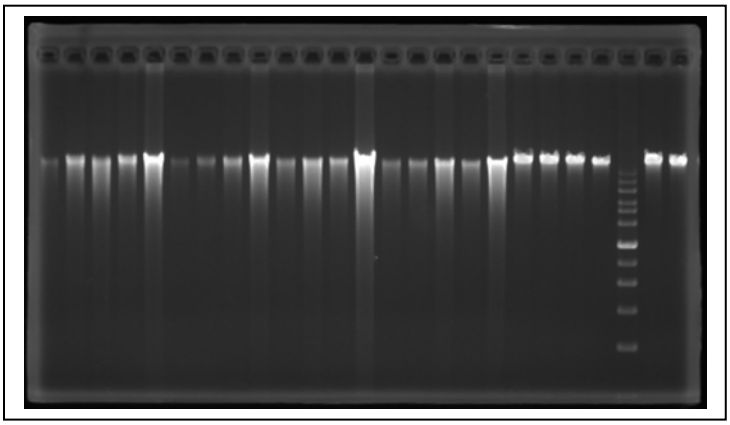
APPENDIX D: DNA SAMPLES

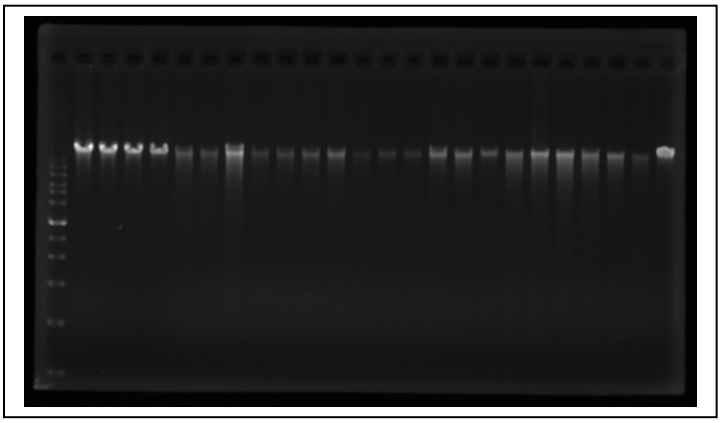
DNA Set 1 Samples	Experiment Label
CF16	1
CF17	2
CF48	3
CF49	4
TB00342	5
TB00349	6
TB00350	7
TB00520	8
TB00571	9
TB00603	10
TB00707	11
TB00709	12
TB00761	13
TB00799	14
TB00800	15
TB00815	16
TB00823	17
TB00824	18

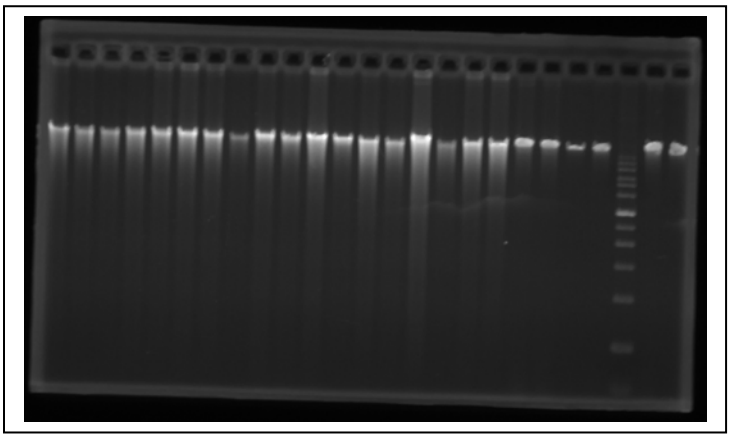
DNA Set 2 Samples	Experiment Label
BB006	*NA
BB113	19
CF10	20
CF28	21
CF34	22
CF35	*NA
CF36	23
CF37	24
CF38	25
CF39	26
CF40	27
CF41	28
CF42	29
CF43	30
CF44	31
CF45	32
CF47	33
MC27	34

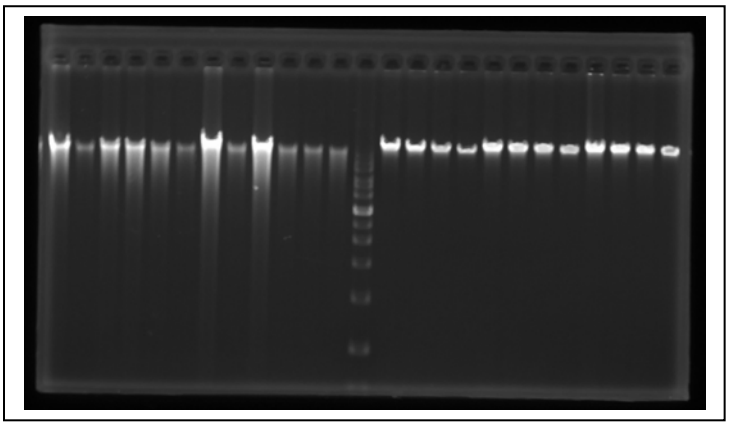
*After the samples were taken out of the freeze dryer, the DNA pellet from CF35 had fallen into the tube containing the DNA pellet of BB006. Thus, both of the samples were eliminated, leaving only 16 DNA samples total from the second set.

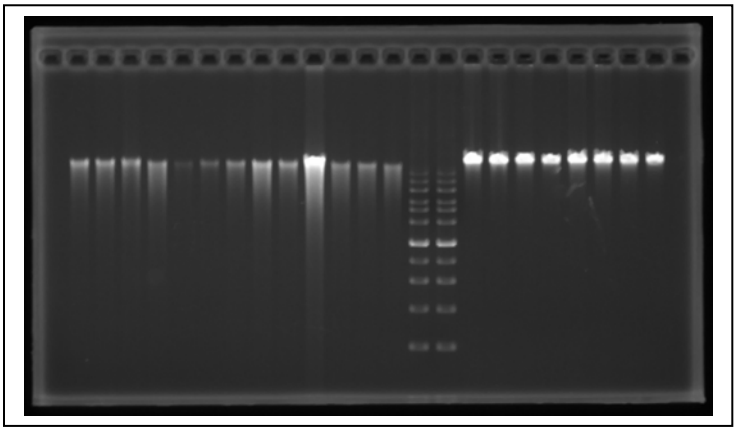
APPENDIX E: GEL PHOTOGRAPHS FROM QUANTIFICATION ANALYSIS OF DNA SET 1

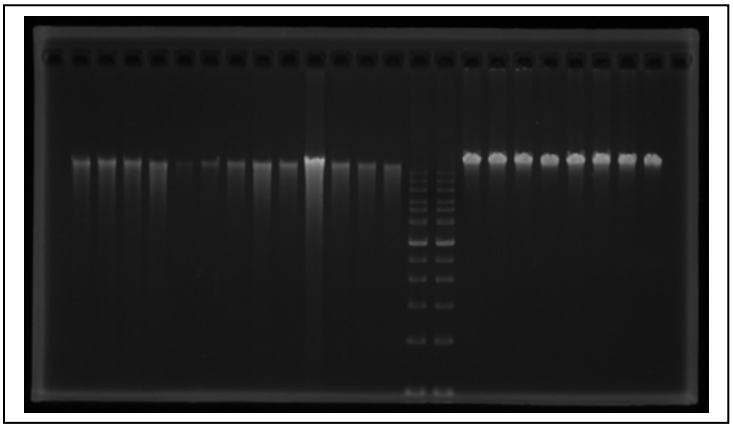




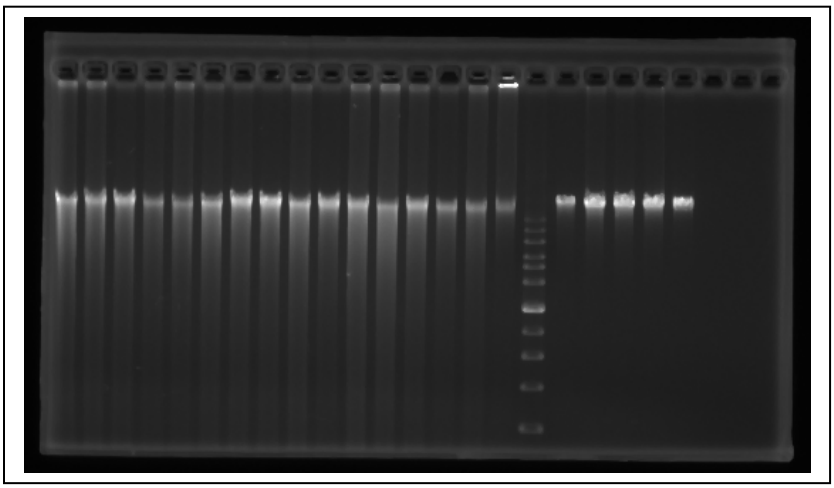


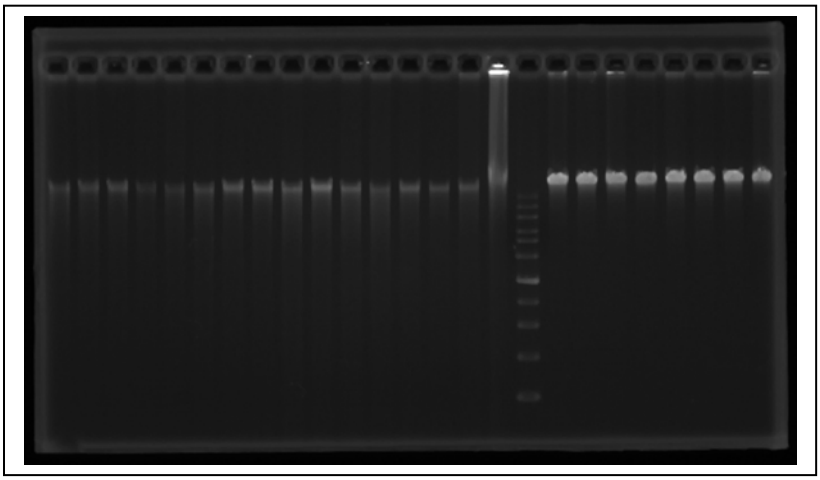


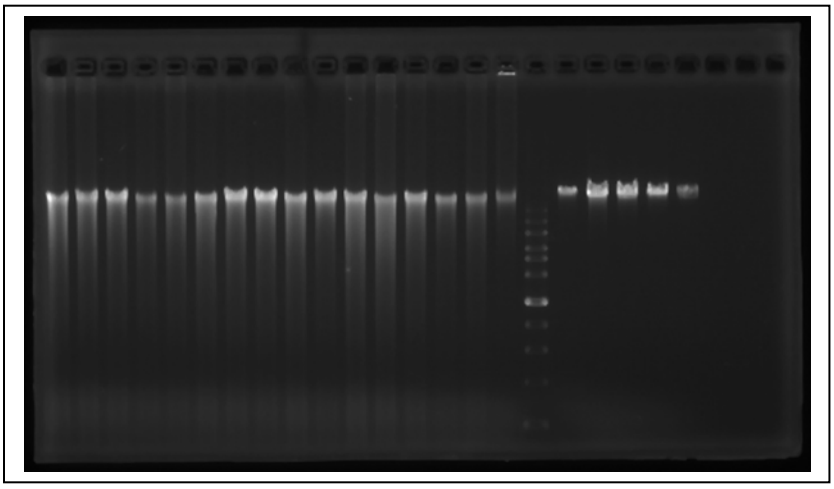




APPENDIX F: GEL PHOTOGRAPHS FROM QUANTIFICATION ANALYSIS OF DNA SET 2







APPENDIX G: ORIGINAL DNA SAMPLE CONCENTRATIONS

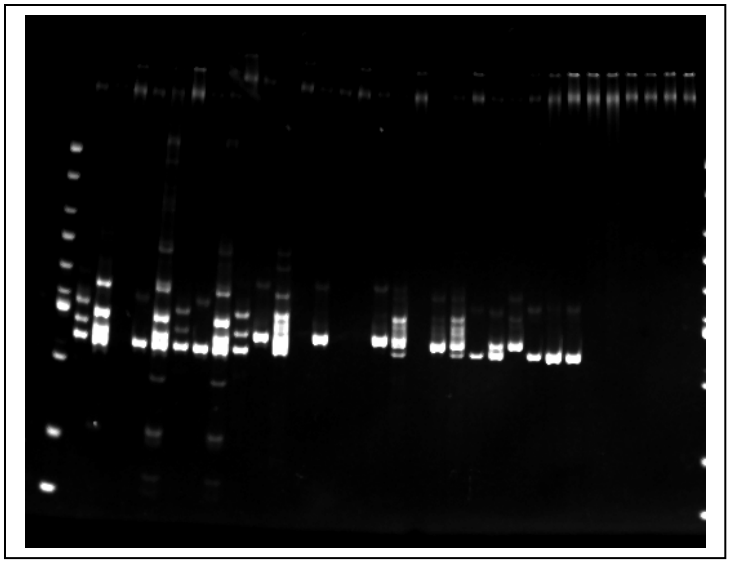
Original Concentration of DNA Samples:

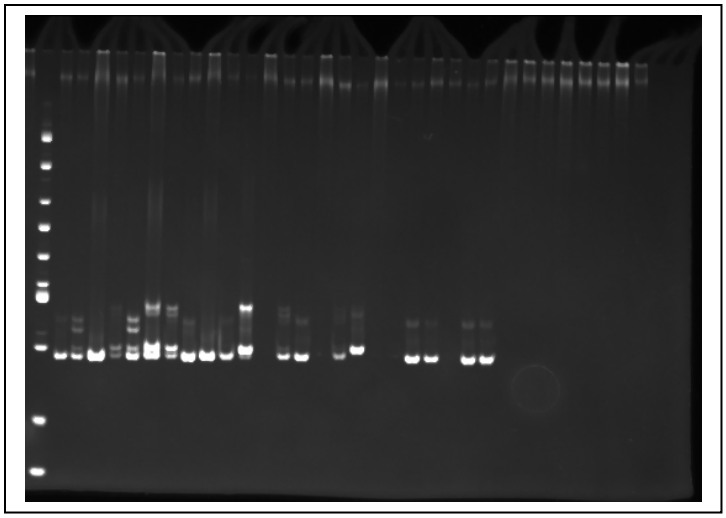
Sample Label	Original Concentration (in ng/μL)
1	176.59
2	51.816
3	Inconclusive--Eliminated
4	136.644
5	173.106
6	297.201
7	191.308
8	142.299
9	202.263
10	170.884
11	262.686
12	189.910
13	328.932
14	156.214
15	Inconclusive--Eliminated
16	145.476
17	265.452
18	360.256
19	46.456
20	37.000
21	46.262
22	25.628
23	24.364
24	31.694
25	71.079
26	87.996
27	40.846
28	43.851
29	44.344
30	25.194
31	35.753
32	24.356
33	25.238
34	33.321

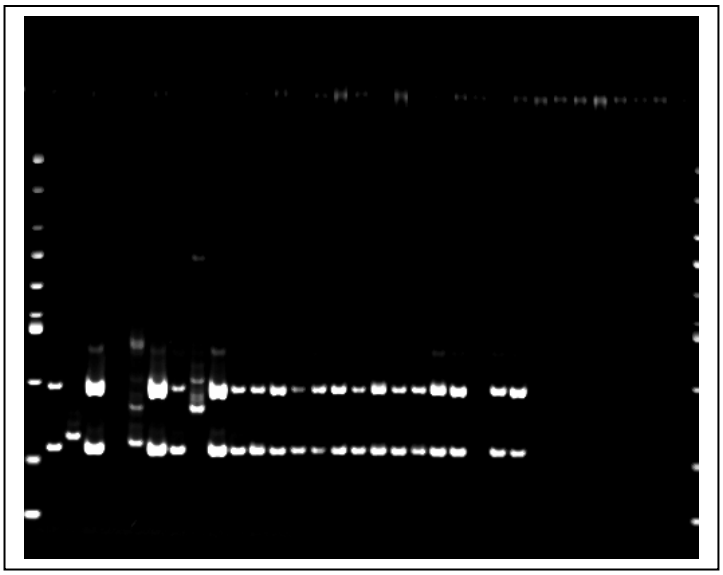
APPENDIX H: PREPARATION OF DNA WORKING SOLUTIONS

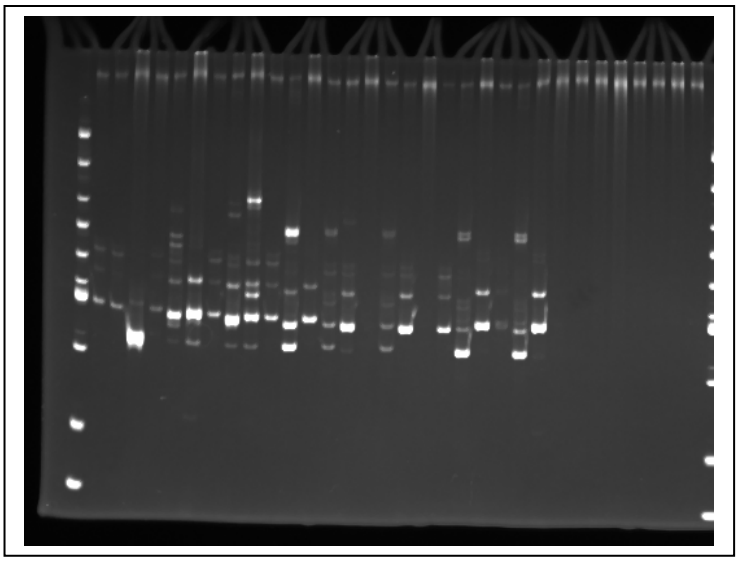
Sample Number	Amount of Original gDNA Needed	Amount of Original gDNA Added	Amount of MQ Water Added
1	14.157 µL	14.16 µL	235.84 µL
2	48.248 µL	48.25 µL	201.75 µL
4	18.296 µL	18.30 µL	231.70 µL
5	14.442 µL	14.44 µL	235.56 µL
6	8.412 µL	8.41 µL	241.59 µL
7	13.068 µL	13.07 µL	236.93 µL
8	17.569 µL	17.57 µL	232.43 µL
9	12.360 µL	12.36 µL	237.64 µL
10	14.630 µL	14.63 µL	235.37 µL
11	9.517 µL	9.52 µL	240.48 µL
12	13.164 µL	13.16 µL	236.84 µL
13	7.600 µL	7.60 µL	242.40 µL
14	16.004 µL	16.00 µL	234 µL
16	17.185 µL	17.19 µL	232.81 µL
17	9.418 µL	9.42 µL	240.58 µL
18	6.940 µL	6.94 µL	243.06 µL
19	53.71 µL	53.71 µL	196.29 µL
20	67.568 µL	67.57 µL	182.43 µL
21	54.040 µL	54.04 µL	195.96 µL
22	97.550 µL	97.55 µL	152.45 µL
23	102.610 µL	102.61 µL	147.39 µL
24	78.879 µL	78.88 µL	171.12 µL
25	35.172 µL	35.17 µL	214.83 µL
26	28.410 µL	28.41 µL	221.59 µL
27	61.206 µL	61.21 µL	188.79 µL
28	57.011 µL	57.01 µL	192.99 µL
29	56.377 µL	56.38 µL	193.62 µL
30	99.230 µL	99.23 µL	150.77 µL
31	69.924 µL	69.92 µL	180.08 µL
32	102.644 µL	102.64 µL	147.36 µL
33	99.057 µL	99.06 µL	150.94 µL
34	75.028 µL	75.03 µL	174.97 µL

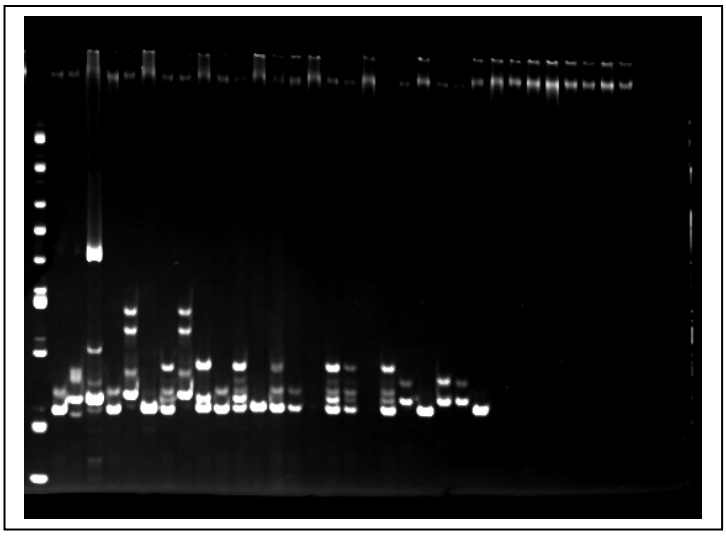
APPENDIX I: PHOTOGRAPHS OF SCORED ACRYLAMIDE GELS

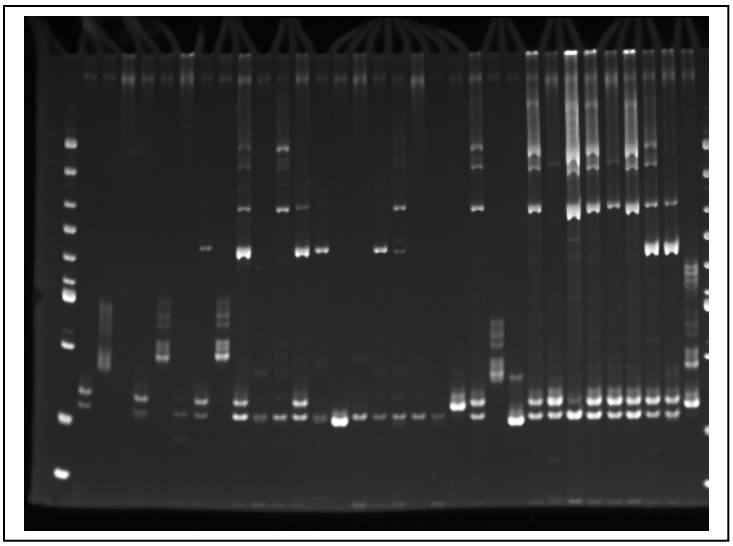


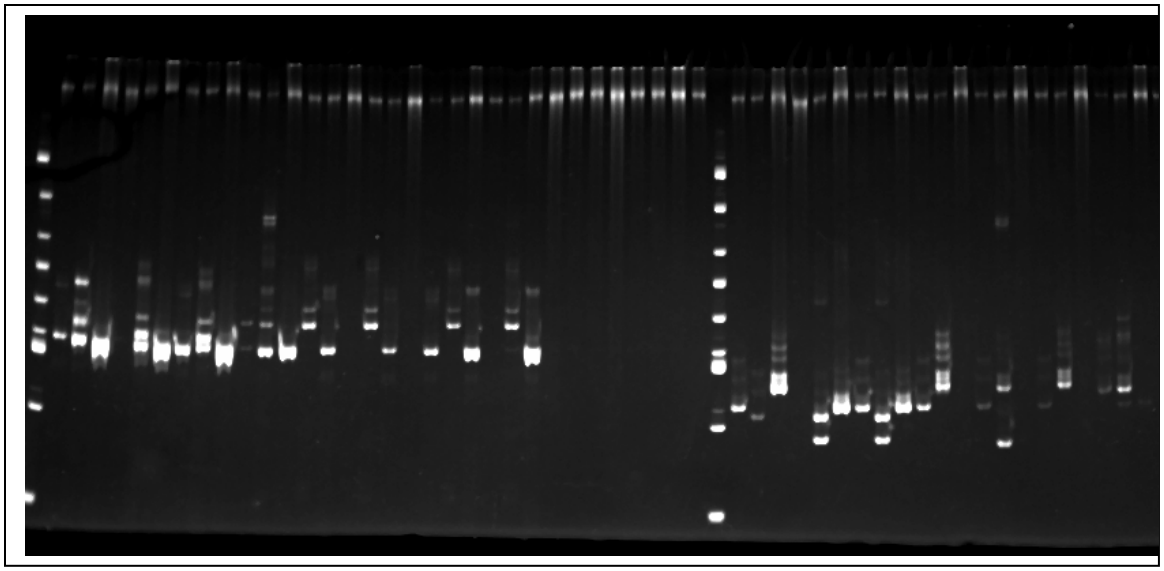


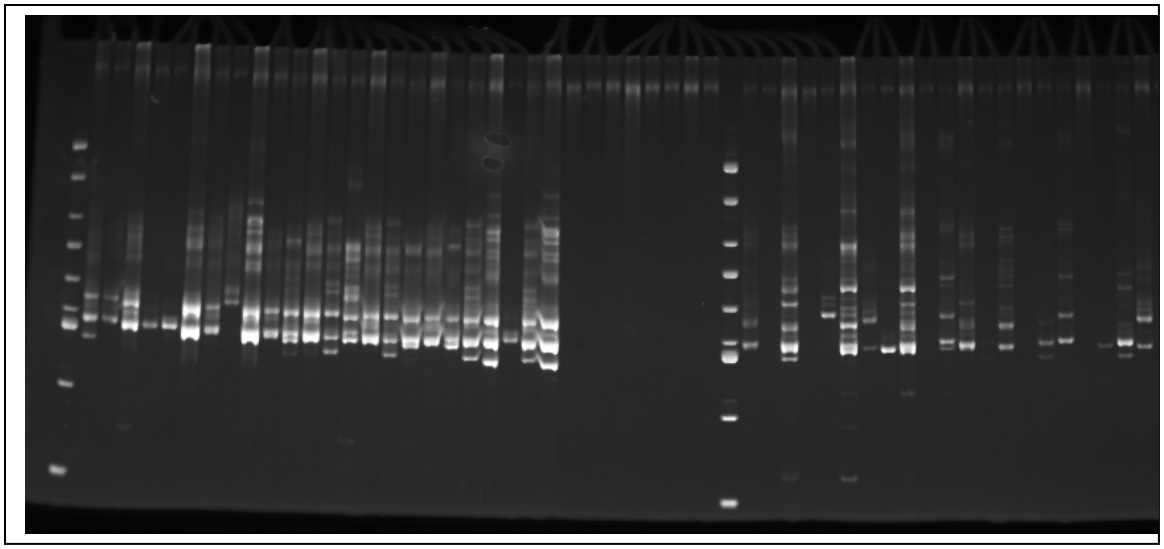












APPENDIX J: SSR PRIMER INFORMATION

Total Markers Screened: 20

Marker Label:	BrassicaDB ref.
1	Na10-E02
6	Na12-C08
8	Na12-F12
10	Na14-C12
17	0110-B01
18	0110-F11
21	0110-H04
25	0111-G11
26	0111-H02
27	0111-H06
29	0112-E03
31	0112-F11
35	0113-E08
40	Ra2-E03
41	Ra2-E04
44	Ra2-E12
45	Ra2-F11
46	Ra2-G09
49	Ra3-E05
50	Ra3-H10

Primers Scored:
8
18
25
26
29
31
35
41
45
46
50

Primers NOT Scored:
1
6
10
17
21
27
40
44
49

These are the markers with fewer than 18 amplified DNA samples.

APPENDIX K: SCORING OF PRIMERS

Primer	Sample	1	10	19	2	11	20	4	12	21	5	13	22	6	14	23	7	16	24	8	17	25	9	18	26	27	28	29	30	31	32	33	34
	Gell Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
8	BSSR801	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR802	1	0	0	0	0	1	0	0	1	0	9	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR803	0	1	0	0	1	0	0	1	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR804	1	0	0	0	0	9	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
	BSSR805	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR806	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	0	0	0	0	0
	BSSR807	0	1	0	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR808	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR809	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	BSSR1801	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1802	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1803	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1804	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1805	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1806	0	0	0	1	0	0	1	0	0	1	9	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR1807	1	1	1	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
25	BSSR2501	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2502	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2503	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2504	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2505	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	BSSR2506	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR2507	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	BSSR2508	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	BSSR2509	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR2510	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0
26	BSSR2601	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2602	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2603	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR2604	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2605	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2606	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2607	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2608	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2609	0	0	0	0	1	0	0	1	1	9	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
	BSSR2610	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2611	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2612	1	1	9	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2613	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0
	BSSR2614	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	BSSR2615	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2616	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
29	BSSR2901	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2902	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2903	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2904	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2905	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2906	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2907	0	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
	BSSR2908	1	0	0	1	0	0	1	0	0	1	9	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2909	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR2910	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
	BSSR2911	1	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0
31	BSSR3101	0	0	0	0	0	0	0	0	0	0	9	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR3102	0	1	0	0	1	0	0	1	0	9	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR3103	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR3104	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
35	BSSR3501	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR3502	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR3503	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR3504	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	BSSR3505	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR3506	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR3507	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	BSSR4101	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4102	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4103	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4104	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4105	1	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4106	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
	BSSR4107	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4108	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4109	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4110	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4111	1	0	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0
45	BSSR4501	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
	BSSR4502	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR4503	0	0	0	0	0	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4504	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4505	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0
	BSSR4506	1	1	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
	BSSR4507	0	0	0	0	0	1	0	0	1	0	1	1	1	1	0	1	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0
	BSSR4508	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR4509	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0

46	BSSR4601	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4602	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	BSSR2603	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	BSSR4604	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4605	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4606	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4607	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0
	BSSR4608	0	0	1	0	0	1	0	0	9	0	0	9	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	BSSR4609	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	BSSR4610	0	0	1	0	0	1	9	1	1	0	9	1	0	1	0	0	0	0	9	0	1	0	0	1	1	1	0	0	0	0	0	0
	BSSR4611	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4612	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4613	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR4614	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	BSSR5001	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	0
	BSSR5002	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	0
	BSSR5003	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	1	1	1	1	1	1	0
	BSSR5004	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BSSR5005	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
	BSSR5006	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	BSSR5007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	BSSR5008	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0
	BSSR5009	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	BSSR5010	0	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	0
	BSSR5011	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

APPENDIX L: SAMPLE NTSYS MATHETMATICAL CALCULATIONS

Matrix: C:\Documents and Settings\boss\Desktop\hemalisim1.xls
Type: Symmetric (dissimilarity)
With 32 rows and 32 columns

	1	10	19	2	11
1	0.00000000000000E+000	1.28093384546206E+000	1.50253180049144E+000	9.34360255182092E-001	2.39995713139030E+000
10	1.28093384546206E+000	0.00000000000000E+000	1.75990156203500E+000	1.15750380649630E+000	6.95209039151876E-001
19	1.50253180049144E+000	1.75990156203500E+000	0.00000000000000E+000	2.51194026042314E+000	2.42699074202544E+000
2	9.34360255182092E-001	1.15750380649630E+000	2.51194026042314E+000	0.00000000000000E+000	2.05338354111033E+000
11	2.39995713139030E+000	6.95209039151876E-001	2.42699074202544E+000	2.05338354111033E+000	0.00000000000000E+000
20	1.19259431076463E+000	1.91432069824455E+000	7.37381504553749E-001	1.56774710796457E+000	2.52251836040679E+000
4	6.93147180559945E-001	1.09861228866811E+000	2.16864537041624E+000	4.64356625936356E-001	2.39995713139030E+000
12	3.07423414795882E+000	9.94792606278987E-001	2.40812057803402E+000	2.72766055767885E+000	3.32527722846465E-001
21	9.80829253011726E-001	1.56861591791385E+000	8.75468737353900E-001	1.44518587894808E+000	2.28217409573392E+000
5	5.67489966419492E-001	1.34984097571585E+000	1.65608187964588E+000	6.26381484247684E-001	2.92896657724173E+000
13	1.44295870377339E+000	1.13679877806040E+000	2.01267584536757E+000	1.54044504094715E+000	1.50339105487029E+000
22	1.07820129140798E+000	1.99449202328214E+000	1.25783915422738E+000	9.54771252442219E-001	2.89037175789616E+000
6	1.10871364232687E+000	1.95601150271407E+000	2.38851022145738E+000	1.32175583998232E+000	2.56420916487632E+000
14	9.71495219475989E-001	1.53111100741141E+000	9.98528830111127E-001	1.18453741713144E+000	2.02152563391728E+000
23	1.03972077083992E+000	2.13833305950803E+000	1.47221948958322E+000	1.79175946922805E+000	0.00000000000000E+000
7	1.14719416289493E+000	1.70680995083036E+000	2.39995713139030E+000	1.36023636055038E+000	2.60268968544438E+000
16	7.70222520473575E-001	2.27429991724985E+000	2.30133352788499E+000	1.52226121886171E+000	2.47703247130393E+000
24	1.03972077083992E+000	2.13833305950803E+000	1.47221948958322E+000	1.79175946922805E+000	0.00000000000000E+000
8	1.09861228866811E+000	1.28093384546206E+000	1.79175946922805E+000	1.44518587894808E+000	1.70680995083036E+000
17	8.88005556129958E-001	1.58115273668990E+000	1.15129254649702E+000	0.00000000000000E+000	3.17017965186388E+000
25	9.97141866669765E-001	2.24990483516513E+000	1.36064771392612E+000	1.49786613677700E+000	2.45263738921921E+000
9	1.64791843300216E+000	1.24245332489400E+000	2.27693844580027E+000	1.99449202328214E+000	1.85065098705625E+000
18	1.12832399890351E+000	1.46477623552472E+000	1.00221586033748E+000	0.00000000000000E+000	2.58381952145296E+000
26	8.88005556129958E-001	2.27429991724985E+000	1.38504279601083E+000	1.52226121886171E+000	2.47703247130393E+000
27	1.15129254649702E+000	0.00000000000000E+000	2.27693844580027E+000	1.21018406432521E+000	0.00000000000000E+000
28	1.54552122667916E+000	0.00000000000000E+000	2.67116712598241E+000	1.60441274450735E+000	0.00000000000000E+000
29	1.64791843300216E+000	0.00000000000000E+000	0.00000000000000E+000	1.30134484272219E+000	0.00000000000000E+000
30	1.99449202328214E+000	0.00000000000000E+000	0.00000000000000E+000	9.54771252442219E-001	0.00000000000000E+000
31	2.34106561356211E+000	0.00000000000000E+000	0.00000000000000E+000	1.30134484272219E+000	0.00000000000000E+000
32	2.34106561356211E+000	0.00000000000000E+000	0.00000000000000E+000	1.30134484272219E+000	0.00000000000000E+000
33	2.13833305950803E+000	0.00000000000000E+000	0.00000000000000E+000	1.09861228866811E+000	0.00000000000000E+000
34	1.79175946922805E+000	0.00000000000000E+000	0.00000000000000E+000	0.00000000000000E+000	1.99449202328214E+000
	20	4	12	21	5
20	0.00000000000000E+000	1.19894763639919E+000	1.81050101585543E+000	4.29301484865515E-001	2.27783786033810E+000
4	1.19894763639919E+000	0.00000000000000E+000	2.36147661082224E+000	1.08273793951082E+000	5.67489966419492E-001
12	1.81050101585543E+000	2.36147661082224E+000	0.00000000000000E+000	1.75247830797650E+000	0.00000000000000E+000
21	4.29301484865515E-001	1.08273793951082E+000	1.75247830797650E+000	0.00000000000000E+000	1.09061211799489E+000
5	1.27783786033810E+000	5.67489966419492E-001	0.00000000000000E+000	1.09061211799489E+000	0.00000000000000E+000
13	1.10892193226948E+000	8.55172038871273E-001	1.30921902120626E+000	8.81794296130679E-001	1.09861228866811E+000
22	8.42064402517845E-001	8.52374046119213E-001	2.17835441334480E+000	5.77426003495493E-001	9.09038388772714E-001
6	1.23127487134336E+000	8.57399214045963E-001	2.13987389277673E+000	1.26286432215413E+000	7.11331002645383E-001
14	9.60525055867959E-001	8.10930216216329E-001	1.68793978683893E+000	1.23214368129263E+000	8.45838005335536E-001
23	1.16228199985641E+000	7.52038698388137E-001	0.00000000000000E+000	1.32740284329170E+000	6.26381484247684E-001
7	1.42390607173868E+000	8.95879734614027E-001	2.17835441334480E+000	1.18356180706581E+000	7.51352356482151E-001
16	1.27385377551352E+000	8.88005556129958E-001	2.45816230731251E+000	9.52544077267529E-001	8.95879734614027E-001
24	1.16228199985641E+000	7.52038698388137E-001	0.00000000000000E+000	1.32740284329170E+000	6.26381484247684E-001
8	1.60441274450735E+000	9.44461608840851E-001	1.95601150271407E+000	1.37042001196260E+000	6.56693795155901E-001
17	1.25162789422810E+000	2.27429991724985E+000	3.15130948787245E+000	1.29334467204897E+000	2.14864270310940E+000
25	8.68388667405351E-001	1.12564589930325E+000	2.02830211711963E+000	5.66030111367825E-001	1.18231024195675E+000
9	0.00000000000000E+000	0.00000000000000E+000	2.52492800362477E+000	2.62874768601389E+000	2.21540839942166E+000
18	1.23127487134336E+000	2.38108696739888E+000	2.56494935746154E+000	1.26286432215413E+000	1.83035428857362E+000
26	7.63028151747525E-001	1.15129254649702E+000	2.05269719920434E+000	4.58145365937077E-001	1.02563533235657E+000
27	1.67931888362168E+000	1.03972077083992E+000	2.43376722522779E+000	1.43897461894880E+000	2.12424762102468E+000
28	2.07354756380381E+000	1.21018406432521E+000	2.82799590540993E+000	1.09240102866883E+000	2.51847630120681E+000
29	2.46362684257860E+000	1.64791843300216E+000	0.00000000000000E+000	1.15129254649702E+000	2.21540839942166E+000
30	2.11705325229863E+000	1.30134484272219E+000	0.00000000000000E+000	1.18356180706581E+000	2.86883480914168E+000
31	2.46362684257860E+000	1.64791843300216E+000	0.00000000000000E+000	9.28148995182813E-001	1.52226121886171E+000
32	2.46362684257860E+000	1.64791843300216E+000	0.00000000000000E+000	9.28148995182813E-001	2.21540839942166E+000
33	2.26089428852452E+000	1.44518587894808E+000	0.00000000000000E+000	1.03972077083992E+000	2.01267584536757E+000
34	0.00000000000000E+000	0.00000000000000E+000	1.97562185929071E+000	0.00000000000000E+000	0.00000000000000E+000
	13	22	6	14	23
13	0.00000000000000E+000	1.07329042225873E+000	7.26716831978760E-001	8.85484349779491E-001	9.72955074527657E-001
22	1.07329042225873E+000	0.00000000000000E+000	1.24245332489400E+000	8.95879734614027E-001	8.36988216785836E-001
6	7.26716831978760E-001	1.24245332489400E+000	0.00000000000000E+000	1.13574725296201E+000	9.16290731874155E-001
14	8.85484349779491E-001	8.95879734614027E-001	1.13574725296201E+000	0.00000000000000E+000	7.79072309023275E-001
23	9.72955074527657E-001	8.36988216785836E-001	9.16290731874155E-001	7.79072309023275E-001	0.00000000000000E+000
7	5.47078969159365E-001	1.28093384546206E+000	7.93025150883193E-002	1.17422777353007E+000	9.54771252442219E-001
16	3.65733022431041E-001	9.72955074527657E-001	9.34474553959592E-001	6.91895615450886E-001	8.29114038301766E-001
24	9.72955074527657E-001	8.36988216785836E-001	9.16290731874155E-001	7.79072309023275E-001	0.00000000000000E+000
8	5.97802277327710E-001	1.25783915422738E+000	4.89674433920646E-001	6.93147180559945E-001	1.03972077083992E+000
17	1.34656227544277E+000	1.66610225508760E+000	8.29114038301766E-001	1.38504279601083E+000	1.52226121886171E+000
25	1.05888020299099E+000	4.37734368676950E-001	1.31554457998304E+000	1.02417547730490E+000	1.09240102866883E+000
9	2.56198198970163E+000	0.00000000000000E+000	0.00000000000000E+000	2.36809922419725E+000	0.00000000000000E+000
18	1.07831559018548E+000	1.75327894865999E+000	8.40590908646471E-001	1.15535760953865E+000	1.62904826901074E+000
26	1.08452685018476E+000	4.62129450761666E-001	1.18578898224050E+000	1.04857055938962E+000	1.11679611075355E+000
27	2.35924943564755E+000	9.48559992442941E-001	2.41415686865115E+000	1.58379126524032E+000	1.49786613677700E+000
28	1.71878251291652E+000	1.05510660017329E+000	2.11523836827334E+000	1.57255483731430E+000	1.89209481695913E+000
29	1.46336970103352E+000	1.03972077083992E+000	2.50531764704813E+000	2.36809922419725E+000	1.58902691517397E+000
30	1.52226121886171E+000	6.93147180559945E-001	2.15874405676816E+000	2.02152563391728E+000	1.24245332489400E+000
31	1.17568762858174E+000	1.03972077083992E+000	2.50531764704813E+000	2.36809922419725E+000	1.58902691517397E+000
32	1.46336970103352E+000	7.52038698388137E-001	2.50531764704813E+000	2.36809922419725E+000	1.58902691517397E+000
33	1.66610225508760E+000	5.49306144334055E-001	2.30258509299405E+000	2.16536667014317E+000	1.38629436111989E+000
34	0.00000000000000E+000	0.00000000000000E+000	0.00000000000000E+000	0.00000000000000E+000	0.00000000000000E+000