# Exploring the Breeding Process


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## Personal Reflection

The two months I spent interning at the World Vegetable Center in Shanhua, Taiwan, profoundly impacted me both academically and personally. Arriving in early June and departing in August, I left as a changed person, carrying invaluable experiences that I might not have gained otherwise.

Initially, I did not experience culture shock; however, I did deal with severe jet lag. The stress of traveling solo was challenging, but I was quickly put in laboratory work by the third day. It was not until halfway through the internship that I fully realized the significance of being far from home. My busy schedule had kept me focused on my work, and it took a bit longer for me to acclimate to my new environment and accept Taiwan as my reality for the coming weeks.

The Center, though English-speaking, had staff with varying fluency in other languages like Chinese and Hindi, which occasionally created communication barriers. Nevertheless, mutual effort made overcoming these challenges manageable.

Socially, I became increasingly aware of the differences between Taiwan and my hometown of Houston. Initially, I was overly cautious, feeling that danger I felt in the States would also be a similar experience in Taiwan. Over time, I learned that not all places are as dangerous as I had been led to always perceive them to be and that being aware is good but not everyone poses a threat. I also noticed a significant shift in the nature of my relationships. In the fast-paced environment of my school in the United States, forming meaningful connections outside of work or school was difficult. However, at the Center, I was able to build genuine relationships and establish a balanced lifestyle that combined my professional and personal life—a feat I struggled to achieve back in the States.

The work environment in Taiwan was remarkably peaceful compared to my usual fast-paced life. There was a distinct lack of rush and stress, with a greater focus on personal well-being. The people, both known and strangers, exhibited unmatched kindness, which was a refreshingly new experience.

My typical workday began around 7 AM with breakfast and a start at the office by 8 AM. My tasks varied depending on the department, ranging from work in greenhouses and fields to laboratory tasks. After finishing work around 5 PM, I would unwind by playing volleyball or exploring the city by bike. On the weekends, I would take the train with other interns to explore neighboring cities like Kaohsiung and Taipei. My more favorite local experiences included visiting morning and night markets and enjoying evening tea walks.

Throughout the internship, I gained hands-on experience with techniques like PCR and RNA extraction, which were new to me beyond theoretical knowledge. This practical experience highlighted the interconnectedness of scientific disciplines, a perspective I plan to apply in my future career.

Overall, this internship has been a transformative experience, offering a new environment and purpose. It has reshaped my perspective and inspired me to pursue a career that integrates plant sciences with medicine. The personal and professional growth I've achieved will guide my future endeavors.

**Abstract**

Prebreeding is a crucial component of modern agricultural breeding, bridging traditional and contemporary methods to introduce novel traits from germplasm banks into cultivar development. The World Vegetable Center aims to alleviate poverty and malnutrition by promoting vegetable cultivation and consumption, focusing on the Solanaceae family (potatoes, eggplants, capsicums, tomatoes) and integrating breeding with education to address market and environmental challenges. An eight-week internship at the Center provided a holistic understanding of the breeding process through exposure to all eight departments. The internship included activities such as PCR and RNA extraction, exploring Seed Health and Quarantine, conventional and molecular breeding, disease management, and pest control. Key areas covered included seed purity, disease resistance, germplasm management, integrated pest management, and nutritional education. This comprehensive experience offered valuable insights into prebreeding processes and their practical applications in enhancing agricultural practices.

## Introduction

Prebreeding plays a pivotal role in modern agricultural breeding by introducing novel traits from germplasm banks into contemporary breeding practices, effectively bridging traditional methods with modern approaches. This process is essential for developing new cultivars that meet evolving market demands and environmental conditions. The World Vegetable Center, dedicated to addressing poverty and malnutrition, focuses on promoting the cultivation and consumption of vegetables, especially in low- and middle-income countries. Their work primarily targets the Solanaceae family, which includes potatoes, eggplants, capsicums, and tomatoes. By integrating breeding with cultivation practices and educational initiatives, the Center aims to enhance both the quality and productivity of these essential crops. An internship at the World Vegetable Center offers a unique opportunity to gain in-depth knowledge of the breeding process, encompassing a range of activities from seed health and quarantine to molecular breeding and pest management. This comprehensive exposure provides a valuable understanding of how prebreeding and various departmental operations contribute to advancing agricultural practices and improving food security.

## Methodology and Departments

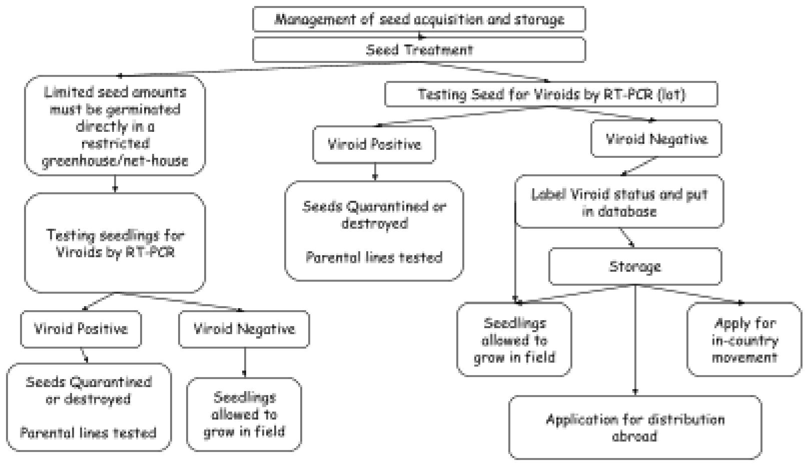
### Seed Health and Quarantine

Seed Health and Quarantine (SHQ) handles seed testing and screening, focusing on two main areas: health and quarantine. In seed health, scientists ensure seeds are disease-free and prevent pathogen spread through seed treatment and quality testing. The quarantine aspect involves regulating and monitoring seed movement to maintain high standards. At the WorldVeg Center, SHQ primarily focuses on detecting viroids in Solanaceous crops.

Although SHQ focuses on viroid detection, they are also responsible for working with different departments on other seed borne pathogens that include: fungi, bacteria, nematodes, viruses, and concomitant contamination. They work to mitigate these phytosanitary problems in relation to the control of plant disease spread.

In order to enhance the germination rates of seeds, the contaminants are removed, thereby reducing the competition between viable and non-viable seeds which helps in disease spread prevention. In the center, 10% of the seedlings must be tested for viroids as per SHQ export quarantine procedures. Surface contamination with viroids can be combated through seed treatment ; this, however, can only be done on small lots of tomato and pepper seeds. Seeds are washed in cycles of HCl, water, and TSP. It is optional to dry the tomato seeds at 80C for 24 hours, but doing the same procedure on pepper seeds reduces its viability. Seed cleaning is done in order to produce non-contaminated seeds for use and is done in the following order: Seed Disinfection (physical contaminants are removed), Seed Testing (Virus and Viroid testing)

Seedline Testing (Seedlings are tested for any disease causing pathogens), Healthy Seedlings (Seedlings grow to produce seeds that are confidently free of any pathogens), and finally the gathering of clean seeds from a pathogen free crop.




***Viroid Detection Lab (Seeds)***

1. Background information

Viroids are single-stranded RNA molecules without protein coats, potentially impacting crop health. At SHQ, top viroids screened include PSTVd, TCDVd, PCFVd, TASVd, TPMVd, and CLVd. The experiment aims to master RNA extraction, gel electrophoresis, PCR, and data analysis using a Nanodrop analyzer. The methodology involves homogenizing seeds with plastic beads (instead of a steel homogenizer), followed by RNA extraction, which includes lysis, phase separation, and careful handling to avoid degradation.

The purpose of this experiment is to learn the methodology behind RNA extraction, be able to perform Gel Electrophoresis and PCR, and to understand the statistical significance of the data from the Nanodrop analyzer. No viroids were tested during this experiment and it was done in order to be able to successfully complete RNA extraction in a laboratory setting.

2. Materials for precipitation for RNA Extraction

Prepare the materials needed for RNA extraction. The buffers have been premade.

Grinding Buffer 1 (premade)

| Reagent | Final Concentration | Amount required for 1L |
|---|---|---|
| 1.0M Tris-HCl pH8.0 | 0.2 M | 200 ml |
| NaCl | 1.0 M | 58.4 g |

Grinding Buffer 2 (premade)

| Reagent | Final Concentration | Amount required for 1L |
|---|---|---|
| 0.5 M EDTA pH 8.0 | 0.1 M | 200ml |
| Sodium lauryl sulfate (SDS) | 2.5g/100 ml | 25g |
| PVP-40 | 6.6 g/100 ml | 66g |

| | |
|---|---|
| Extraction Buffer (premade)<br>Mix equal parts (1:1) buffer 1 & 2 and mix evenly | 5M potassium acetate (KoAc) |
| Isopropyl alcohol (IPA) | 70% ethanol (EtOH) |
| 2 mercaptoethanol | RNase-free water |

3. Homogenizing Seed

There are 3 crops with 4 repetitions each to minimize bias and error in the data, totaling at 12 1g samples. Each sample was weighed at approximately 1g each and placed into a steel homogenizer. The seeds were homogenized after being briefly placed in liquid nitrogen. The seeds had been dehydrated beforehand to remove as much water from the samples before grinding in order to achieve a powder condition. Previous protocol required that each sample for RNA extraction was done by the amount of seeds; however, due to the nature of this experiment and closeness in weight, each sample was weighed out at approximately 1g.

| Crops | Seeds |
|---|---|
| Tomato (#1-4) | 1g x 4 (400) |
| Pepper (#5-8) | 1g x 4 (200) |
| Eggplant (#9-12) | 1g x 4 (1g) |

The seeds were ground to a powder using a homogenizer for approximately 2 minutes. Subsequently, 5 mL of Grinding Buffer 1 was introduced into the steel homogenizer and mixed thoroughly. Following this, 4 mL of Grinding Buffer 2, characterized by its higher viscosity, was added and mixed. After ensuring complete homogenization, the mixture was transferred to a plastic bottle. To this, 50 mL of Extraction Buffer was added and mixed gently to avoid bubble formation. The KoAC in the extraction buffer removes protein, stabilizes the RNA, and removes heavy metals.

4. RNA Extraction

The key takeaway to RNA extraction is first the lysis of the cell. The cell must be properly lysed at the molecular level to ensure that the genetic material from the nucleus is obtained. Next, phase separation of plant material from genetic material. Finally, there is extraction with an elution buffer, being careful not to degrade the material of which is to be extracted.

Take note that the order of the laboratory is to go from clean to dirty in order to reduce contamination and to both double glove and wear a mask for safety.

Take 1.5 mL of the sample from the plastic bottle and transfer it into a 2 mL tube, then place the tube on ice. Incubate the sample at 65°C for 10 minutes using either a water bath or heating block. After incubation, add 500 µL of 5 M potassium acetate to the tube and mix thoroughly. Place the tube on ice for 30 minutes. In preparation, set aside 540 µL of isopropyl alcohol in a

1.5 mL tube. Centrifuge the sample at 100,000 rpm for 10 minutes at 0°C. Carefully remove the supernatant and rinse the pellet with 700 µL of 70% ethanol. Centrifuge again at 10,000 rpm for 5 minutes at 0°C, then remove all supernatants. Perform an additional centrifugation at 10,000 rpm for 1 minute at 0°C, repeating if necessary to ensure the complete removal of the supernatant before air drying. Remove any residual supernatant with a pipette and allow the pellet to air-dry briefly for 15 to 30 minutes. Finally, re-dissolve the pellet in 50 µL of RNase-free water or elution buffer and mix thoroughly.

5. RT-PCR

In this experiment, Real-Time Qualitative PCR was used in order to amplify the amount of RNA present in the sample. Since DNA is not the one directly being copied, RNA serves as a template for cDNA in the process known as reverse transcriptase and then PCR occurs to create copies of that cDNA. For PCR, $2^{n-1}$ is used for the amount of cycles completed.

In the elution buffer, add in Diaster 2x one step RT-PCR smart mix (SolGent Company) for reverse transcriptase that include the PCR and dye, 5x Band Doctor, Rnase-free water, and forward and reverse primers. In this experiment, we used the Nad mRNA MT-F2 and Nad mRNA MT-R1 primers as they are always expressed in successful RNA extraction.

| | |
|---|---|
| RNA | 1 ul |
| H2O | 4.2 ul |
| Primer F | 0.4 ul |
| Primer R | 0.4 ul |
| Diaster 2X one step RT-PCR smart mix | 6 ul |

| | |
|---|---|
| Total | 12 ul |

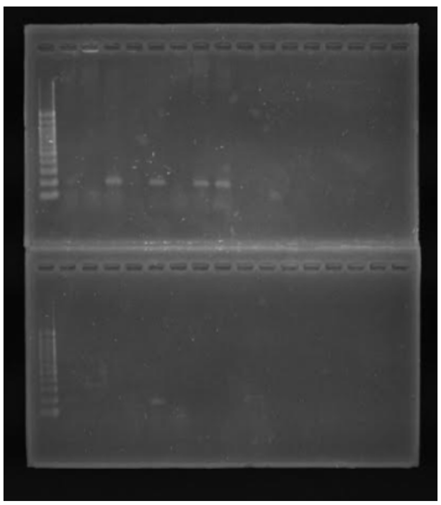
There are 3 stages to PCR: Stage 1 is the cDNA stage consisting of 50C for 10 minutes and 94C for 2 minutes; Stage 2 consists of denaturing and annealing for 35 cycles of 94C for 30 seconds, 58C for 30 seconds, and 72C for one minutes; Stage 3 is responsible for the extension of the strands at 72C for 5 minutes. The whole process took a total of 2 hours.

6. Gel Electrophoresis

To prepare for gel electrophoresis, first gather the necessary materials: agarose, TBE buffer, and DNA view dye (ethidium bromide from BioTools Corporation), which binds to DNA strands and fluoresces under UV light. Begin by preparing a 2% agarose gel by mixing 2 grams of agarose with 100 mL of TBE buffer. Gently stir the mixture for 30 to 40 minutes to ensure the dye is well incorporated. Once the gel is prepared, perform gel electrophoresis by running the gel for 30 minutes in a 0.5x TBE buffer. This process will separate the DNA fragments, allowing for visualization under UV light.

During gel electrophoresis, be aware that the orientation of the charges at the nucleus is positively charged meaning that the gel must be run from positive to negative. The higher the voltage, the faster the samples move through the gel, being careful to make sure the get is not left too long that it “jumps into the sea”. The higher the percentage of agarose, the thicker the get and the more visible the smaller fragments. Depending on the amount of base pairs that are to be viewed, the thickness of the gel must be adjusted.

On the left most part of the gel is the ruler, used to have in comparison the relative size of the base pairs. The ruler is sized at 100 base pairs meaning that the expected size of the pairs is between the first 2 bottom lines at around 185 base pairs. The sample testing was overall unsuccessful due to human error. The gel shows that we were not able to successfully extract the RNA samples as it was only expressed in 25% of the experiment. When loading the samples into the gel, there must be a positive and negative control. The positive control contains the RNA, viroids, and miRNA while the negative control just contains RNA free water, which are both used in the comparison of the results. The dye itself was made twice of 50 ml and autoclaved water.

| | |
|---|---|
|  | It is expected that the cells did not lyse correctly and therefore were unable to extract the RNA from the cells. The samples for the eggplants were not homogenized correctly. The pepper samples 3 & 4 were not properly homogenized and centrifuged.<br>The gel did not load properly on 3,4,7, and 8. |

7. Nanodrop

For RNA qualitative analysis, utilize the RNA Nanodrop to assess the concentration of genetic material. Place 2 µL of the RNA sample on the pedestal of the Nanodrop, which employs UV-Vis light absorbance to measure RNA concentration. The ratio of A260/A280 is used to evaluate the purity of the RNA extraction, with ideal values typically ranging between 1.8 and 2.0, indicating good quality. Additionally, the A260/A230 ratio provides insights into secondary metabolic processes and potential contaminants.

| Sample Name | Nucleic Acid(ng/uL) | A260/A280 | A260/A230 |
|---|---|---|---|
| Sample 1 | 215.145 | 1.679 | 0.675 |
| Sample 2 | 337.217 | 1.143 | 0.271 |
| Sample 3 | 17.395 | 1.994 | 0.519 |
| Sample 4 | 15.914 | 1.869 | 0.39 |
| Sample 5 | 177.138 | 1.716 | 0.655 |
| Sample 6 | 94.184 | 1.898 | 0.798 |
| Sample 7 | 152.43 | 1.589 | 0.46 |
| Sample 8 | 197.886 | 1.603 | 0.553 |
| Sample 9 | 68.489 | 1.541 | 0.427 |
| Sample 10 | 134.631 | 1.533 | 0.486 |
| Sample 11 | 90.807 | 1.761 | 0.65 |
| Sample 12 | 338.268 | 1.465 | 0.422 |
| w 1 | 0.192 | -0.262 | 0.332 |

W1 serves as the control for the nanodrop analysis check that the system is still working. The nucleic acid content was very inconsistent. A260/A280 ratio showed that although a lot of genetic material was extracted, it was not all purely RNA meaning that there is some kind of contamination. The A260/A230 ratio confirms that there is contamination, especially with secondary metabolic processes.

***Viroid Detection Lab (Leaves)***

| Sample Name | Nucleic Acid(ng/uL) | A260/A280 | A260/A230 |
|---|---|---|---|
| Sample 1 | 292.121 | 2.085 | 1.898 |
| Sample 2 | 25.421 | 1.99 | 1.241 |
| w 1 | 0.091 | 0.257 | 0.31 |

For leaf extraction, first, ensure that the leaves are maintained at a pH between 2 and 7 and stored at -20°C, similar to seeds. However, for a more effective preservation of metabolic processes, storing at -80°C is preferable. Begin the extraction by adding 0.7 µL of 2-mercaptoethanol (2ME) to a mixture of Buffer 1 (125 µL), Buffer 2 (125 µL), and Extraction Buffer (125 µL). Add 500 µL of potassium acetate (KoA) to the mixture and vortex thoroughly. Place the mixture on ice for 20 minutes. Afterward, remove the supernatant and rinse the pellet with 70% ethanol. Pour out the ethanol and dry the pellet at 70°C. Note that while this procedure is similar to seed extraction, the key difference is the use of plastic beads for homogenization instead of a steel homogenizer. The A260/A230 once again shows that there is a high possibility of contamination.

## Pepper Breeding Programs

Peppers are highly valued in cuisines around the globe as a contributor of flavor and spice to dishes. A biodiverse crop, the Pepper Breeding Program aims to identify and select the traits of the crop that can be used in an adaptation to a changing environment, resistant to emerging pests, and desirable in new environments. This research involves the selection of agronomic traits that contribute to the plant's productivity and vigor. By selectively breeding and studying their genetic diversity, breeders are able to uncover the resilient characteristics of pepper crops that support food security and sustainable agricultural practices.

### *Common Pepper Diseases*

The more common of the pepper diseases are virus and fungal infections, many of which are insect borne. The following diseases are commonly found in diseased pepper plants, but are not limited to that crop. Such infection can possibly be identified by the state of the leaves and on the fruit. Discoloration of the leaf and brown discoloration on the surface of the plant that does not penetrate the rest of the pepper indicates a viral infection. Brown spots on leaves and indentation on the fruits that go through the skin of the pepper indicates the presence of fungus or bugs. Bruising can occur; oftentimes, molds form because of the openings made by bugs and insects.

1. Cucumber Mosaic Virus (CMV) is most identified by yellow molting on leaves and stunted growth of the plants, passed on mechanically through sap or by aphids. This is most prevalent during the spring and summer.
2. Chilli Veinal Mottle Virus (ChiVM) is potyvirus transmitted by aphids and like species and results in vein banding, chlorosis (insufficient chlorophyll giving a lighter “sickly” plant color), and leaf molting.
3. Bacterial Wilt (BW) enters through a plant through wounds either by the surface of the plant, infected soil, or through insect vectors. The effects of the virus are most visible on the leaves of the plant; the effects on the roots and stem are prominent, but not as visible.
4. Leaf Curl Virus (LCV) affects the orientation of the leaves. The curling of the leaves does not allow the plant to obtain the amount of sunlight needed for photosynthesis and thus resulting in stunted growth. This capsicum curling (PepLCV) is a DNA virus genus begomovirus that is transmitted by an insect vector and plant resistance is based on the genetic level.
5. Anthracnose is a fungal infection most common in warm and humid areas. Although they can infect all parts of the plant, they target the shoots, leaves, and fruit of the plant that causes plant necrosis.
6. Phytophthora cinnamomi (PC3) is a soil borne water mold that causes the rotting of roots and stem necrosis. The damage to the roots does not allow for sufficient water intake.

***Introduction to Pepper Breeding and Scoring***

Breeding deals with both hot chili and sweet peppers. Common peppers include bell, banana, cherry, habanero, hungarian wax, chocolate, and guajillo peppers. The testing, sowing, and collection of peppers depends largely on season. Spring and Summer are referred to as the wet months from February to August; Fall to Winter is from September to January and are considered the dry months. Pepper plants in particular are sown at the beginning of spring and tested between June and August for heat and wet tolerance.

Peppers are ranked on a 0 to 5 scale of goodness. During a testing period, pepper plants can be scored from high to low and vice versa. A plant that receives a lower score on the first round and higher scoring the second round of the same season is not better or worse than another plant that might receive a higher and later a lower rating; the interpretation of the score is dependent on the quality of which the plant is being bred for. Breeding is specialized and done by regions; breeders are often well versed in all plants but specialize is the selection of crops suited for the climate and market of a certain country. Plant selection is largely scored and based on its marketability.

Scoring of peppers includes both in-field crop yield evaluation and in-lab single crop evaluation. In-field evaluation takes a look at overall crop yield, plant characteristics, and plant health in exposed conditions - in particular chili pepper. The field included both open and closed pollination conditions. Open pollination conditions means that the pepper plants were exposed to all the elements and are susceptible to insects and cross pollination by bees. Closed pollination areas are covered by nets that still allow for water and sunlight to come in but prevent the interaction of insect vectors and outside pollination; this particular area housed only the hybrids. In the lab, the bell peppers from both the hybrid and field lines were evaluated. The quality of the fruit. Field line peppers were the ones that were exposed to all the elements, openly pollinated, and were susceptible to insect vectors. These lines contain a variety of colors. Fruit scoring is based on the length and width of the fruit, thickness of the pericarp at its thickest point, and the number of cells it contains which can be determined from the bottom of the fruit. Colors include green, dark green, red, yellow, and orange. However, a color like orange is not considered a true color as the fruit is not completely ripe. It is also considered if the color of the fruit is brown; a brown tinge indicates discoloration due to age but spots of brown tend to indicate fungus, which can be due to insufficient storage and age, but more often outside infection. Silvering of bell peppers is common - similar to the 'stretch marks' of plants, cells adjacent to the cuticle of the fruits separate resulting in dull colored lines. Detected in fruit, this does not indicate diseases, it is just not physically appealing to the market. Seed grade of the fruit is dependent on the color and amount, also being scored on a 0 to 5 scale. 0 to 1 indicates that there are little to no seeds. 2 also indicates that there are not enough seeds but including browning. A score of 4 or 5 shows a high yield and many seeds in high quality. It is important to look both at the fruit and seeds of bell pepper - having a healthy looking fruit does not always accurately depict the overall health of the plant. Low seed grade can indicate a diseased plant but also does not allow for farmers to obtain a large yield of fruit in the future.

The hybrid lines that came from the covered area of the field were mainly yellow or red. Their crops contain a 2-stem system that cannot be found in-field (single plant with 2 main stems only). Unlike the non-hybrid lines, these bell peppers were evaluated only by their outside appearance. A majority of the time was spent in using a chart that provided the family's parent plants and seeing which parent could have possibly been responsible for a certain characteristic. For example, a crop that appears to be resistant to a certain disease may share a parent with another whose offspring is not resistant to it; therefore, the uncommon parent is most likely responsible for the disease resistance trait. The types of characteristics that are focused on are the indentations, shapes of parents and cells. The indentations at the bottom are related to the cell numbers of the plant and are important in the storage of bell peppers. On average, more desirable bell pepper plants will have between 3 to 5 cells in order to fit market standards, stand on its own, and be easily stored. The hybrid's shape is best determined by its parent shapes that are dominantly characterized as a small and rounded apple or larger and more elongated pear-like shape.

***F2 Generation Selection***

Successive generations are labeled from F1 and onward. At the center, it is more common to deal with the F7 generation maximum as the ultimate hybrid families. The F1 generation is considered the pure, starting generation. When crossing the first generation, the parents (P1 and P2) are cross pollinated. F1 seeds are produced: only the plants that are selected for desirable traits during the F2 generation will be crossed and grown in the F3 generation, and so forth. Consecutive generations show more consistent traits that become stabilized after following crosses. Because the F2 generation has the most diversity of the crop after planting, it is used for trait selection for the F3 generation. The various plots are checked mainly for plant health and its pericarp. Breeders look at certain characteristics based on its marketability to the target market. A thin pericarp is desirable for drying spices. Clusters and if the fruit is upturned or pendant is desired by farmers in terms of yield and feasibility of harvest. Color of peppers are also very important as some markets favor one color over another - though uncommon colors such as purple or brown may not indicate disease, but are not often desirable.

***Cross Pollination***

Within enclosed greenhouses, the hybrids are housed for artificial cross pollination. The enclosed environment allows the control of crossing without accidental pollination or entrance of disease vectors. To cross pollinate a plant, the plant must be assigned as male or female at time of cross pollination - each plant is assigned a number. The top number on the plant card is the female while the bottom number is the male. The date of the most recent cross pollination is also on the card. Although the pepper plants are considered to have both male and female parts, for the sake of crossing, the pollen donor is male and the plant recipient is female.

A flower that is close to blooming but has not yet opened is selected as the recipient. The number of petals of the flower indicates the number of anthers; both must be removed in order to expose the pistil. The pollen from the male plant's flower is used and rubbed along the pistil, making sure there is sufficient pollen surrounding it. If a majority of the plant seems to reject the cross pollination indicated by the pollinated flower drying and falling off, the selected plants are then reverse pollinated. In this case, the female plant will now be the male plant and vice versa for the previously male plant. This is due in part to the problem in the metabolism of organelles that are passed down from the maternal parent.

***Disease Inoculation***

Peppers are purposefully inoculated with diseases to identify disease resistant lines.

Anthracnose screening involves various pepper lines from the field that had been previously inoculated by the fruit by injecting the pathogen via syringe and putting it in an incubator for about 3 days. The diameter of necrosis was recorded, checking for the extent of resistance; necrosis diameter of 2mm or less is considered a resistant line. Some of the peppers had secondary infections and were not able to be accurately evaluated due to fruit rot.

Pepper seedlings were inoculated with Phytophthora cinnamomi (PC3)a week prior and checked for stem necrosis and root rot. This is most visible as a stem infection. The plants were considered resistant if they were scored a 0 and susceptible between the scores of 1 and 4 (4 being the most severe and the plant is almost dead). Stem necrosis is checked by the half height to the cotyledons; roots are gauged if they were browning, exhibiting necrosis, or appeared weak. Pepper seedlings in a separate greenhouse were inoculated with Chili Veinal Mottle Virus (CVMV) and Cucumber Mosaic Virus (CMV). Though both types of diseased plants were located within the same greenhouse, they were separated by a dark room that discouraged bugs to follow through in order to prevent cross contamination and insect vectors from entering. The leaves 4 main leaves, considered the “true leaves” and first 2 leaves to bloom were inoculated with a green liquid containing the plant virus. The liquid was spread on the leaves thoroughly with a cotton pad and left to sit in the sun for 10 minutes, by which it is then washed off to prevent a smell from occurring.

***RNA Extraction of Pepper Leaves***

A side experiment was observed regarding heat tolerant pepper plant lines. The experiment had 2 chambers, a control chamber and a heat tolerance chamber. Both chambers had the same humidity level, light intensity and timing, as well as amount for watering the plants. The pepper leaves from close-to-flowering plants were collected for RNA extraction using an RNA extraction kit from Qiagen. The purpose of this experiment is to determine gene response memory, trying to find the specific protein that may be responsible for heat tolerance. The data collected from these trials from the RNA extracted from the pepper leaves and newly bloomed flowers will be used for more specific experiments regarding the molecular mechanisms in relation to genetic structure.

The primary concept of RNA extraction using a Qiagen kit is similar to “old-school” RNA extraction. The main difference is that most of the materials and chemicals are already prepared in the collection tubes, saving time. First, the leaves were broken in a hemolyzer after being frozen and stored in liquid nitrogen. The concept of centrifugation to separate the supernatants, impurities, and finally only extracting RNA remains the same - rapid purification.

The kit contains a purple and a pink centrifuge tube that is used to separate the supernatants, respectively.

**Disease Resistance**

Plant diseases, categorized as fungal, bacterial, or viral, pose significant threats to agriculture and crop productivity. The Disease Resistance Department focuses on plant pathologies and developing effective prevention strategies. This department explores pathogen interactions and improving disease management to support sustainable agricultural practices.

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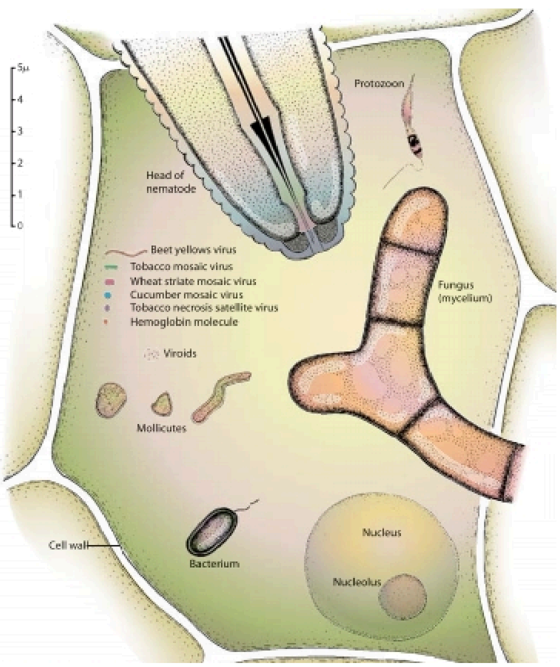

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“Types of Plant Diseases.” Agriculture Information Bank, 5 Apr. 2015, agrinfobank.blogspot.com/2013/04/types-of-plant-diseases.html.<br><br>
This department focuses on the identification, treatment, and prevention of biotic diseases that are showcased by relative sizes in the image on the right. Non-infectious diseases include those that are caused by an insufficient environment.
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Diseases caused by viruses and viroids hack into the metabolism of the plant while diseases caused by fungi, prokaryotes, protozoa, and nematodes tend to create a more physically oriented damage to the plant. Damage done by these organisms can make it easier for secondary infections to occur.

***Virology***

Viruses exhibit remarkable complexity and have significant impacts on plant health. Recent advances in molecular and genomic techniques have deepened our understanding of viral mechanisms and interactions in light of emerging infectious diseases. Viruses function by infecting a host plant and using the host's metabolism and resources to make more viruses, essentially hijacking the host organism.

A gel electrophoresis lab was conducted to demonstrate how to do virus detection and to test a primer for pod pepper vein yellows virus (PeVYV). Because what was being tested was a virus, Reverse-Transcriptase PCR was used. Overall, virus detection using the new primer was unsuccessful. The band widths of which was expected to detect the virus was not seen.

***Mycology***

Mycology unit includes both pathogenic and non-pathogenic fungi. Not all fungi are pathogenic and can even aid in pest and disease management. Whether or not fungus is pathogenic or not is determined by culturing.

Fungus preservation can be effectively managed using various methods to maintain viability over long periods. One common approach involves using silica gel, which is both inexpensive and simple. This method employs silica and colored silica indicator beads to absorb moisture from the fungus and the surrounding environment, ensuring that water levels do not fluctuate in a way that could impact growth. Typically, this method uses standard appliance freezers and requires one tube per culture, with storage at a consistent temperature of -20°C. Long-term preservation is highly dependent on the quality of the seal. Alternatively, mineral oil can be used to maintain a moist environment. This technique involves storing fungus on slant agar covered with mineral oil, which prevents dehydration and provides an aerobic environment conducive to a dormant state. Mineral oil storage can be maintained at room temperature and is suitable for long-term preservation, even for several decades, especially for fungi that cannot tolerate freeze-drying or cryogenic methods.

Jana's experiment on Fusarium wilt, a disease that causes entire plants to turn yellow and wilt, focuses on understanding its spread and preservation techniques. Fusarium wilt is particularly problematic for solanaceous crops and can be introduced through infected transplants or spread via contaminated equipment. Favorable conditions for this disease include warmer weather and acidic soil, with its most common entry points being through cultivation practices or nematode feeding. In her experiment, Jana filled tubes with silica gel and indicator beads. The silica gel helps in moisture control, while the purple beads serve as an important indicator of moisture content. Notably, Jana's work does not require the use of gloves, as the materials involved are not toxic, differing from protocols in other departments. The purpose of this is for temporary storage of the fungus and later, incubation.

As noted earlier, not all fungus is pathogenic and can even be used for pest and disease management, known as entomopathogenic fungi - which will be covered in the Integrated Pest Management unit. The basis of many of the lab procedures in mycology is based around three different skills : fungal suspension, spore counting, and determining spore concentration.

1. Fungal suspension is used for the creation of inoculation liquid that is used to inoculate healthy plants for disease screening. Fungal spores that put on media that are incubated until sufficient growth. Most of the media that is used for fungal incubation is potato dextrose. Once there is sufficient growth of the fungus and spores are being produced, the plates are then “washed” with water: how this works is that around 20 ml of water is put in a plate, and collectively, that water and a glass slide is used to scrape off the fungus into a beaker for collection. The collected fungus is strained through a cheesecloth as to not include the agarose or any other impurities.
2. Spore counting is a very important step after fungal suspension and is vital for accuracy in determining spore concentration before finally inoculating plants. This is done using a hemocytometer which is a thick glass microscope slide with grid prints. The way to count the spores depends

| Fungal Isolate | Spore concentration | Final concentration |
|---|---|---|
| CC213 | $5.175 \times 10^8$ | $7.01 \times 10^7$ |
| Bb141 | $2 \times 10^8$ | |
| Bb143 | $1.275 \times 10^8$ | |
| Bb153 | $1.57 \times 10^7$ | |
| Bb157 | $3.75 \times 10^7$ | |
| Ma69 | $1.1 \times 10^8$ | |
| Mp125 | $7.75 \times 10^7$ | |
| HA023 | $2.87 \times 10^8$ | |
| HA041 | $8.25 \times 10^8$ | |
| HA088 | $2.35 \times 10^8$ | |
| TM212 | $4.58 \times 10^8$ | |

***Agroecology***

Agroecology is the combination of the study of crops, soil, and ecology. In the duration of the internship, there were 4 ongoing in-field experiments.

1. Different Tillage and Mulching Practices
2. Mungbean Biofertilizer experiment
3. Biochar experiment
4. Intercropping

All of these experiments are meant to produce results that analyze the effectiveness of various soil practices that optimize nutrient abso[tion of plants and help to find solutions in the prevention of overuse of both fertilizers and insecticides. The intercropping experiment was the most focused during the week at the agroecology department. This deals with the planting of okra and mungbean simultaneously. The mung bean is a legume that provides the means of nitrogen fixation to the okra, eliminating the need for excessive fertilizer.

As agroecology deals with the interconnectedness of biotechnology, nanotechnology, and ecology, various instruments are used. These measure the health and state of the plants to analyze the state of the plant. The studies are not limited to just the health and state of the plant but also the interactions with other biological factors. The following most used are listed below:

| | |
|---|---|
| SEM | Standard Electron Microscope<br>a. Simple microscope<br>b. Compound microscope |
| ICP | Inductively Coupled Plasmolysis |
| (old) AAS | Automatic Absorption Spectroscopy |

| | |
|---|---|
| FTIR | Fourier Transformation Infrared Analyses |
| PCR | Polymerase Chain Reaction |
| GCMS | Gas Chromatography Mass Spectroscopy |
| LCMS | Liquid Chromatography Mass Spectroscopy |
| HPLC | High Performance Liquid Chromatography |
| LI -Cor | 6800 - measures photosynthetic systems<br>7810 - Methane/CO2 analyses<br>7820 - NO2 analyses<br>3100 - leaf area meter |

**Genetic Resources**

The Gene Bank department at the World Vegetable Center plays a crucial role in conserving the genetic diversity of vegetable crops. This department maintains a comprehensive collection of seeds and plant materials, preserving valuable genetic traits that can be crucial for developing new crop varieties. By safeguarding these genetic resources, the department ensures that future breeding programs have access to a diverse pool of traits that can be used to improve crop resilience, yield, and quality. Their work supports long-term agricultural sustainability and helps protect against the loss of genetic diversity due to environmental changes or other threats.

***Interviews***

World Vegetable Center also serves as a seed distributor globally and there are various departments that must be gone through before seed distribution as well as containment. Seeds both get sent out and brought into the center, all of which have to go through quality control.

1. Management

Every department must follow a protocol that ensures quality assurance and control. Quality assurance deals with how to manage the seeds and germ plants as well as implementation or the conservation of such. It all starts at the first level in headquarters where materials are collected, acquired, and then conserved. From conservation, there can be three separate pathways; characterization of the plants, regeneration of the germplasm so that more seeds can be produced, or distribution or the seeds from storage.

2. Inventory and Distribution

Inventory at the center has changed over the years from paper records to digitize markets. The main goal of the gene bank is to conserve plant material for future use, but has expanded to global markets and various scientific researchers. On the website, a person may request a seed or use the international database called Genesys.

There is a process of managing all the requests and outgoing. First, the request is processed, then background information about the requester is obtained, the local government criteria is viewed, and the material is sent after all approval checks are marked off.

Not all the seeds are used in research. Some serve as seed kits that are given in light of natural disasters or as traditional landraces that are given to individual households. The main goals of this is so that not only are people in various regions educated, but households and farms can sustain themselves in the long run with minimal researcher input in their practices.

The gene bank itself houses a variety of plant materials. Short term storgate is meant for almost immediate destruction, -5C storage is meant for distribution but with a longer wait time (most often the distribution is set within the next few months to years while -18C storage is long term that is meant to last for years.

3. Research Associates

There is a separate genebank in Tanzania that works directly with Headquarters. In addition to being a separate center, they focus on direct cultivation, local education, and reduction of poverty. Because they are in a separate region, they focus their seed studies on more localized plants. There is still coordinated activity between the centers such as germplasm collection and making sure that agriculture is sustainable enough to create an income generation.

4. Curators

Plant curators manage and preserve diverse collections of vegetable germplasm to support agricultural research and global food security. Their work involves documenting species, evaluating genetic diversity and plant traits. Their research plays a crucial role in improving crop yields, enhancing climate resilience, and contributing to sustainable agriculture worldwide. They are usually assigned to certain plant species to focus their attention to. Their work includes characterization, germination testing, and other plant passport research. The following results often coincide with plant education and testing.

5. Landscape Genomics and Crop Adaptation

Landscape genomics are more focused on the diversity analyses of plants and most often direct their attention to only particular regions and resources. The meaning of diversity in this field is in terms of breeding and conservation, often characterizing resources on environmental data. They use future dada to create conservation techniques with the best strategies, with local adaptations having a home advantage for those plants.

***Practical Training***

1. Germplasm collection of garlic

Some plants are non seeding and therefore must collect the germplasm of these plants. 20 cloves of V1039675 were collected and membranes removed. The cloves were sterilized with 2% sodium hypochlorite and shook for 15 minutes. The cloves were then cleaned and sterilized with water for 5 minutes, then 10 minutes, then 15 minutes. The shoot tip was cut with a scalpel at approximately 2mm. After the shoot tip was collected, it was put in a 30ml sucrose and 25 g/L sorbitol medium for storage.

2. Germination testing

Germination testing occurs to see what proportion of seeds are still viable either in regards to storage length or just overall seed health. Sometimes, the seed must first be physically abraded with sandpaper or scalpel in order to make sure that the water can penetrate properly through the seed. Other times, an ultrasonic bath may be used to stimulate the seed for growth.

***Technical Review Paper* - Harnessing Genebanks in Healthcare**

*Introduction*

In 2015, all United Nations Member States adopted the 2030 Agenda for Sustainable Development. At its core, it serves as a recognition to create global partnerships to tackle the growing problem of the 21st century in both developed and developing countries. The Agenda outlines 17 goals that target social, economic, and environmental problems. However, as the world approaches 2030, the progress towards eradicating such obstacles begins to stagnate and sometimes regress. The United Nations submits an annual report detailing the progress of its programs; however, in 2024, the report reveals that only 17% of the sustainable development goals are on track to be achieved, and about 50% are showing subpar progress. Over 30% have halted or declined. This is partially due to the global pandemic but a majority of such regressions can be attributed to the growing geopolitical conflicts, international tensions, and drastically worsening effects of climate change (The 17 Goals).

Within the area of plant biodiversity, the biggest threats are climate change, disease, pollution, and changes in land usage. Two of the seventeen goals of the Agenda are to achieve a “Zero Hunger” society and to promote good health and well-being - both of which can be achieved through food security. Over 800 million people worldwide go hungry, while around 1.3 billion suffer from silent hunger. This number continues to exponentially increase as populations are not able to continuously consume nutritious and sufficient foods (Over 820 Million People Suffering from Hunger). In this fight against hunger and the declining health of the world's population, genebanks provide a way to secure the world's food supply, creating food security that can be used in distribution and research. Countries continue to make progress in the ratification of agreements about access and benefit-sharing instruments; by 2023, up to 93 counties had consistently been reporting their policies under the International Treaty on Plant Genetic Resources for Food and Agriculture (The 17 Goals) making a testament to advancements to creating food security through securing the world's plant biodiversity.

*Genebanks*

A prime example of biodiversity and food security is the Svalbard Global Seed Vault, the largest seed storage in the world, built within the Mountains of Spitsbergen in the Svalbard Islands which is endorsed by over 100 countries and supported by hundreds more of seed donors and organizations. This vault was designed to store and protect the genetic diversity of food crops from global catastrophes as well as a resource for breeding in light of a rapidly changing environment and the needs of humanity (Svalbard). Many more agricultural organizations store their seeds and genetic material for cultivation and research. By understanding the properties of plants, scientists can provide farmers with the right crops to plant in a changing environment while ensuring that the people that they are feeding are consuming highly nutritious food.

Genebanks store seeds and various plant materials to protect and preserve the genetic diversity of crops. With climate change, pests, and diseases, the world's crop diversity is quickly diminishing and food security and humanity's nutrition are becoming threatened. By allowing access to this diverse genetic resource, researchers and breeders can develop new crop varieties with improved characteristics such as yield for a growing population, disease resistance from pests and pathogens, environmental resistance from climate change, and nutritional contents responsible for silent hunger (Guardians). Conservation of genetic diversity is vital to achieving food and nutritional security worldwide.

Most crops produce orthodox seeds that can be easily stored. However, some other crops produce recalcitrant seeds that cannot be easily stored or some crops do not produce seeds at all. For the variety of necessary conditions, there are different types of preservation protocols. For those plants that cannot produce seeds that can withstand vault storage in liquid nitrogen storage or low temperatures, some genebanks will conserve plants in their natural habitats in controlled cultivated fields or in vitro collections where plant material will be preserved via cultures (Panis). All germplasms have a specific phenotypic and passport information that identifies their characteristics to anyone requesting such genetic material. Recently, there has been a push to also include the genotypic characterization of accessions (Signh).

Ongoing research has continuously proved that plant nutrition is vital to human health, and has even become a growing aspect in healthcare. Medicine has been turning more towards natural and personalized healthcare. Most of which has been focused on phytotherapy - using the medicinal properties of plants. The phytonutritional approach in healthcare uses the phytonutritional content of various plants in the diet that can help create a more natural approach to healthy diets and managing various health conditions. The bioactive compounds, phytonutrients, have a strong positive correlation in the contribution of potentially high value of nutrition and health (Monjotin). By protecting genetic diversity, there is an assurance of a diverse nutritional crop pool that can be both bred for optimal plant cultivation and distributed to sufficiently feed needed populations. Diverse germplasm stored in ex-situ, in vivo, and in vitro genebanks are excellent resources for breeding. Many of the beneficial genetic bases have been discovered through routine genebank activities such as seed regeneration and characterization (Nguyen).

*Screening for Pytonutrients*

Phytonutrients provide health benefits beyond basic nutrition through their biological properties. Potential in alternative medicines, they provide many antioxidant, anti-inflammatory, and anti-cancer properties in their therapeutic effects (Gupta). These phytonutrients affect the body through interference of gene expression modulation and interaction with certain cellular signaling pathways that either hinder or promote a cellular response that helps regulate the body back to homeostasis.  The top seven phytonutrients include: phenolic acids, caffeine, tannins, organosulfur, flavonoids, carotenoids, and anthocyanidins - all of which can be naturally found in plants. These phytonutrients found in studied diets demonstrated significant therapeutic potential (Monjotin).

Currently, about 20% of known plants have been used in studies for their pharmaceutical properties in treating cancer and harmful diseases. These plants have high concentrations of phytochemicals that protect against free radical damage- or in other words, the destruction and degradation of healthy cells. Common ethnic foods, including tamarind, lemon grass, and other spices and herbs, already have natural antioxidants. Highlighting this health benefit of plants can help reduce the use of synthetic products for dietary supplements. In addition to health benefits, a growing concern about food pathogens has increased the demand for nontoxic and natural preservatives. These natural plants already have certain antimicrobial and antifungal properties, even without enhancement (Altemimi).

Consumption of such bioactive compounds has shown a high positive correlation in between health and phenolic contents; although the measurement of antioxidant activity has been focused on the activity of phytonutrients rather than the antioxidant isolation of such materials. Many essential nutrients are found in large quantities of plants, such as monocot species, vegetables, and legumes. In general, vegetable consumption has been shown to reduce the risk of cancer and cardiovascular diseases in their antioxidant, anti-inflammatory, and anticoagulant properties that help in the regulation of blood glucose, blood pressure, and reversing aging biomarkers (Altemimi).

Genebanks often contain the genomic data of the crops that they conserve. Using this information, genes that are either known or suspected to be related to the regulation of phytonutritional content of plants can be utilized. A combination of genetic screening, biochemical analysis, and comparative studies assists in phytonutritional screening of various plant species.

### *Techniques and Approaches*

Genetic resource management along with crop improvement impacts global agriculture and nutrition. Many of the staple crops in developing countries, such as maize, wheat, and rice, lack important nutrients. In fact, rice in particular, is a prime staple food in more than 30 developing countries, but lacks many important micro- and macro- nutrients. Improving the nutritional value of these staple crops will help in the sufficient nutritional development of the populations that depend on them. These phytonutrients are found in a range of plants, but contain them in altered levels (CGIAR).

Once the nutritional content of plants are evaluated, they can be classified as high, sufficient, or low nutrition. The identification of any nutritional deficiency is done either by genotyping or phenotyping showing if the plant is deficient in micronutrients, amino acids, or vitamins - such information can be provided by genebanks (CGIAR). The development of improved crop varieties can be done through conventional breeding or biotechnological approaches. In conventional breeding, crops are selected and cross bred with higher nutritional values. Biotechnological approaches include various methods of genetic modifications and screening (International).

Various phenotyping techniques like remote sensing, imaging, and automated data collections have been used to enhance genotype-phenotype associations (Nguyen). Phytonutrient screening can be done through chromatography, spectroscopy, and bioassays. Isolation of these phytochemicals is most often done through chromatography where individual compounds in a mixture can be separated and identified. To directly identify compounds, spectroscopic techniques and bioassays are the most often used. Spectroscopy uses electromagnetic radiation to identify structures and properties of media, and bioassays use living materials to detect various targeted substances (Altemimi).

It must be noted that the similar plants across the same species often vary in their characteristics - often exacerbated both directly and indirectly by climate change. These plants exhibit adaptive genetic variation across geographical areas that prompt the preservation of their biodiversity and management interventions. In particular, a study done on Tetrastigma hemsleyanum, a herbal plant native to mainland China, shows that genetic divergence of populations across the landscape are inevitable and affects genome-wide variations. There is a broad, but strong, correlation between genetic and regional climatic differences within species that account for the shifting of ranges, and loss of genetic diversity and fitness (Wang). Through the utilization of landscape genomics, scientists are able to identify and predict characteristics of plants despite the changing environment to make use of in breeding for superior characteristics. This particular plant is rare but has a high traditional value due to historical use in treatment of various illnesses. It has been documented to be used in treatment of fever, pneumonia, asthma, and rheumatism - to name a few. Despite its centralized use in China, its therapeutic content varies due to the different adaptations of the plant due to drastic changes in temperature and rainfall as well as topographical ranges. A similar concept can be applied to all types of plants.

The basis of crop improvement is on the understanding of genetic diversity, variability, and the selection of superior properties. Identifying desirable traits both on the genotypic and phenotypic level allows for the successful selection of traits through successive generations. However, even within the same breeding lines, there is a large variability of genotypes and traits due to prior breeding, either intentional or unintentional. It is vital to have consistent screenings for such crops to maintain crop viability and their desirable traits.

### *Applications in Medicine and Health Products*

There is increasing evidence of the superior potential of phytonutrients from plants in human health. These phytonutrients exert their mechanisms through the application and alteration of naturally occurring biological mechanisms such as gene expression and enzyme inhibition. Certain daily activities have the ability to disrupt the body’s homeostasis, especially when such actions are not done in moderation or in consideration to underlying health concerns. For example, consuming heavily processed foods can accumulate lipid content in arteries and it can be difficult to reverse once started. However, consumption of phytonutrients can curb the feeling of need to consume such products as well as reverse the damage. Medical benefits of including phytonutrients in the diet include the prevention and even treatment of physiological disorders allowing for an alternative to synthetic and commercialized medicines.

Phytonutrients have been proven to maintain and even modulate immune functions to prevent specific diseases. This shows that consistent consumption of such nutrients can help in preventative care as well as roads to recovery without the use of high grade medical drugs long-term. Specific biological activities are targeted in order to support human health like antimicrobial, anti-oxidant, anti-inflammatory, neuroprotective, and immuno-modulation that focus on maintaining overall cell health and life (Gupta). In particular, the molecular pathways of which these phytonutrients exert their benefits improve cardiometabolic and neurological health, decrease chances of cancer, and increase immune function. Given these observed benefits, it has become increasingly recommended to integrate phytonutrient-rich foods into dietary and clinical practices to optimize health outcomes (Monjotin).

Due to their more natural characteristic, phytonutrient implementation into diet has the ability to become a cheaper alternative to healthy lifestyle, alongside other practices. Most medical facilities focus on the treatment of the consequences of lifestyle choices. However, through preventative care, people are able to take control of their lifestyle choices- in particular, a more deliberate and healthier diet- without expensive treatments and medication. Implementing a highly nutritious diet is key to preventing many modern chronic diseases.

It has become an increasingly popular recommendation by healthcare professionals to implement plant-based nutrition to prevent and treat chronic diseases. Cardiovascular disease remains the world’s leading cause of death, with many of the early deaths being preventable and nutrition-related. In the United States, the most important dietary risk is a diet that is low in nutritional value that lacks fruit, nuts, vegetables, and seeds, being high in sodium and trans fat. Plant-based diets are associated with lowering mortality rates and improving health by reducing medication needs and high-risk ailments (Hever). There is a high correlation in the consumption of more vitamins, minerals, and fibers with greater plant consumption.

*Case Studies*

Efficient curation of these plant species helps in the conservation of such varieties as well as enhances access to genetic material. Germplasms can be optimally conserved through the resource allocation in genebanks while the unique variety of genetic resources for research can be accessed (Singh). With human populations projected to grow over nine billion by 2050, there is a growing concern about increased food insecurity and challenges to meeting the global food supply.

The primary plants that were commercially modified for mass consumption were the staple crops such as wheat, maize, and rice, as they are of high importance in many developing countries' diets. Rice in particular, can be found to be consumed all over the globe, especially in Asian countries. It is a key resource of carbohydrates and vitamin B, but it still needs enhancement in its nutritional value-related genes as it is not sufficient to get necessary nutrients from the consumption of rice alone. Many of the breeding strategies address the benefits of genetic manipulation of gene expression to combat malnutrition and improvement of dietary quality (CGIAR).

Biofortification of staple crops are the main goals of breeders that focus on the enhancement of their nutritional quality. Such breeding and biotechnological interventions address malnutrition and aim to improve health outcomes. These genetic enhancements reduce micronutrient deficiencies which ultimately improves cognitive development and enhances overall health outcomes for all populations. Organizations such as the International Rice Research Institute hold the largest and most diverse of these genetically enhanced crops such as stacked beta-carotene, iron, and zinc lines as well as high folate, lysine, or leucine rice. Such enhancements target specific nutrient deficiencies of vulnerable populations (International).

Because rice is one of the versatile models for cereals and an economically relevant food crop, it is the most suitable for molecular characterization and a common target for biofortification. It has its centrality as a model for the Poaceae species, with there being significant efforts to dissect genes and quantitate the trait loci. The process of such integrates genomic information into breeding strategies to develop nutrient rich varieties that ultimately improve the nutritional intake of vulnerable populations (Swamy).

In particular, Golden Rice has saved millions of people from malnutrition, especially South and Southeast Asian Countries whose half daily caloric intake is solely from rice. Named for its golden hue, scientists inserted genes responsible for the beta-carotene production in daffodils and added it to the DNA of rice, thus modifying its nutritional content on a molecular level. Rice quality is primarily assessed based on physical properties such as shape, grain color, and something aroma, but is now leaning more towards the assessment of its basic nutritional content - such traits controlled by major and minor quantitative trait loci. Development of Golden Rice varieties have moved away slightly from the original to make it more suitable for cultivation by smallholder farmers in various countries to account for differences in environment to ensure the same quality of rice for differing markets (International).

A study done on the phytonutrient and nutraceutical action against the SARS Covid-19 was done in Wuhan China and used the potential or medicinal herbs and their actions. The results are linked to the understanding of the different mechanisms of these plants that suggest a promising potential in prevention or treatment of diseases as the first line of defense. The phytonutrient component of these herbs blocked enzyme pathways that were responsible for the SARS-CoV adhesion to cells. Since the virus could no longer attach themselves to the host cell, the virus had little to no effect on a person's health and led to a speedy recovery. Further studies on the extent of recovery are currently under investigation. Various studies on the use of natural and plant medicines on viral infections show that there is a possibility of using specific plant compounds in the prevention and complementary treatment of diseases (Pastor).

*Challenges and Considerations*

Despite all these advancements in technology, genebanks still face many challenges in the storage and utilization of germplasm. The conservation of genetic diversity is the key to a nutritious future, and multiple treaties have been made to safeguard access to such genetic material between many countries (Guardians). Most of the barriers to a truly collaborative network are addressing the regulatory and safety concerns that come with the exchange of germ plants across different regions and nations (International).

Conservation in itself is a challenge as germplasm requires very specific conditions in order to be stored. If not stored correctly, genebanks are vulnerable to genetic erosion of which genetic drift and loss of diversity can occur or to environmental factors such as climate change and diseases. Most of all, there are resource constraints that limit funding and therefore the sufficient structures and personnel for operations (Panis).

It is not enough to just hold the germplasm for safekeeping, but also having it available to the public. Although there is passport data that details a plants' characteristics, there is a lack of data integration of genomic data as well as the standardization of requesting material for those outside of internal genebank operations (Singh). Tackling conservation concerns as well as adapting to modern technologies can help in the advancement of plant screening and utilization of more specific characteristics in breeding for optimal conditions in extraction by having available both genomic and phenotypic data.

### *Future Direction*

The future of healthcare is still heavily dependent on pharmaceuticals, but is slowly moving towards the use of nutraceuticals and studies in phytonutrient utilization. Many times, nutraceuticals and phytonutrition are used interchangeably, but there are subtle differences in these types of medications. Pharmaceuticals refer to a medicine or any substance used as medication that is used in both the prevention and treatment of a disease (Pastor). Nutraceutical is considered a nourishing food component (Pastor) and not necessarily to fulfill human nutritional needs (Nasri). Phytonutrients are substances that are derived specifically from plants to elucidate a health benefit such as the aiding of metabolism after eating. Nutraceuticals are specifically compounds or mixtures that are present in foods or supplements that exert a therapeutic effect of which the source could be animal or plant based (Pastor).

Phytonutrients play a vital role in both pharmaceutical and nutraceutical industries. Plant biology and research contributes to drug discovery and developments due to their diverse health benefits and therapeutic potential. Because of its natural nature and promising evidence of prevention and treatment of disease, phytonutrients serve a dual function that has become more attractive in the medical field. Phytonutrient utilization is being widely utilized in dietary supplements for their health promoting properties and an increasing demand for more natural products in the market.

Research on phytonutrient extraction in the short term focuses on the extraction efficiency and effects of various phytochemical compounds on biochemical pathways (Altemi). With continued innovation in technology and data analysis, phytonutrients of screening can become an integral part of healthcare. Long term, plant sciences will become more important in the realm of natural medicine as people lean further away from pharmaceuticals. By understanding the metabolic properties of what we consume, it can come to understand that people can use less synthetically made products and rather work with natural mechanisms to achieve optimal health. Synthetically made products more often than not world in the mitigation of a symptom and biohacking a biological system into healing. Rather, with natural medicines, minimal synthetic material is introduced to biological systems and can be used to prevent and heal before major damage has occured. Instead, phytonutrients aid in preventative care which is much cheaper, convenient, and more often than not healthier long term.

*Conclusion*

Genebanks serve the purpose of storing and maintaining the world's variety of germplasm. Each of these unique plants hold health potential that is yet to be utilized through breeding and further research. There is strong evidence that a plant-based diet can be beneficial throughout a person's lifespan, decreasing chances of getting certain chronic diseases and maintaining health (Hever). Health is a large aspect of someone's life and is greatly influenced by diet. By screening for the best nutrients to consume in higher quantities, people are able to make more informed and deliberate decisions on how they are to attain the optimal health. Having a range of genetic variation allows for the utilization of therapeutic and healing properties from plant nutrients. The screening of phytonutrients by genebanks allows for the optimization of breeding and genetic techniques of the future, revolutionizing the image of what healthcare will look like.

**Integrated Pest Management**

The Integrated Pest Management (IPM) department focuses on developing and implementing strategies to control pests and diseases affecting vegetable crops in an environmentally and economically sustainable manner. By combining biological, cultural, mechanical, and chemical control methods, the department aims to reduce pest populations while minimizing negative impacts on the environment and human health. Their approach emphasizes prevention, monitoring, and intervention, ensuring that pest management practices are both effective and aligned with best practices in sustainable agriculture.

***Bacteriology***

Bacteriology often deals with disease diagnosis in the following capacities; observation in the field, infected plants, seasons dn time of occurrences, and application of practices before disease occurrence. The trifecta for any susceptible plant is for a host, pathogen, and right environment for that pathogen to fluoride, Disease control can be chemical, biological, physical methods, and integrated control practices. The focus of the bacteriology unit is the identification and prevention of diseases. A very important practice is inoculation and culture.

The process of inoculation involved diagnosis, cultring, and isolation. A bacteria is screened on a petri dish and tested for morphology. Another way that the plate is streaked is for the increasing volume in order to obtain enough bacteria to be used for insulation.

***Entomology and Insectary***

The department studies pest behavior, biology, and ecology to develop sustainable pest management strategies that reduce crop damage and improve yields. Their work involves identifying insect pests and their natural enemies, researching biological control methods, and assessing the impact of climate change on pest populations. The most common pests that studies are Phthorimaea absoluta, Bamisia tabaci, aphid species, Amrasca biguttula, and Thrips palmi. The damage of these insects are inspected and studied in order to figure out their patterns and create prevention protocols that stop, or at least slow, their growth into adult stages. Interestingly, some practices that would have been thought to be harmful may actually be beneficial. For example, fungus can be used as a natural pesticide that does not necessarily harm plants. These fungi can be studied to serve as a coating for plants that rejects and repels the most common to pests such as thrips and whiteflies. In order to study the behaviors of these pests, bugs must also be cultured. This is done by puttinging them in confined containers with treated leaves. The nymphs and larva are observed (in their respective instar stages) for growth and development. These bugs can either be individually student or released into a controlled environment for observation.

**Tomato Breeding Programs**

The tomato breeding programs focus on developing improved tomato varieties to address various agricultural and market needs. The program aims to enhance disease resistance, improve fruit quality, and increase yield by incorporating resistance genes and selecting for desirable traits. It also emphasizes adaptation to local environmental conditions, including drought tolerance and heat resistance.

***Root Washing Protocol***

Sharukh Pasha, a fellow intern at WorldVeg is doing his research on the architecture of the tomato roots in response to heat stress. He developed a new root washing protocol that would preserve the architecture of the roots, keeping them intact and unbroken. Previous protocol cut the pot and beat the top of the soil, leaving broken roots. With the new protocol, there is minimal root destruction. Plant height, leaf temperature, SPAD, and stem width were taken before cutting the top from the root and applying the root washing protocol.

The root wash protocol required that the top of the soil be loose with water. In order to get the roots out of the pot, water was pushed in through the bottom until the soil loosened and came out. Following this, the roots were soaked  and sprayed with water to remove the surrounding soil. Rocks and other debris would be manually picked out. Dry and fresh weight of the roots and stem were taken.

The root washing protocol was created to study the underground part of plant systems which are a vital part of how plants obtain their nutrition. By studying the tertiary and minute roots, the health of the plant can be determined on a basis more than just its yield and appearance above ground.

It should be noted that the growth of these tomatoes was limited. The pot effect can be observed in some of the data discrepancies as the roots were only allowed to grow in the space provided for them.

***Heat Tolerance Screening of Tomato***

Under heat stress, tomatoes tend to have more lateral roots and a weaker tap-root. Most often, the regular plants have the tap roots; the more fibrous roots are a result of the plant looking for more water and grounding in its surroundings. Leaves tend to curl under the intense heat in order to reduce evapotranspiration and conserve their water.

Plant height, root length, and root area tend to be positively correlated with plant health. The susceptible lines of tomato tend to be shorter and weaker roots. The shoot and root fresh and dry weight are compared to see how well tolerated these tomatoes are to heat. When the fresh and dry weight are closer in number, that means that despite losing water, the plant protects itself well and maintains its overall structure. It can be observed from the heat tolerant lines that they maintained an overall higher fresh and dry weight. After 2 days in the oven for drying, their phloem also remained thick in comparison to susceptible lines that became brittle very quickly. The SPAD value determines the relative amount of chlorophyll present in an active leaf, giving an idea of how much photosynthesis is being done in a plant. The more chlorophyll present, the higher the SPAD value, the more photosynthesis is able to happen, which is associated with a healthy plant.

Using an photosynthetically active plant, the LI-COR machine was used to measure a variety of parameters. It is a portable photosynthesis system that takes a plant leaf, and runs through a chamber that will measure things such as humidity, temperature, and $CO_2$ values which all measure photosynthetic rate. Measuring the rate of photosynthesis with these values gives insight on the overall health of the plants that cannot otherwise be seen with the naked eye. Heather plants tend to have a higher and more stable rate of photosynthesis than sickly plants as the plant metabolism is more adapted to the harsher conditions.

**Healthy Diets**

The Nutrition Lab at the World Vegetable Center is dedicated to studying the nutritional benefits of vegetables and promoting healthy eating habits. This department conducts research on the nutritional composition of various vegetable crops and their impact on human health. By analyzing how different vegetables contribute to a balanced diet, the lab aims to provide evidence-based recommendations for improving dietary practices and addressing global nutritional challenges. Their work supports public health initiatives and helps to raise awareness about the importance of incorporating vegetables into daily diets.

**Molecular Breeding**

The Molecular Breeding and Biotechnology Lab focuses on advancing crop improvement through modern biotechnological methods. This department utilizes techniques such as genetic mapping, marker-assisted selection, and genetic engineering to enhance vegetable crops' traits, such as disease resistance, drought tolerance, and yield. By integrating molecular tools with traditional breeding practices, the lab aims to accelerate the development of new and improved vegetable varieties. Their research contributes to increasing agricultural productivity and addressing the challenges posed by climate change and other factors affecting food security.

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