The Germination of Legume Crops to Understand Seed Longevity

The World Vegetable Center
Tainan, Taiwan

Tyler Reid 2018 Borlaug-Ruan International Intern
Accokeek, Maryland

Table of Contents:

Acknowledgements……2
Reflection:.....2
Credits:.....4

Abstract……6

Introduction……7

Methodology…… 9
Apparatus and Materials:.....9
Procedures:.....10

Results and Discussion……11
Result Analysis on Soybeans:.....11
Result Analysis on Mung beans:.....14
Discussion:.....16

References……18

Photos……19-20

Acknowledgements

Reflection:

Four years ago when I was beginning my high school career, if someone would have told me that I would be spending the summer before my freshman year in college in Taiwan participating in an internship. It is very possible I would have looked at you like you were crazy I might have even laughed a little. The journey towards obtaining the beautiful opportunity all started in May of 2017 when I attended the first Maryland Youth Institute at the University of Maryland Eastern Shore. Myself along with dozens of other youth were in attendance, we presented our papers and had open discussions. Fast forward to June of 2017 I found out that my research paper was one of the papers selected, meaning I would be able to Participate in the Global Youth Institute in October. While attending I heard about the Borlaug-Ruan International Institute one of my group leaders had even participated in it during the summer of 2017. When the application opened I applied and in April of 2018 I was delivered the news that I was officially accepted as a 2018 Borlaug-Ruan summer intern. At the same time I found out that I had been placed at the World Vegetable Center in Shanhua, Taiwan, which made me excited instantly I had never traveled to Asia before. Once I learned more about the country of Taiwan, their culture and of course the World Vegetable center that just added to my excitement. My almost twenty-four hour journey to Taiwan began on June 3rd, I landed in Kaohsiung, Taiwan on June 4th. One thing that took some time to get used to was the twelve hour time difference between Taiwan and Maryland. A continuous obstacle that I faced throughout my time in Taiwan was the language barrier. In

Taiwan they speak Mandarin I had learned a few words before arriving in Taiwan, but I didn't speak it fluently. On the World Vegetable center campus mostly everyone spoke english, although there were times where there was a challenge communicating. In some of those instances instead of trying to communicate verbally the communication happened through visual representations or demonstrations. Another challenge was keeping myself from getting to homesick of which is to be expected. Being gone for two months was not only the longest I had been away from home but it is also the longest trip I have taken away from my family and close friends. Of course staying in touch and communicating with family and friends helped, but also keeping myself busy helped to, being busy makes time feel like it is going by faster.As well as branching out and making new friends it which aided in me not feeling lonely all the time. Branching out was also beneficial because I got the chance to learn about other countries and cultures through the new people I met. At the beginning of my internship my mentor suggested two topics for me to think about for my research. Those two topics were, spatial diversity analysis of traditionally African leafy vegetables and the germination of legume crops to understand their seed longevity. One of the other interns in the genebank Shin-yee was focusing a project topic similar to the first topic suggested to me, but she had knowledge and experience concerning both topics. I talked to her several time to help me get a better understanding of both topics. The first topic I would be learning to use a new software called GIS. With the second topic I would be learning how to conduct and analyze germination test. Ultimately I chose to go with the second topic for the reason that it was more hands on and would allow me the chance to do more lab related work.

Credits:

This was a once in a lifetime opportunity that I am eternally grateful for and will forever hold close to my heart. I am overwhelming grateful the following people for their continuous encouragement and support throughout my journey in Taiwan.

First and foremost, to Norman Borlaug who was not only a trailblazer and helped to bring forth many victories in the fight against hunger on a global scale but the man responsible for setting the stage for this internship to exist. A great thanks goes to Mr. John Ruan for seeing what the World Food Prize stood for and its potential along with making sure the rest of the world got to see it to. A special thanks for Ambassador Kenneth M. Quinn on continuing his dedication to the World Food Prize organization and all the programs within it.

Next, the highest thanks to Ms. Crystal Harris for the continous work she put forth in efforts of bringing my internship into forwishen along with the other interns. I am truly thankful for the foundation and support you provided throughout this entire process from the application process to making sure my travel to and from Taiwan went as smoothly as possible. I am extremely thankful for you and the selection committee seeing the potential in me and allowing to partake in this wonderful opportunity.

To the World Vegetable Center staff, thank you for providing a remarkable and life changing internship experience for me while I was on campus. A special thanks to Dr. Maarten van Zonneveld, Ms. Miao-rong Yan, Mr. Yung-kuang Huang and the rest of the GRSU staff for

welcoming me with open arms it enhanced my experience for the better. Along with the unwavering support and guidance provided to me throughout the conduction of my research. A deep thanks for Ms. Aileen Kuo for constantly checking on me to make sure that I was okay and enjoying my time in Taiwan and at the World Vegetable Center. The friendly faces and people that I came in contact with while there made the experience that much more worthwhile and will forever hold a place in my heart and my memories.

To Ms. Gudderra, Mr.Belle, and Mr.Binns , I am eternally grateful for the guidance you gave me up to this point in my life. Exposing me to the possibilities and world of agriculture of which now holds a special place in my life. For pushing me to step out of my comfort zone while continuing to better myself and build towards my future. Thank you for the constant encouragement and motivation throughout my high school experience. You all have reshaped my life for the better and for that I am grateful.

To my mom, sister and the rest of my family and other supporters, who have really helped to keep me going throughout this experience. Mom, thank you for believing in me to take on this opportunity and supporting me in every you knew how to. The consistent Whatsapp calls from you and Tia made me feel like I had little piece of home with me in Taiwan. For my entire support system thank you for the consistent support whether it reading my most recent blog post or sending me a message to check on me and see how I was doing.

Abstract

Legume crops are the main focus of the research more specifically soybeans along with mung beans. There are many different kinds of legume crops but generally they produce a pod that rips right down the middle equally, for instance soybeans and mung beans. Not all legume crops come in the form of pods such as clover, alfalfa. While the pods of legume crops are low on the glycemic index they are high in protein and fiber. Legume crops are popular and are present in most areas in the world. These crops also grow at a fast rate and are inexpensive. The lifespan or longevity of seeds in storage like settings vary, not all seeds last for the same amount of time before needing to regenerated. The seeds that don't have a long seed longevity, after a certain amount of time they begin to decline in a way, such as soybeans. Then there are other seeds that do have a longer seed longevity like mung beans for instance. Germination is the process of development of a seed or similar structure. In order to determine which germination test method is going to be used solely depends on the size of the seed. Before starting the actual germination test, the seed moisture content of the seeds was measured, which is an indicator of seed decay.

Introduction

The World Vegetable Center genebank contains four hundred and thirty-nine different vegetable species from one and fifty-one different countries. As well as, holding fifty-nine thousand nine hundred and sixty-seven active accessions. An accession is a specific breeding line of plant material. At the World Vegetable Center they store seeds in either short, medium or long term. Short term storage maintain seeds at fifteen degrees celsius for five to ten years. While medium term stores material at five degrees celsius for twenty to fifty years. Then long terms stores material at negative fifteen degrees celsius for fifty to one hundred years. Gene banks also play a critical role because they can store and preserve seeds and other plant like material for varying amounts of time. Furthermore, this brings the importance of seed saving and preserving to the forefront. The practice of preserving seeds has been going on years, which is how the crops that hold high popularity now came to be. Over time there has been a decline in genetic diversity in crops. Leaving us with a smaller selection of crops to rely on, which in turn makes it more difficult to adapt to changes occuring due to climate, pests and diseases. Making it more critical to preserve heirloom and other seeds that may not be present in the main selection of seeds.

Seed saving was an essential activity when the first humans started to farm land 10,000 years ago, and still is a foundation for sustainable agriculture and food security. In terms of food security the preservation and saving of seeds could play a major role in combating the ever evolving issue. The main test method in my research is germination test hold significance because they show if certain seeds will still germinate even in a controlled setting, along with the

fact that it can help to determine if a particular group seeds need to be woken up or regenerated. Germination test are beneficial due to the fact, that the results gained from them can be used to estimate how successful they would be if planted in the field. There is more than one type of germination, but in the lab there are two that are mainly used. Those two types are the petri dish method and the paper method. When it comes to distinguishing which type of methods is going to be used it all comes down to the size of the seed. If the seeds are of a smaller size than the petri dish method will be used, but the seeds are of a larger size the paper method will be used. Peppers, eggplant, and tomato seeds fall under the smaller seed category. While okra, bitter gourd, soybean, and mung bean seeds fall under the larger seeds category. In my research I conducted germination test on soybeans and mung beans, which means I used the paper method to execute my germination test. Prior to taking the seeds through the germination test they have to go through a moisture test as well. One of the purposes of conducting the moisture test is to reduce oxidation, decomposition or the loss of other crucial material all while assuring the elimination of as much moisture as possible. The results that are obtained from moisture test can also start to show you signs if the seeds are still on a healthy track or not. With soybeans if the moisture test levels where over 8 that was a sign that the seeds could possibly beginning to go down hill. Legume crops are the main focus of the research more specifically soybeans along with mung beans. Commonly, legume crops produce pods that split down the middle evenly, such as soybeans, mung beans, and peas. Even though there are some legume crops that exist that don't produce pods like alfalfa, and clover. Legume crops are popular on a global scale, putting them in a position to be available in a lot of different areas. This specific group of crops are also rather inexpensive, are high of protein, tasty, fixate nitrogen, and they grow quickly. The

longevity of seeds varies depending on the crop, some have longer seed lifespan than others. The seeds that have a shorter seed longevity decline earlier and have to be regenerated more often. Soybeans have a reputation for having a shorter seed longevity, unlike mung beans which are known to have longer ones. The main claim for my research is to see if the seeds are a affected at all by being kept in storage for a certain amount of time, and if they how long should they be kept in storage before needing to be regenerated.

Methodology

The germination test were conducted on soybeans and mung beans. Soybean seeds were collected from four different years, and each year have twenty accessions. While mung bean seeds were gathered from three different years and there were ten accessions per year. With both soybeans and mung beans each accession had three replication meaning the germination test conducted on each accession was repeated three times.

Apparatus and Materials:

When sorting the seeds out into their replications you need a marker and the spreadsheet stating the order of each replication. The moisture content of the seeds is taken by the hygrolab C1 Bench-Top Indicator, and then the seeds are poured into a container, and set on a scale to collect the overall weight of the pack of the seeds. Once all of the results from the moisture test which include temperature, moisture, weight are collected, they are inputted into a database to come up the seed eq. In order to begin conducting the actual germination test process sheets of plastic,

paper towels, distilled water, a marker, a pen, labels, and a germination test record sheet is needed.

Procedures:

My research uses the experimental design structure of RCBD which stands for randomized complete block design. The experimental method of germination test more specifically the paper technique. The dependent variable is the amount of years the seeds have been kept in storage. While the independent variable is the survival rates of the seeds. Finally, the controlled variable is the number of accessions for every replication. To start off you have to collect all the seeds from the different storage units. The soybean seeds were pulled from the medium term storage unit while the mung beans were pulled from the short term storage unit. Once the seeds have been pulled they must be organized and sorted out by year and replication. The next step is to conduct the moisture test, but the moisture test only needs to be conducted on one replication per year. After that is completed I moved on to completing the actual germination test. Which begins with getting one sheet of plastic, then getting two pieces of paper towel and laying them on top of the sheet of plastic and pouring some distilled water onto the paper towel. Next, I put the label which has the accession number written on it on top of the paper towel. Coupled with the placement of the seeds, every germination test has to contain at least fifty seeds. Again after the seeds have been placed another two paper towels are put over top, along with more distilled water. In addition, after the distilled water is poured on top, I then began to roll the plastic sheet and paper towels. Furthermore, once everything it is rolled it is put into a cup where more water is poured into it, but only up until the a little bit of the paper towel is submerged into water. This

process is repeated until all of the accessions for that replication are done. Once they are finished the accessions are placed in a incubator, where the will checked on after seven days of incubating. Throughout the seven days the replications get taken out once or twice to add more water into the cups. Moreover, after seven days have passed the replications are taken out of the incubator and analyzed to figure out how many are healthy, abnormal, or dead. In some cases if a seed is still in a hard seed state it placed on a new sheet of plastic with paper towel on it with distilled water and placed back into the incubator for a couple a days, to see if anything changes. All of the results are written down on a record sheet specifically for germination test.

Normal

Abnormal

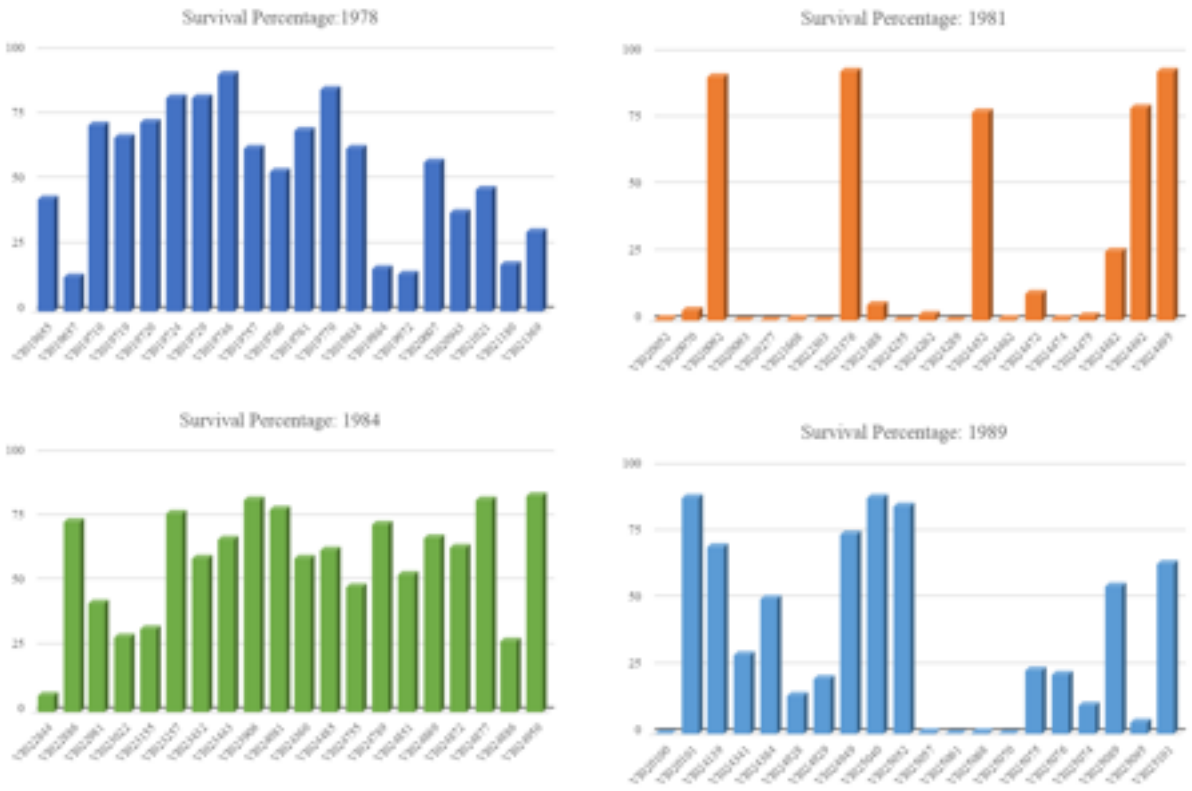
Dead

Results and Discussion

Result Analysis on Soybeans:

Once again the main focus of my research is to see if seeds are affected by being kept in storage for a certain amount of time, coupled with how long should they be kept in storage before being regenerated. I previously, stated how seed longevity varies depending on the crop these test put the exact statement to the test. With the results collected from the germination test I was tracking

the survival rate, that was done by counting the amount of seeds that were normal, abnormal, and dead. With soybeans it was expected for the results to fluctuate at time especially as the seeds grew older, meaning they were in storage for a longer period of time. For the soybean seeds there were twenty accessions pulled from four different years. The four years that seeds were collected from were 1978, 1981, 1984, and 1989. Going into the experiment it was expected that the later years like 1984, 1989 were going to have the higher survival. Unlike 1978 where it was expected that the survival rate was going to lower and 1981 was going to be somewhere in middle. These assumptions were drawn due to the fact that soybeans are known for having lower survival percentages if it is kept in storage for a longer period of time.

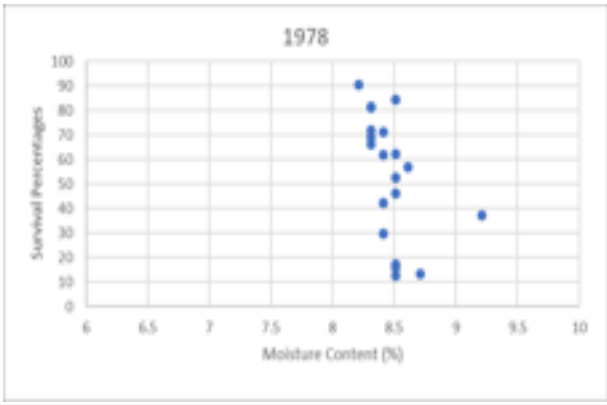


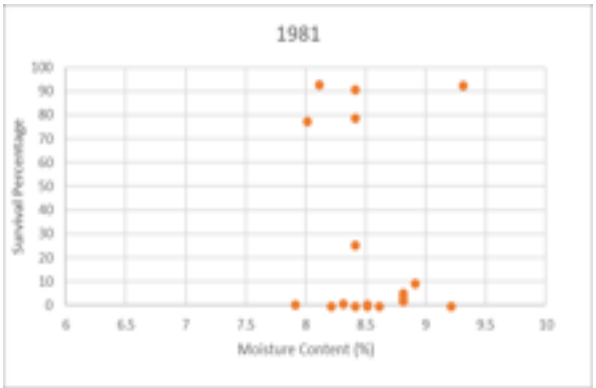
As shown from the graphics above the pre-drawn conclusions made at the beginning of the experiment proved to be wrong. The poorest seed survival percentages resulted in 1981 and 1989, which were expected to have a higher outcome. Unlike, the years 1984 and 1978 which ended up having the best seed survival percentage rates.

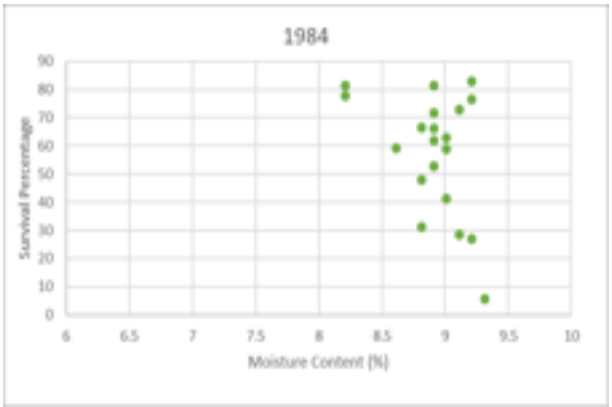
	1978	1981	1984	1989
Average:	53.8	24.3	58.3	35.1
Max:	91.0	93.4	83.5	88.3
Min:	13.2	0	6.4	0

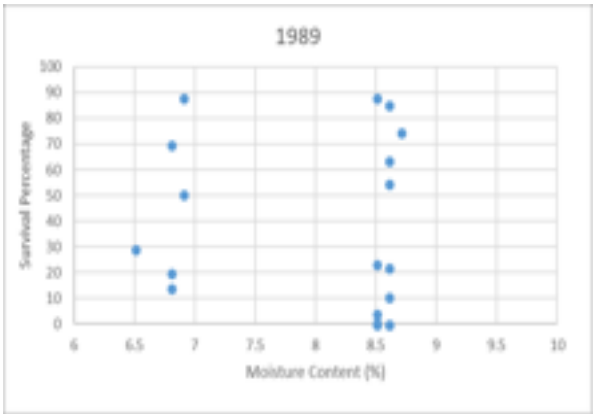
Prior to conducting the germination test the seeds were examined in the area of moisture content. Moisture content could play a possible role in the outcome of a seed germination. In the process of finding a reason for these unexpected outcomes the moisture content results were re-examined, as a possible contributing factor. The overall moisture content results were rather high.

Moisture Content Results









Result Analysis on Mung beans:

Mung beans and soybeans are very similar, so the process used to conduct germination test on soybeans is the same process used on mung beans. Once the germination test were complete the mung beans were categorized the same as soybeans, they were either normal, abnormal, or dead. Similar to the soybeans the survival percentage of the mung beans was what was being measured. One difference with the mung bean seeds was the fact that the seed were only pulled from three different years, which were 1975, 1982, and 1990. Another difference with the mung bean seeds also was that I only had ten accessions.

	1975	**1982**	**1990**
Average:	67.76	60.85	69.50
Max:	79.85	69.92	76.98
Min:	49.64	39.76	61.12

Just from looking at the averages from each year for the mung bean seeds, you can see that they had better results as a whole. Mung beans have a reputation for being more constant in results produced from

germination tests than soybeans do. Beginning the experiment it was expected that the mung beans would have a more steady result pattern. It was expected that the results would be on the higher side of seed viability, due to mung beans having a better reputation with germination test.

Discussion:

When it comes to soybeans it was expected that 1978 would have the lowest survival rate but after analyzing the data that wasn't the case. The expected ranking for the survival rates from highest to lowest was 1989, 1984, 1981, and 1978. The ranking after looking at the results from highest to lowest is 1984, 1978, 1989, and 1981. The results that ended up being produced weren't expected. The next question that was asked was how did this happened? What factors

contributed to these unexpected results? A potential reason is that high moisture content was related to low seed survival percentages. High moisture content results explained how the results were produced for 1978 and 1984, but they did not explain the results composed for the 1981 and 1989. So the moisture content results only partially explain the outcomes. Prior handling of the seeds before entering into storage could also be a factor. The outcomes of the mung beans came out as expected. The results were mostly high and steady, there were a few drops but not often and not that low. The mung beans lived up to their reputation of being more stable.

There are a few conclusions that can be drawn from the experiments conducted. One them being that seed viability is not related to the duration of seed storage. Along with, seed moisture content does play a role just not a dominant one. It can therefore be deduced that the poor seed handling prior to seed drying could have led to the lower survival percentages in 1981 and 1989. The overall main conclusion is that previous seed handling does play a important role in seed viability. Since 2010, the World Vegetable Center has established two new seed dryers to improve seed drying and handling procedures. It would be recommended to start keeping and maintaining documentation about seed harvesting and handling prior to entering into storage for tracing back possible causes of seed decay. In addition, it would be recommended to also use a non destructive seed viability test similar to taking the seed moisture content. There is a non destructive seed viability test the measures the amount of Chlorophyll in seeds, which could also serve as useful in determining seeds germination potential

References

Grant, B. L. (2012, June 14). Popular Legume Plants: What Are The Different Types Of Legumes. Retrieved from https://www.gardeningknowhow.com/edible/vegetables/vgen/what-are-legumes.htm

International Seed Testing Association. (2014). *International rules for seed testing: Adopted at the ordinary meeting ... to become effective on*. Bassersdorf, Switerland: ISTA.

Lima, J., Buitink, J., Rossi, R. F., Pelletier, S., Silva, E., & Leprince, O. (2017, July 12). Molecular characterization of the acquisition of longevity during seed maturation in soybean. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5507495/

Photos


World Vegetable Center
The Germination of Legume Crops to determine their seed longevity
THE WORLD FOOD PRIZE
PIC•COLLAGE

Photos