Resisting Waterlogging in Maize
China Agricultural University
Beijing, China
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Acknowledgements

The opportunity to travel to the other side of the globe without having to spend a dollar was something that I will never forget. While at first, I was very appreciative, I didn't truly understand the magnitude of the gift I'd been given. It was when I landed in Beijing and began to experience the life changing nature of travel that the grandeur of the Borlaug-Ruan International Internship dawned on me. My view of the world has been significantly altered due to this internship. While I was quite familiar with the way I lived, I knew virtually nothing about how people lived outside of my home country besides what I had read online or seen on t.v. To be candid, I didn't think about it very often anyway. I'm sure there are a lot of people in the world that are the same way. These people-like me-don't ever really stop to consider the sheer vastness that is the Earth. If you were to travel just five miles in any given direction, you'd find that this new place is unlike where your journey began. Travel five more miles and you'll encounter further unfamiliarity. No place is ever like the other. The extent of what you know decreases as the distance increases. Traveling to Beijing has made me immensely more cognizant of the fact that there is so much more in this world than the portion I live on. My once one-dimensional perspective of life as a whole has morphed into an ever-changing complex of thoughts, experiences and interactions. My newfound view has come with the knowledge that while there truly is no place like home, that is not a bad thing at all. There are billions of people populating this planet, all of which are capable of making any place home for you. It's not the slab of earth that you live on that makes it a home, it's the people that live there that bestow this title upon it. Surroundings and people vary all throughout the expanse of the globe, but what does not change is something visceral. In its most raw form, humanity is something that we all possess. Often

times this can be hard to see. We mask it with the clothes we wear, the language we speak, our mindsets, our demeanor, and the lives we live. If you were to peel back all these things you'd discover something unsullied that isn't unknown to you at all- a human. At our core we all want and need similar things: food, water, shelter, happiness, camaraderie, people with which we can transpose thoughts, and for lack of a better word, life. In the eight-week duration of this internship I have discovered more about myself and the world than I have in the previous eighteen years of my life. The most important realization for me was that although I may look at the world and see unknown places full of nameless faces, there is something that we already have in common- our humanity. Another gift afforded to me as a result of this internship is the practical skills I was able to develop by working in the lab every day. The lab is no longer unfamiliar territory in my mind, it is actually quite the contrary now. The lab is a place where I feel comfortable and focused. If I wasn't sure before, now there isn't a doubt in my mind that a career in biochemistry is something that I'll find enthralling.

None of this would have occurred without the unparalleled leadership of Ambassador Quinn. Ambassador Quinn has altered the course of so many students' lives through his constant strides towards a better tomorrow. I would like to say that I am immensely thankful for having been able to experience something so unique because of your efforts. Another person who is deserving of much praise is Crystal Harris. Crystal has shown me that superwomen do exist. To this day I am unsure how one person can do so much and so well. She is truly the backbone of the entire internship and I would like to thank her for taking such good care of all of us interns and ensuring we were all well looked after. The next people I am grateful are all of the members of the lab at China Agricultural University. My experience would not have been anywhere near

as fantastic as it was without all of the students and professors that made adjusting to the unfamiliar terrain so much easier. The one person there who deserves the most credit of all is Professor Zhou. She was like a mother to me all summer and always made sure I was taken care of. Without her Beijing wouldn't have been the same at all.

I would like to dedicate this project to all of my friends in China who made me feel welcome and made sure I never felt alone. Even more than just that, they are the ones who showed me around the campus and went in depth with me in the lab. Without them none of the data gathered would have been recorded and my overall experience would not have been half as spectacular as it was.

Abstract

While at China Agricultural University I conducted research on transgenic maize plants. The plants that were the object of my study were modified to have two genes respectively that may or may not confer waterlogging resistance. So, there were two groups of fourteen pots. One group was for the GRMZM2G341959 gene and the other one was for the GRMZM2G040673 gene. After the cultivation and study of the modified maize seedlings we were able to conclude that both genes do in fact play a role in waterlogging resistance. We were able to come to this conclusion based off of the phenotypic and genotypic characteristics of the samples. By the completion of my project we could deduce that the genes could play a previously unheard-of role in resisting water logged conditions.

Introduction

The main issue casting a shadow on this entire generation is that the Earth is dying, and humans will meet their demise along with it. Various factors such as such as rapid population growth and climate change are responsible for the gradual decimation of the planet. These two factors in particular are the reason for this paper. Combined, the growing population of over seven billion and the ever-erratic climate put severe stress on the availability of food in the world. The number of hungry people in the world is growing with each passing year. 821 million people do not get enough food to eat(fao.org). A specific subtopic of food insecurity that is especially prevalent in southern China is water logging of crops. What does it mean for a crop to be water logged? Waterlogging occurs whenever the soil is so wet that there is insufficient oxygen in the pore space for plant roots to be able to adequately perform respiration. There are also many other gases detrimental to root growth such as carbon dioxide and ethylene, that accumulate in the root zone and negatively affect the plants as a result of this. What makes combating water logging so difficult is that plants differ in their demand for oxygen. There is no one level of soil oxygen that can conclusively determine waterlogged conditions for all plants. In addition, a plant's need for oxygen in its root zone will vary even further depending on its stage of growth(soilquality.org).

Lack of oxygen in the root zone of plants for an extended period of time will cause the root tissues to decompose. Typically, this will manifest itself in the tips of roots first, thus causing roots to appear as if they have been cut. The consequence is that the crop's growth and development come to halt. If the oxygen deficient conditions continue for a great deal of time the

plant eventually dies. Most often, waterlogged conditions do not last long enough for the plant to die. Once a waterlogging event has passed, plants recommence respiring. As long as soil conditions are moist, the older roots close to the surface allow the plant to survive. However, further waterlogging-induced root pruning and/or dry conditions may weaken the plant to the extent that it will be very poorly productive and may eventually die. Many farmers do not realize that a site is waterlogged until water appears on the soil surface. However, by this stage, plant roots may already be damaged and yield potential severely affected.

In the lab at China Agricultural University two unstudied genes that may play a role in resisting water logging in maize plants were studied and are the topic of this project. The purpose of this research is to determine whether or not the two genes of interest will aid plants facing waterlogging.

Method

I. Participants:

Elijah J. Ortiz with help from Peng Chuanxi

II. Apparatus/ Materials

Sample Prep/ Container Set up:

- 14 groups of 20 modified maize seeds
- 14 Plastic containers

- Net Bags
- Aluminum foil

RNA Extraction and qPCR:

- qPCR machine
- 10-100 µl Pipette
- Mortar
- Pestle
- Well Plate
- Ice
- Styrofoam Box
- Liquid Nitrogen
- Nano Drop 2000 Spectrophotometer
- Nuclease Free Water
- Primer F and R
- Master Mix

III. Procedure:

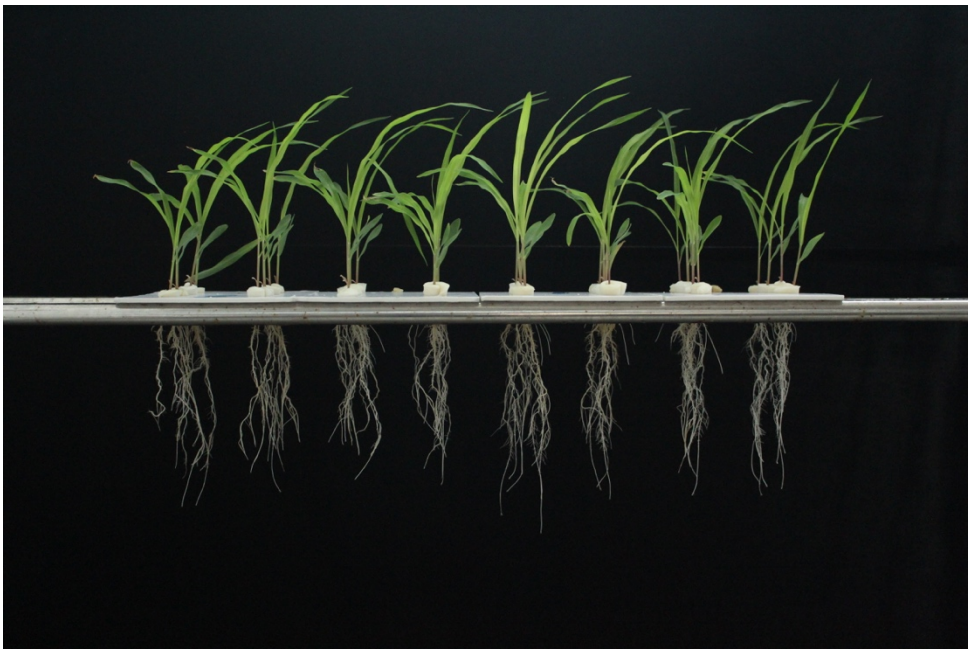
Experimental Design:

For this experiment it was required that participants grow the maize seedlings that were subjected to either waterlogged or normal conditions. After a period of about four weeks samples

were taken from all seedlings and their gene expression levels were then calculated using various formulas. In this experiment the control group is labeled actin in the charts below. The control group was a line of maize seedlings that did not possess any additional genes. The experimental groups were GRMZM2G040673(Gene 1) and GRMZM2G341959(Gene 2).

Prepare the samples:

The beginning of the experiment was field work heavy. Before any real analysis could occur, all of the maize seeds needed to be planted and cultivated. The transgenic maize had already been pre-prepared for the experiment before it began due to the process of creating and inserting a vector being a long one. First, the seeds needed to be separated into 14 groups of 20 keeping in mind their genetic lines and group number. After this they were put into net bags. The seeds needed to be cleaned before the experiment began so they were washed once using a diluted alcohol solution and then washed two subsequent times using water. Once this step was



completed the seeds needed to be planted. They were first planted in sand and allowed to germinate for 4–5 days.

Set Up Containers:

After the germination period the seedlings needed to be moved. They were put into containers filled with water and wrapped in aluminum foil to block out light. The bases of the plants were wrapped with soft sponge and inserted them into a piece of cardboard with pre-cut holes in it. This cardboard piece is then placed on top of the container. The seedlings were allowed to grow for about two weeks. It is important to note that each group consists of two containers with six seedlings respectively. One container had a tube supplying air to the water thereby simulating normal conditions. The other container did not have this tube so that there would be low oxygen in the water thereby simulating water logged conditions.

Gather Data:

Every three days photos of the phenotype (physical appearance) of the maize were taken. After the final phenotype photos were taken all of the plants were cut in half and put into paper bags so the leaves and roots could be weighed. The weight of all 112 of the seedlings was measured wet and then left out over night to dry. The dry measurements were taken the next day. Before the maize was weighed, a piece of every plant's leaf and root was extracted for gene analysis. First the samples needed to be ground up into a fine powder and then RNA was extracted from them.

RNA Extraction:

Protocol with RNA Lysis Solution B-

1. Weigh no more than 100mg of fresh tissue, or frozen tissue and homogenize it under liquid nitrogen.
2. Immediately transfer the homogenate to a microcentrifuge tube and add 450 µl of Plant RNA Lysis Solution, vortex vigorously and incubate at 60℃ for 5-10 minutes.
3. Add 150 µl of Protein Precipitation Solution into the lysate. Mix well and incubate on ice for 5 minutes, then spin at top speed for 5 minutes at room temperature.
4. Transfer the supernatant to a RNA Spin Filter (with yellow ring) inserted in a clean 2 ml Collection Tube. Spin at top speed for 2 minutes.
5. Transfer the flow through from the collection tube to a new 1.5ml micocentrifuge tube, avoiding disturbing and pipetting the pellet.
6. Add 1.5 volumes of RNA Binding Solution/ Ethanol Mixture to the lysate (flow through), and mix well by vortexing or pipetting.
7. Load the lysate/Binding/ Ethanol Mixture to the RNA Spin Column inserted in a 2ml collection tube, spin at top speed for 1 minute and discard the flow through.
8. Transfer the RNA Spin Column into the original collection tube and add 500 µl RNA Wash Solution I, spin at top speed for 1 minute and discard the flow through.
9. DNase I Digestion: For each isolation reaction premix 80 µl of DNase I Incubation Buffer with 2 µl DNase I in a new sterile tube (Mix by flicking or inverting the tube, do

not vortex.). Add 82 µl of the solution to the center of the RNA Spin Column membrane, and incubate at room temperature for 15 minutes.

10. Add 500 µl f RNA Wash Solution I to the RNA Spin Column, spin at top speed for 1 minute, and discard the flow through.
11. Transfer the RNA Spin Column to the original collection tube. Add 600 µl of RNA Wash Solution II, spin at top speed for 1 minute and the discard the flow through. Repeat this step once more.
12. Place the RNA Spin Column into the original collection tube and spin at top speed for 3 minutes to remove any residual ethanol. Transfer the RNA Spin Column to a clean 1.5 ml microcentrifuge tube.
13. Add 30-50 µl of Nuclease Free Water into the center of the RNA Spin Column membrane and let stand for 1 minute. Centrifuge for 1 minute at top speed and store the RNA sample at -70℃.

Acquire Concentration of RNA in samples using Nano Drop 2000 Spectrophotometer:

1. Reset machine by putting a drop of nuclease free water on the sensor then scan.
2. Drop 1 µl from each sample on the sensor and scan to calculate concentration.

qPCR (Quantitative Polymerase Chain Reaction):

This step “amplifies” or replicates the cDNA many times so the expression of the gene of interest can be calculated later on. QPCR is carried out by a thermal cycling machine which

exposes the samples to varying temperatures for varying amounts of time. This machine also monitors how many cycles it takes for the samples to be amplified to the maximum point. This information can then be used to calculate the gene expression level which was done at the conclusion of the process.

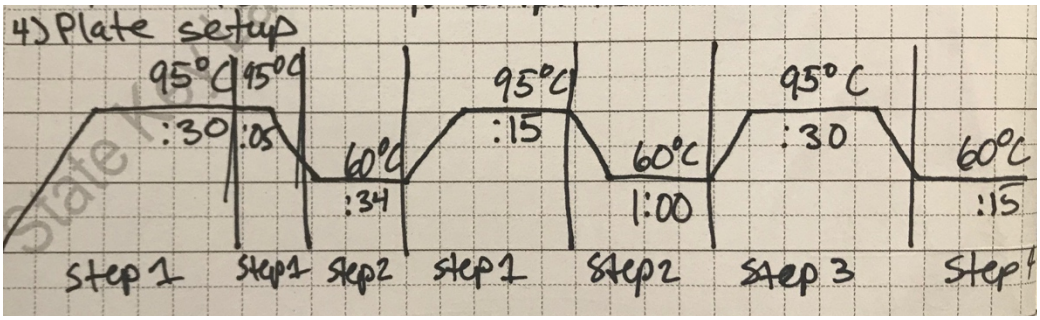
For qPCR the following mixture needs to be made-

		Gene 1	**Gene 2**
	x1	x40	x30
Water	5.1µl	204µl	153µl
SYBR- Taq (Fluorescence)	7.5µl	300µl	225µl
Primer F	.3µl	12µl	9µl
Primer R	.3µl	12µl	9µl
Dye II	.3µl	12µl	9µl
	13.5 µl+ 1.5 µl cDNA= 15 µl		

qPCR Set Up:

1. Continue/ start up
2. Advanced set up
3. 7500 fast (96 wells)
    a. Quantification- Comparative CT (ΔΔCT)
    b. SYBR Green Reagents
    c. Standard (2 hours to complete a run)

4. Plate set up-



Results

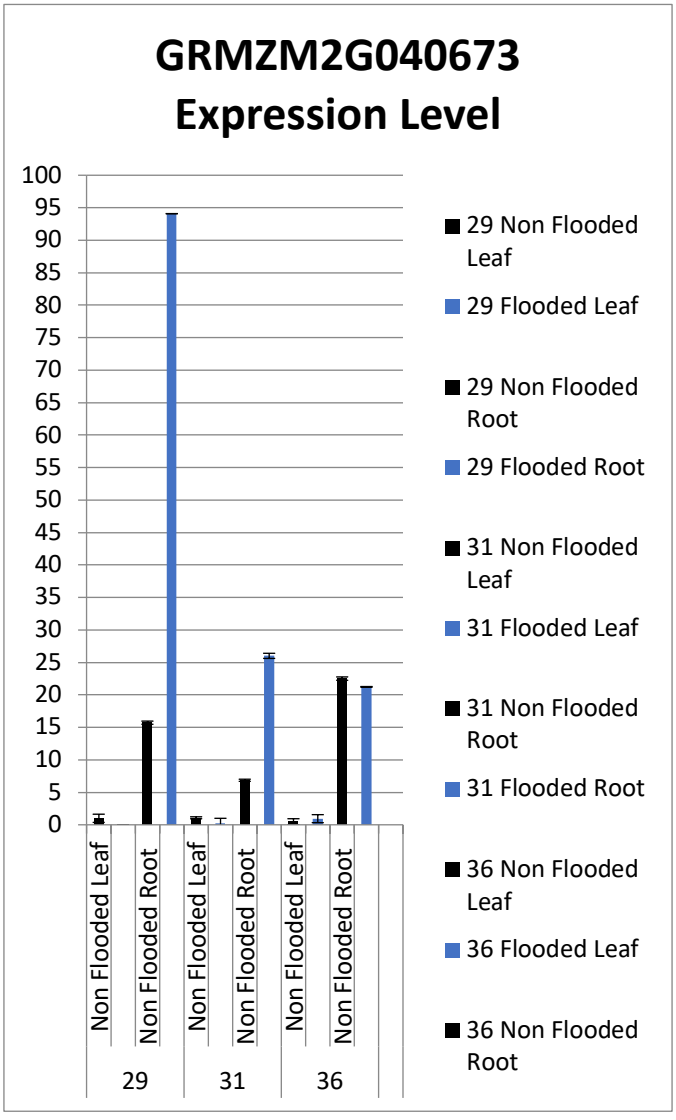
	NUMBER OF CYCLES TO REACH MAX AMPLIFICATION											
	29				31				36			
	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root
actin	25.715	und	26.776	36.516	27.082	26.692	25.918	27.406	25.584	26.802	26.623	25.07
actin	25.829	36.507	26.866	36.696	27.04	26.776	25.913	27.694	25.631	26.651	26.865	25.199
GRMZM2G040673	32.557	und	30.01	36.964	/	36.722	30.075	29.44	34.033	33.219	29.461	27.689
GRMZM2G040673	33.373	und	29.75	und	33.762	35.265	29.878	29.647	33.65	33.539	29.023	27.639
GRMZM2G040673	32.128	und	29.521	und	33.975	35.514	30.204	30.202	33.116	34.409	29.014	27.592
GRMZM2G341959	und	34.739	28.803	und	37.036	und	26.093	26.687	36.798	35.209	26.31	25.22
GRMZM2G341959	und	und	28.3	38.73	und	und	26.117	26.732	und	34.976	26.453	25.169
GRMZM2G341959	und	und	28.828	36.391	und	und	25.951	26.506	34.654	/	26.121	24.658

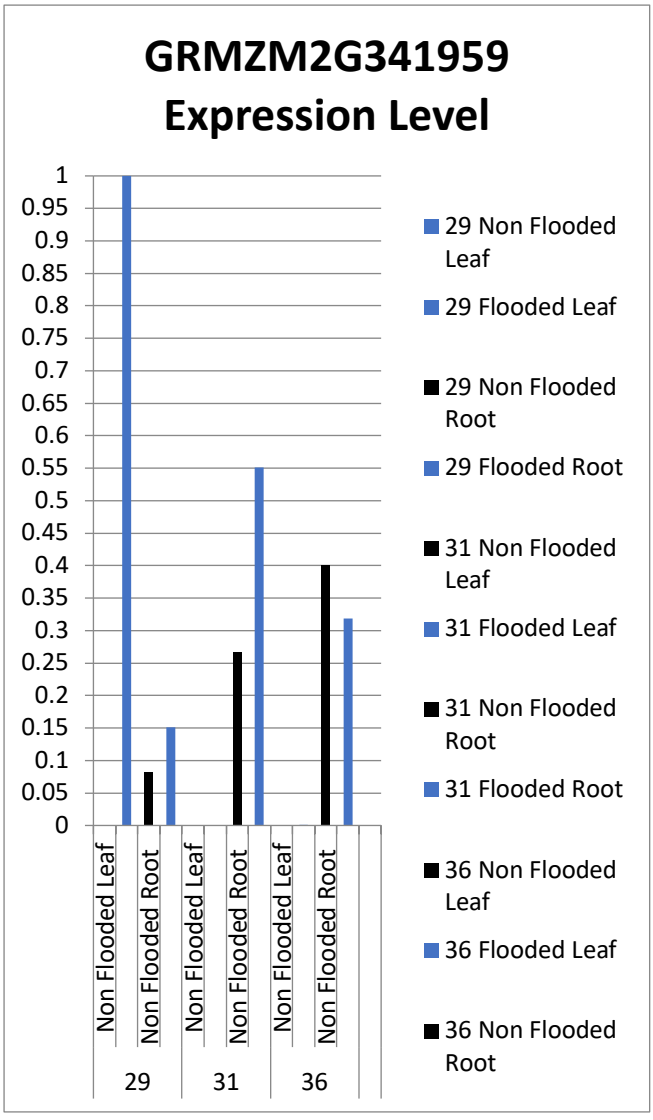
	AVERAGE NUMBER OF CYCLES											
	29				31				36			
	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root
actin	25.772	36.507	26.821	36.606	27.061	26.734	25.9155	27.55	25.6075	26.7265	26.744	25.1345
GRMZM2G040673	32.686	und	29.76033333	36.964	33.8685	35.83366667	30.05233333	29.763	33.59966667	33.72233333	29.166	27.64
GRMZM2G341959	und	34.739	28.64366667	37.5605	37.036	und	26.05366667	26.64166667	35.726	35.0925	26.29466667	25.01566667

	GENE EXPRESSION LEVELS											
	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root
GRMZM2G040673	0.008292368819 9	und	0.130368449	0.78024548	0.008927674	0.001822751	0.056844582	0.215685336	0.003927517	0.007835096	0.186597298	0.176104051
GRMZM2G341959	und	3.4058148309958	0.282697951	0.516020398	0.000993633	und	0.908673136	1.876875993	0.000899556	0.00303098	1.365409158	1.085856406

	29				31				36			
	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root
GRMZM2G040673	1.000000002	0	15.72149676	94.09198975	1.076613188	0.219810672	6.855047535	26.01009933	0.473630317	0.944856191	22.50229125	21.236888117
GRMZM2G341959	0	1.00000000003	0.08300449830	0.15151158337	0.00029174590	0.00000000000	0.26680051073	0.55107986973	0.00026412345	0.00088994266	0.40090528280	0.31882426378

	STANDARD DEVIATION											
	29				31				36			
	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root	Non Flooded Leaf	Flooded Leaf	Non Flooded Root	Flooded Root
GRMZM2G040673	0.632445255	UND	0.244663715	UND	0.150613744	0.779328129	0.164177749	0.394021573	0.460567404	0.615819238	0.255517123	0.048507731
GRMZM2G341959	UND	UND	14.32389848	21.71707739	26.18840675	UND	13.027039 3	13.32119142	20.65425403	20.26099926	13.14803643	12.5104073





Discussion

Upon obtaining all of the data required to draw conclusions on the research it became apparent that our hypothesis that the two genes of interest did in fact play a role in increasing the resistance of corn plants to water logging. The participants in the experiment were able to determine this based on the gene expression levels. In both GRMZM2G040673 and GRMZM2G341959 the expression of the respective genes in the modified plants was higher than the levels recorded in the wild type corn in water logged and normal conditions. This means that the genes of interest in both lines showed a higher expression level in the flooded samples. With this knowledge it became clear that the genes being studied could be quite useful in the fight against maize die off due to waterlogging. A lot more research on the genes is necessary to fully understand their function and the implications those functions have on the resistance of the plant and their potential use in the future. However, based on just was able to be deduced from this experiment it is possible to conceive the thought that these genes could have a bigger part to play in the future of alleviating food insecurity. If the world saw in increase the number of corn plants surviving environmental disasters such as hurricanes and tsunamis which can lead to water logging and thus the mass death of maize crops, then perhaps the issue of our growing population and diminishing food sources would see some hope. However, more research is necessary to unequivocally determine how these genes can be used to the advantage of the human race and ending hunger in the world.

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