Rice Blast Resistance in Genes: Utilizing Haplotypes of Resistance Gene *Pid3*

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Abstract

With the world's population steadily rising towards 8 billion, food insecurity remains an essential question to answer. Rice is part of this answer, as it is a staple food to more than half the world. However, rice blast (Magnaporthe orzae), a fungal pathogen, can cause total crop failure and is thus detrimental to achieving global food security. There are several resistance genes in rice that can aid in managing rice blast in rice production. One such resistance gene is *Pid3*, of which there are 40 haplotypes. Almost a third of these are nonfunctional.

The purpose of this study was to determine the presence of novel alleles within a target haplotype group of the resistance gene *Pid3*, as well as confirm that the sequencing data of the 3000 Rice Genomes Project is accurate. Through the use of dCAPS markers in PCR and sequence analysis, we found one novel allele in our target group. We also determined that the sequencing data provided from the 3000 Rice Genomes Project is in fact accurate. However, in the future, we should look to wild rice varieties rather than cultivated rice varieties.

I. Introduction

My Roots

If you had asked me ten years ago what I wanted to be when I grew up, I would have answered without hesitation: I wanted to be a veterinarian in a zoo. I always had a passion for the welfare of others, and animals were no exception. I had my entire life planned out by the time I was thirteen with no intention of changing.

This is what led me to take agriculture classes in high school, as our agriculture department offered animal sciences and advanced life courses. I never expected, though, to be quickly and eagerly sucked into the world of FFA. After finding an interest in soil sciences through the "Introduction to Agriculture" class my freshman year, I immediately joined the soils judging team. Soils judging showed me an entire world I had never experienced before, and I wanted more. I acted as president for our parliamentary procedure team, I gave a demonstration on tested nutrient content in soil, and I recited the FFA Creed at leadership contest. FFA helped me grow into the confident, passionate, agriculture-centered person I am today, and it all started with forty-inch pits in the middle of a field.

My first exposure to the World Food Prize was through a mandatory assignment for plant and soil sciences my junior year. Mr. Bowers had discovered the Indiana Youth Institute and decided to add it to our curriculum. Of course, my first reaction was not entirely positive. No one enjoys mandatory writing assignments, after all.

However, what started as just another paper soon became a passion. I spent hours upon hours researching Haiti and its need for public education. I felt restricted by five pages—how could I fit so many lives into such a short space? How could I express the lack of proper education in anything less than a novel? This passion shone through my presentation of my research at Purdue and carried me to Des Moines in October of 2015.

Very quickly I learned that my true interests lie in genetics, specifically those of plants. Every Borlaug Dialogue I attend cemented my need to feed the world through improved crops. Immediately after returning from Des Moines, I applied to the agronomy department at Purdue.

My experience in China has only confirmed that research in genetics is what I truly love doing. I still have a plan for my life, though now it centers around plants instead of animals. I aspire to carry on Norman Borlaug's legacy through improved crop production around the world. I can't imagine doing anything else with my life.

A Stranger at Home

From the moment I landed, I never felt out of place. My mentors lit up as soon as they saw me and immediately I knew this was where I was meant to be. Even at midnight, they were excited to see me and get to know me.

Image 1: At Jiuzhai Valley. From left to right: Tang Li, me, Lv Qiming.

The lab welcomed me just the same, and I was able to share a love of tea and music with people I had the pleasure of working with. Lunch was always a time filled with good food and good conversations, and I enjoyed nothing more than bonding with my now coworkers.

Perhaps the best part, though, was how easily I became a part of my mentors' families. Whether we were visiting a local museum or making dumplings together at Tang Li's house, I always felt like an adopted daughter. Their daughters became my little sisters, and I loved being their older sister. Though I lived on the research campus, I never felt alone because I had the company of my mentors through the weekends. I didn't need a host family to life with as they essentially became one for me.

II. Background

China National Hybrid Rice Research and Development Center (CNHRRDC)

国家杂交水稻工程技术研究中心
湖南杂交水稻研究中心
China National Hybrid Rice R&D Center/Hunan Hybrid Rice Research Center

The Hunan Hybrid Rice Research Center (HHRRC) was founded by Dr. Yuan Longping in 1984 in Changsha, China. In 1995, the China National Hybrid Rice Research and Development Center (CNHRRDC) was founded within HHRRC to ensure national hybrid rice cultivation and promote technology and genetic engineering in breeding programs. Dr. Yuan Longping continues to lead the center after over 62 years of experience in the field of hybrid rice breeding, and is considered the father of hybrid rice (Hunan Hybrid Rice Research Center).

The center is home to a staff of 142 people, of which 56 are senior researchers. Since its founding, the center continues to lead in the discovery of hybrid lines as well as significant scientific achievements. It has played a role in the governing of China by overseeing several

ministerial departments. Dr. Yuan himself has been given over 20 prestigious awards. The center is an important institution and continues to uphold its tradition of "developing hybrid rice for the benefit of the people of the world" (Hunan Hybrid Rice Research Center).

Necessity of Resistance to Rice Blast

Rice blast (*Magnaporthe oryzae*) is a fungal pathogen that causes lesions on the leaves, stems, peduncles, panicles, seeds, and roots of rice. It can lead to total crop failure, which is detrimental to rice farmers. As rice is the staple crop of Asia, as well as other parts of the world, this disease poses a significant threat to food security (TeBeest).

The most cost-efficient and environmentally safe way of combating this catastrophic fungus is through resistance genes. Though there are other management strategies that can be used, such as crop rotation and proper fertilization practices, the utilization of resistance within rice DNA is perhaps the most successful at truly preventing the disease (TeBeest). To date, there are over eighty resistance genes within the rice genome, of which twenty-one have been characterized (Lv 2013).

Pid3 and its Allelic Variation

The resistance gene specifically used in this study was *Pid3*. It was first discovered in the *indica* variety of cultivated rice during a comparison of *indica* and *japonica* varieties. It is located on chromosome 6 and encodes for a nucleotide-binding site leucine-rich repeats (NBS-LRR) protein made up of 924 amino acids and has no intron (Lv 2017).

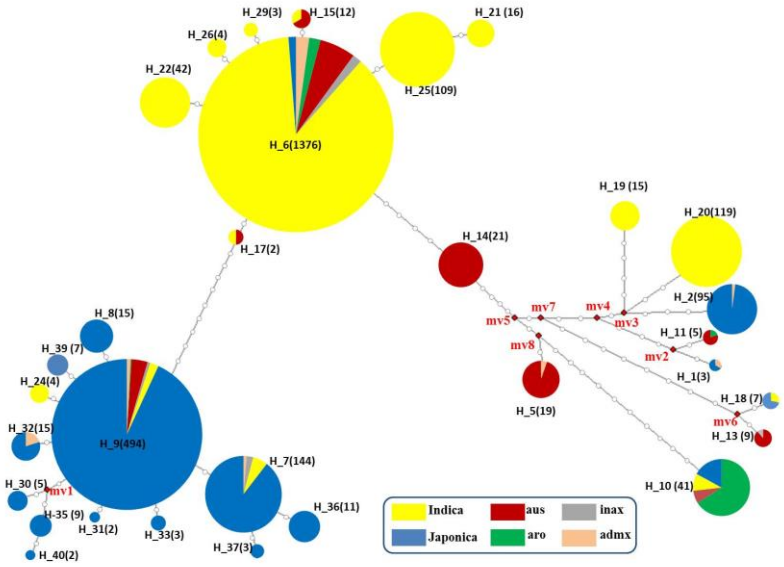

Image 2: Haplotypes of Pid3.

Using the 3000 Rice Genomes Project, 40 haplotypes were classified through 71 single nucleotide polymorphisms (SNPs) within 2621 *Pid3* alleles (Lv 2017). These haplotypes are shown grouped by genetic relation in **Image 2**.

Haplotype 20 has a known successful resistance to rice blast and thus is regarded as the original gene. The majority of *japonica* varieties contain a SNP at 2209 that causes a nonsense mutation;

thus, they are regarded as premature and nonfunctional. Haplotype 6 and the surrounding group contain a SNP at 1874 and though this does not make them premature, it does present a different resistance spectrum that has not yet been characterized. Haplotype 2, though closely related to haplotype 20, contains a SNP at 1766 that causes a nonsense mutation and thus it is also regarded as premature (Lv 2017).

III. Research and Analysis

Objectives

The research of Dr. Lv Qiming and I had two main objectives. The first was to affirm that the sequencing data from the 3000 Rice Genomes Project was accurate and able to be used in future experiments. The second was to find novel alleles of *Pid3* that presented a full resistance spectrum. To obtain this data, we used the 217 minicore subset (Agrama et al), as to reduce our sample size while maintaining the genetic diversity of rice.

Methods and Materials

To find novel alleles of Pid3, we had to first exclude two haplotype groups and one haplotype in our target group through PCR. To achieve this, we used dCAPS markers at 2209 to exclude haplotype 9 and its surrounding group, dCAPS markers at 1874 to exclude haplotype 6 and its surrounding group, and dCAPS markers at 1766 to exclude haplotype 2. I set up many of these PCR reactions, as well as electrophoresed and imaged the results. The results of the PCR performed with the dCAPS markers at 1766 are shown in **Image 3**.

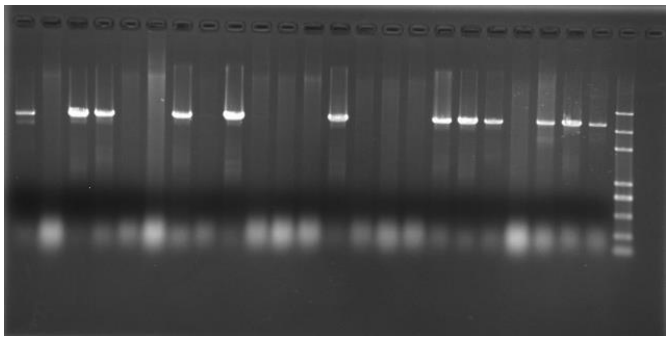

Image 3: Example PCR results. The bands represent samples with the nonsense mutation at 1766.

After removing all but our target group, we performed endonuclease reactions to confirm that the DNA contained the gene variation we were looking for before sending them in for sampling. We also used random excluded samples as controls; haplotype 9 and haplotype 2 were our negative and haplotype 6 was our positive.

We then performed sequence analysis and constructed phylogenetic trees from our results to look for similarities between known sequences and our collected sequences.

Other Responsibilities

Though much of my time was spent performing PCR and sequence analysis for our experiment, I also did other important jobs around the lab. I cultivated rice seedlings from seeds from the minicore subset to be used in resistance tests. I also propagated rice blast colonies to be used in resistance tests. In order to perform the resistance tests, we had to wait until our rice seedlings had grown at least three leaves, as well as wait until our rice blast cultures had begun to release spores. A solution of rice blast spores was sprayed on the leaves and left covered for a week before collecting results. An example of these results is shown in **Image 4**.

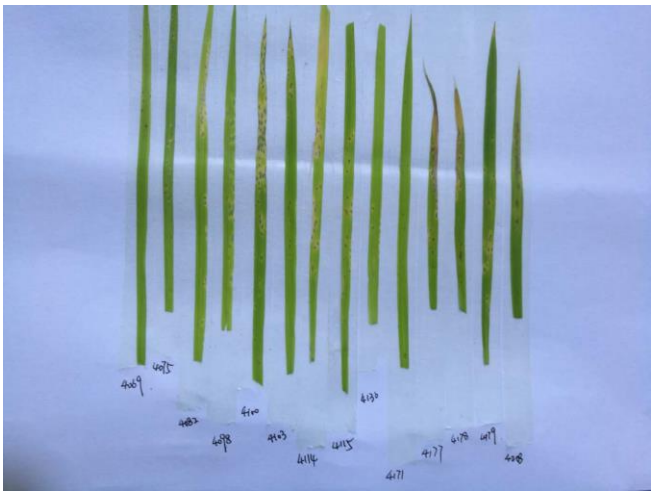

Image 4: Sample results of resistance test.

I also extracted DNA and RNA from the rice seedlings when necessary before spraying with rice blast spores.

I worked in the rice fields a few times as well, gathering rice plants and bundling them to be transported to other research fields. I also escorted my mentor, Dr. Lv Qiming, whenever he needed to check on his plots after a particularly hard rain.

Results

Our dCAPS markers were successful and we were able to remove all samples that were not a part of our target group. This also allowed us to determine which haplotype group each sample belonged to. Roughly 80% of our samples were not within our target group, as shown in **Figure 5** to the right.

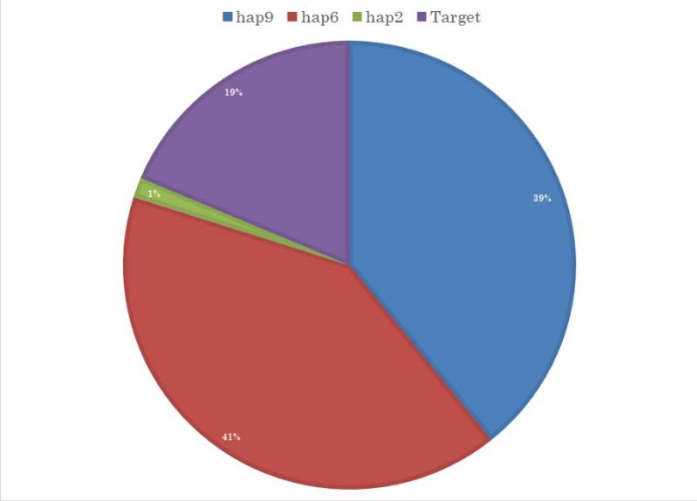

Figure 5: Distribution of samples. 39% fell within hap 9 and the surrounding group, 41% fell within hap 6 and the surrounding group, 1% fell within hap 2, and 19% fell within our target group.

From our sequencing data, we were able to construct a phylogenetic tree, shown by **Image 6**, linking previous haplotypes with haplotypes created from our data.

We were only able to discover one novel allele within our target group of *Pid3* from our samples; the rest were

genetically identical to those created from the 3000 Rice Genomes Project.

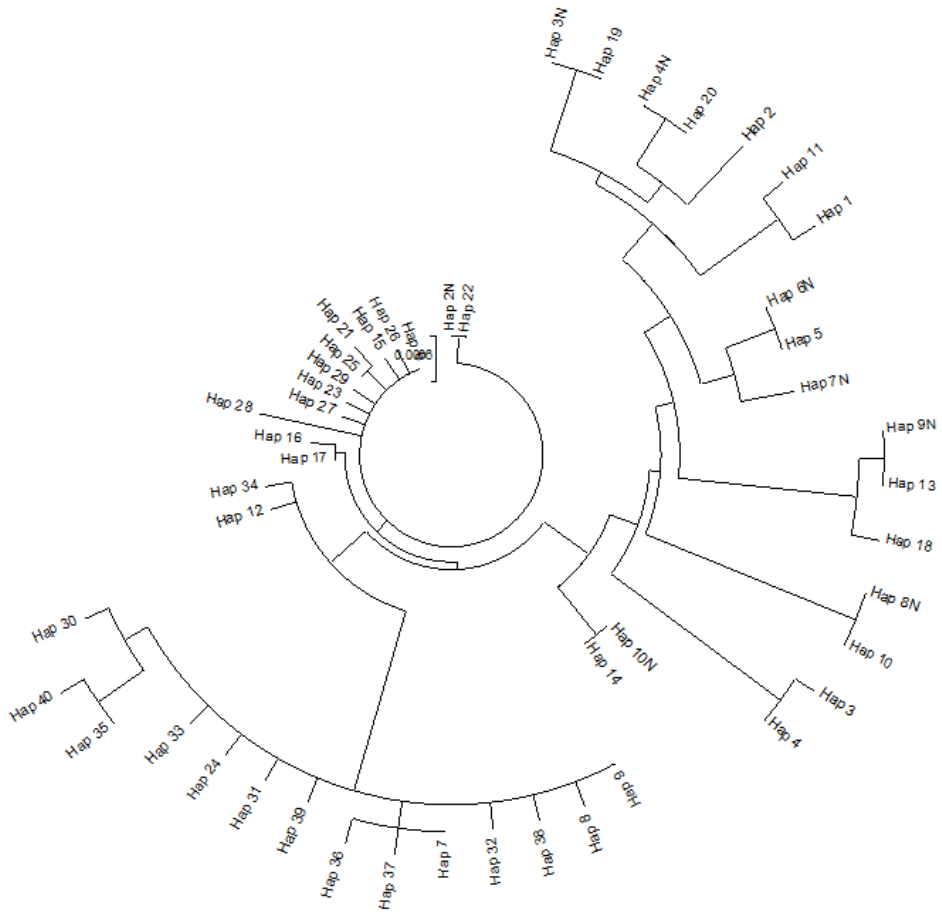

Image 6: Phylogenetic tree of new and previous haplotypes. Haplotypes created from our data are marked with an N. The only novel allele discovered is labeled as "Hap 7N."

Conclusions

From our results, we were able to come to three distinct conclusions, the first being that the sequencing data from the 3000 Rice Genomes Project is accurate. Because our sample distribution matched the haplotype group distribution, and because we only found one novel allele, it is clear that the data provided is correct. Therefore, sequences from the 3000 Rice Genomes Project can be used in the future without fear of them being a variable in the experiment.

The second conclusion is that the template used to design our dCAPS markers is highly successful. Our samples were divided into their respectful haplotype groups through PCR, and the sequences obtained affirmed their haplotyping. Thus, future experiments of this caliber can utilize this template without fear of failure.

Third, and perhaps most important, when looking to resistance genes in the future, wild rice varieties should be considered rather than cultivated rice. Because the data used contained only cultivated rice, we were unable to gather any information on the *Pid3* gene within wild rice varieties. Also, because we only found one novel allele, it is clear that most of the genetic diversity in cultivated rice has been discovered and therefore it is ineffective to continue looking for resistance with *Pid3* through cultivated rice.

IV. Personal Remarks

Through my experience, I have gained a deeper passion for plant genetics. I loved every single second I spent in the lab, and I now know that research is where I belong. Though my sights are set on Africa for my future, I will always consider China and its people a second home. The research campus was never anything but welcoming, and for that I will be forever grateful.

The question now remains: where do we go from here? The ever-present struggle of hunger in a world with a growing population weighs heavy on our minds, and it is important to look to wild plants for a solution. Though cultivated plants play a crucial role as well, wild plants hold an untapped potential in genetic diversity. Whether it be drought tolerance, disease resistance, or photosynthetic efficiency, we should look to native plants for future genetic experimentation.

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