Evaluating the *DRA* Gene in Sterile Rice Using Yeast Two-Hybrid Screening

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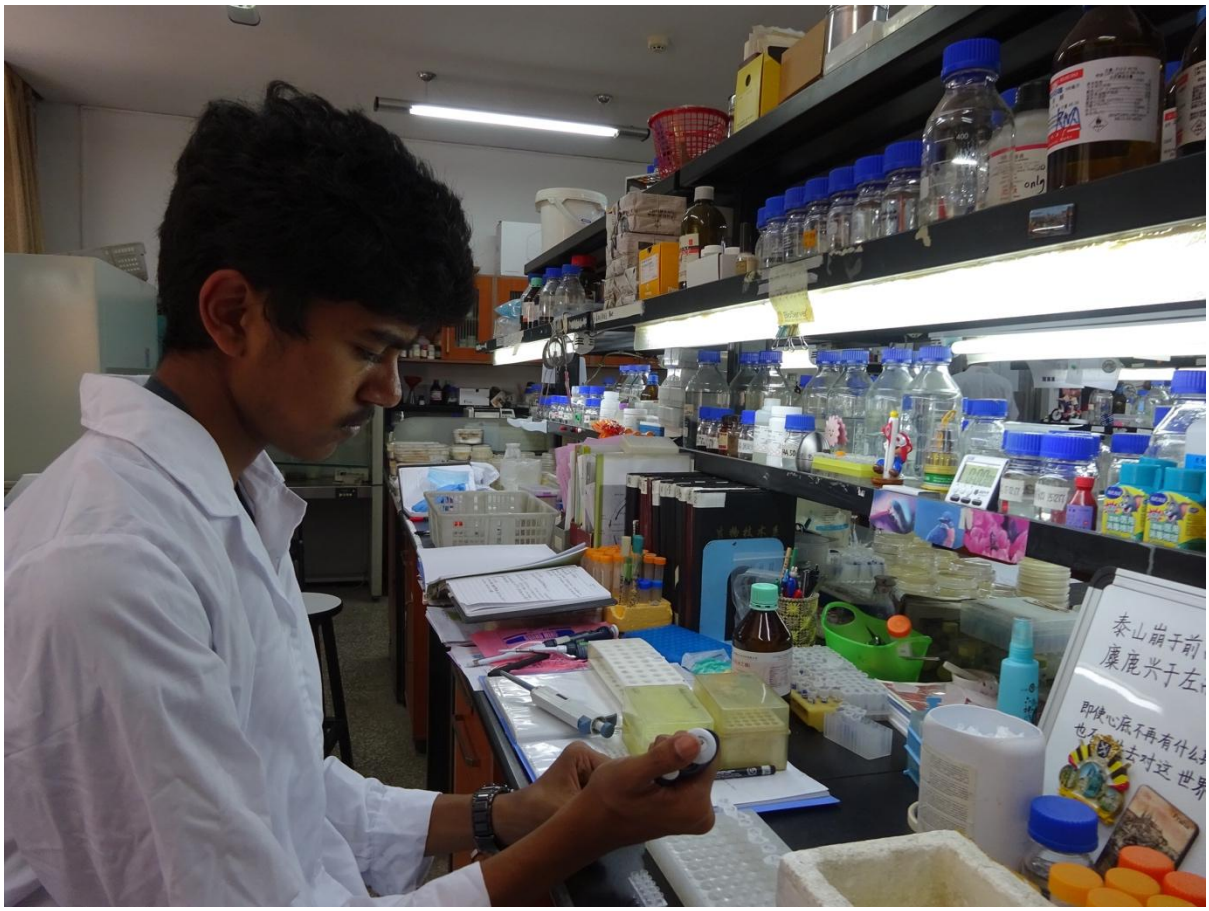

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Table of Contents

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1. Introduction

1.1 A Personal Story

As a child, I was often shuttled to India to visit the hometowns of my parents. They grew up in southern India. I grew up in southern California. Inevitably, my apparent identity as both an American and an Indian began to cause me internal conflict. I was educated in one system yet cultured in the other; I learned the language of one country, yet fed the food of another. But what I came to realize is that the dichotomy I faced brought me a unique perspective to life in both countries. My identity went from being a vulnerability to a gift, and I came to see both countries in a new light.

The summer before I began high school, I departed for India for yet another summer stay, only to find that the India I thought I knew was now different. As it turned out, it hadn't changed at all—it was I who had changed since my last trip. I was becoming more perceptive of societal problems that were impacting India. For one, I knew hunger and malnutrition was out there, and that it was simply a matter of finding it. That summer I found it alive and well in India. I was startled when I saw rampant hunger, spread hand in hand with extraordinary poverty, in parts of my hometown communities. There was no region or age group it spared; the hunger was omnipresent and brutal.

I flew back home to San Diego with a keen awareness of an issue that impacted millions, but I was still unsure what my role was, if any, in solving it. All I knew was that my experience had stirred me. In fact, it had made me profoundly uncomfortable with the extent of hunger in our world. Immediately after the school year began, my biology teacher introduced me to a program meant for students interested in the global hunger situation: The World Food Prize Global Youth Institute. The World Food Prize was offering a challenge to high school students to reflect on the following: *Confronting the Greatest Challenge in History*. With my recent experiences in mind, I immediately became interested in creating a response. With a fuel and a spark, I started writing.

Looking back, that single trip and that first essay took me on an incredible journey through the next few years. I was invited to attend the 2014 Symposium and Global Youth Institute, which brought me in contact with community that revolved around solutions to world hunger. It left me feeling incredibly inspired, and imparted me with a new understanding of this complex, global issue. Most importantly, my participation in the Symposium opened new doors for me through which I could make my own contribution. The Borlaug-Ruan Internship was one of these opportunities, giving students the chance to take part in agricultural or socio-economic research abroad. Encouraged by my time at the GYI, I decided to apply for the internship.

The next thing I know I was boarding a flight to Beijing.

1.2 Peking University

Situated in Beijing's vibrant Haidian District, Peking University is one of China's premier universities. It was founded in 1898 as the Imperial University of Peking, and has shifted to several locations around China in the last century before finally rooting itself in a western suburb of Beijing, China's capital city. Over the years, it garnered recognition as a reputed academic and research center, shaping it into a globally sought after institution for undergraduate, graduate, and post graduate studies. Today, one of China's oldest universities finds itself in the heart of modern developments. Tech companies occupy nearby offices, and subway stations now link the east campus gates to virtually every part of Beijing. Additionally, the university has partnerships with other institutions abroad, hosting international college students to study and experience life at Peking University. Together with nearby Tsinghua University, Peking University is part of a vital academic research network in Beijing

Within the university College of Life Sciences, the State Key Laboratory for Protein and Plant Gene Research is following a mission to expand our understanding of the molecular-level forces that influence different aspects of plants, from development to reproduction. The lab counts dozens of members, including graduate students, post doctorates, and professors. The two story building includes two research wings with equipment rooms for carrying out a variety of biochemistry and genetics experiments related to plant research. The lab grounds also contain several greenhouses and planting areas to grow rice and model Arabidopsis plants.

2. Background Information

2.1 Rice Development and the Role of Anthers

The development of plant organisms is dependent on the interactions between numerous factors. DNA, embedded in every cell, is transcribed and translated by cell machinery, creating proteins that regulate many cell functions. Proteins and the way they interact with one another influence the development of plant structures.

Anthers, the male reproductive structures, undergo rapid development in the early stages of rice plant growth. In proper development, rice anthers grow into straight, slender rods that contain pollen, allowing the plant to successfully reproduce when it matures. As with with

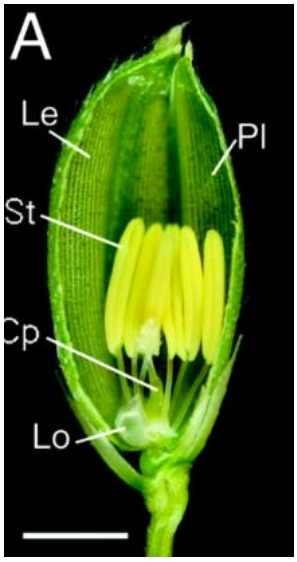

Figure 1: A close up of a rice flower. The set of yellow rods are the anthers.

most plants, rice anthers house pollen grains until they can be used to fertilize a female reproductive cell through pollination.

2.2 The *DRA* Gene

The Deformed Rice Anther gene, or *DRA* gene, dramatically alters the development of rice anthers. When expressed in rice, the plant anthers develop into physically twisted structures that contain no pollen. The disabled anthers make the rice plant sterile and prevent it from reproducing. Ongoing research at Peking University is shedding light on the specific reasons why *DRA* causes rice plant sterility. Research suggests that the protein produced by *DRA* interacts with other proteins to negatively influence anther development (see TCP genes on page 4).

2.3 *TCP* Genes

The *TCP* gene family is a set of genes involved with protein to protein interactions. In rice specifically, *TCP* genes are believed to play a role in regulating genes associated with growth. The family of genes are divided into two main classes. Research suggests that Class 1 genes promote cell proliferation and differentiation while Class 2 alters growth patterns and affects the course of development. As for their relation with the *DRA* gene, is it known that the proteins produced by *TCP* genes interact with the protein produced by *DRA*. These protein-to-protein interactions are responsible for the deformed anthers that appear downstream. Protein-to-protein interactions play a critical role in shaping plant structures, especially in development, and ongoing research is uncovering more about the connection between *TCP* and *DRA* proteins

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3. My Research Goals

The aim of this project is to **find the specific segment of the *DRA* gene that contributes anther deformities.**

DRA is slightly over 1000 base pairs long, but research has not implicated which part of the gene is directly responsible for the deformed anthers. Pinpointing the exact part of the gene involved can help us understand why *DRA* has such a dramatic effect on rice development.

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4. Procedures and Experimentation

4.1 Yeast Two-Hybrid Screening: Testing Protein-to-Protein Interactions

Given that *DRA* proteins and *TCP* protein interactions cause deformities, the crux of the project will involve a test that can detect interactions between proteins. Yeast Two-Hybrid Screening, known as Y2H, is one such test. Y2H is run using yeast cells. It works by pitting two proteins together (known as bait and prey proteins) creating a genetic "on-off" switch inside the yeast. If the bait and prey proteins can interact, the yeast cell will be able to express the reporter gene. If, however, the proteins cannot interact properly, the yeast cells cannot express the reporter gene.

We cannot see the protein interactions with our own eyes, but we can tell if the reporter gene is being expressed rather easily. The reporter gene has functions critical to the survival of yeast cells. If the protein interaction goes awry, the reporter gene will not express, and the yeast colonies will die.

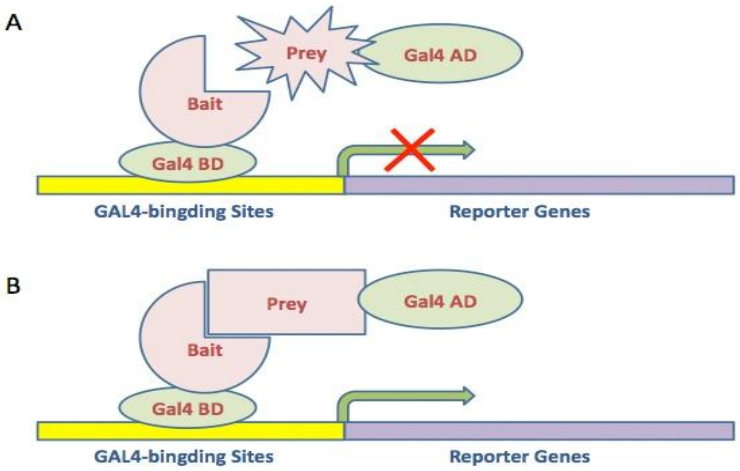


Figure 2: This diagram represents how a Y2H test works. In my project, the bait and prey system will use proteins from *TCP* and *DRA* genes. GAL4 BD is a binding domain and GAL4 AD is activation domain. If the bait and prey interact successfully, the BD and AD connect and the reporter gene is expressed. If the protein interaction is improper, the BD and AD do not couple, and the reporter gene will not be expressed.

In my experiment, a *TCP* gene will produce the bait protein. The variable will be the prey protein, which is to be produced by a different section of the *DRA* gene in each test. Testing each section one by one and running the Y2H test on each fragment will allow me to catalogue all the successful and unsuccessful yeast colonies. When a segment leads unsuccessful yeast colonies, it will indicate a dysfunctional protein interaction occurred. Such improper interactions, when in rice, may contribute to the anther deformities.

The bulk of the project is dedicated to preparing *DRA* for this Y2H test. The test is relatively straightforward, but preparing *DRA* requires time and the completion of several experiments. In the following sections, I will overview the procedures and steps used in preparation for the Y2H testing.

4.2 Preparing *DRA*

After settling on the project idea, my mentor Dongshu Guo introduced me to VECTOR NTI, a computer program created by ThermoFisher Scientific for gene sequence analysis. For our project, we decided we were going to divide the *DRA* gene into 5 different segments, with each segment totaling 210 base pairs long. Using VECTOR NTI, we designed forward and reverse primer sequences, which are small segments of DNA capable of producing segmented sections of DNA. Since the sequence of *DRA* has been digitized, we can refer to the the sequence using the software, allowing us to design new primers accurate to the base pair level. Once designed, these primers can be constructed by a local biotechnology company and delivered to the lab.

Within the lab grounds, several greenhouses and growing areas provide space to grow samples of rice plants that express *DRA*. In order to run the Y2H test I had to collect samples of these plants and isolate DNA from the plant cells en masse. To do this, I followed a DNA extraction protocol. The steps are listed by number, with comments written in light blue.

DNA Extraction Protocol for Rice Plants

1. Using a piece of tape, label the stem of a sample plant. Gently tear approximately an inch off the top of the same plant, and place the sample into an EP tube. Label the tube with the same number used on the label. Repeat as needed. This means our sample contains a substantial amount of cells and contains plenty of genetic material. Tearing from top of a leaf ensures the sample includes the most active areas of cell proliferation in the plant.
2. Prepare the following: a small container of liquid nitrogen, electric drill, and several clean crushing tips.
3. Insert and tighten the drill bit using the drill-specific wrench.
4. Open an EP tube and leave the plant sample inside. Using a pair of tweezers, grab the tube and immerse it in liquid nitrogen. This rapidly freezes the sample, making it incredibly brittle and easy to crush.
5. Place the drill bit into the tube, so as to crush the fracture the plant material. Turn on the drill and crush the sample thoroughly.
6. Add 600 µl of CEG Buffer into the EP tube. This begins the process of separating cell material away from the DNA.
7. Re-run drill. Ensure crushed sample debris is mixed well with CEG Buffer. Close EP Tube lid.
8. Return to step 3 and repeat for all samples.
9. Place all EP Tubes with samples in a 65-degree Celsius incubator for 30 minutes. Every 10 minutes, flick and invert tubes. This exposes as much of the plant material to the CEG Buffer.
10. Add 600 µl chloroform/isoamylol. This is to destroy proteins in the sample.

11. Centrifuge 12,000 RPM for 20 minutes. This separates cell debris from the DNA
12. Remove from centrifuge and pipette the top 400 µl of fluid from the solution into new EP tubes.
13. Add 400 µl of isopropyl alcohol to the tubes. Wait 20 minutes, flick all tubes, then centrifuge for another 15 minutes.
14. Following centrifugation, remove the fluid. The DNA will be pelleted at the bottom. Wash with ethanol, and centrifuge again if needed.

Taking care of the rice plants in the outside garden

Collecting samples from various rice plants

Using the drill to crush frozen rice samples

This protocol helped isolate DNA for future experimentation. With the DNA isolated, I was free to modify, cut, and insert the DNA in future parts of the project.

4.3 Creating Fragments and Verifying Lengths

The custom designed primers described in section 4.2 can be used to cut the *DRA* gene into specific fragments. Having isolated all the plant sample DNA with the DNA extraction protocol, the primers can be applied to the DNA, and specifically the *DRA* gene within it. With Polymerase Chain Reaction (PCR) technology, primers are used in conjunction with the plant DNA to synthesize fragments of the *DRA* gene. The size of the fragment depends on the primer used in the PCR machine. PCR works by synthesizing, then duplicating a specific strand of DNA. As the number of copies grows exponential, PCR can produce millions of copies of a DNA segment within a few hours.

After running the PCR with various primers and letting the PCR cycle, the samples now contained millions of copies of each differently-sized *DRA* gene fragment. As expected, even with millions of copies of the gene in the tube, it is impossible to see DNA with the naked eye. Fortunately, there is an easy way to visually verify the lengths of the DRA segments. Agarose Gels technology is a tool used frequently in the lab for separating DNA strands out by length. Composed mostly of water and a seaweed extract, agarose gels are straight forward and easy tools to work with.

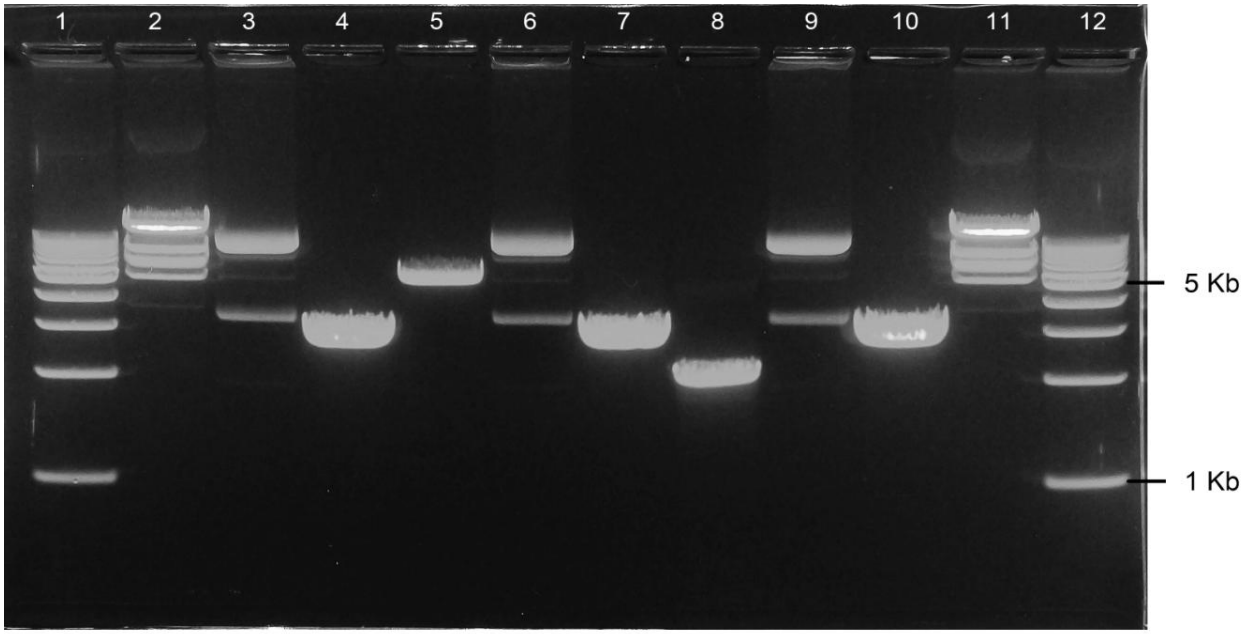


Figure 3: A sample of what an agarose gel looks like under UV light.

The top of an agarose gel contains a row of wells in which DNA, in small amounts, can be placed in. The gel, with DNA loaded, is placed into a setup where an electric current runs through it.

Since DNA has a net negative charge, it gravitates towards the positive end on the opposite side of the gel. As the DNA tries to push itself through the gel material, the shorter strands will have an easier time migrating downwards than the bigger strands will. With time, the strands separate themselves out. The DNA can be run using special buffers that will illuminate the DNA under UV light, making it easy to visually check the lengths of the DNA.

In this project, agarose gels were routinely used at various points to confirm the lengths of DRA fragments.

4.4 Preparing for Yeast Two-Hybrid Testing: Integration into TOPO Vectors and Transformations

Most project time was devoted to preparing the *DRA* gene fragments for Yeast Two-Hybrid testing (section 4.1). While Y2H is a relatively straightforward test, preparing the *DRA* gene fragments for it were not. The first step in preparation was to integrate each fragment into what is known as a TOPO Vector.

TOPO Vectors are circular strands of DNA that can easily be absorbed by competent cells. Placing the *DRA* fragments into vectors makes it easy to insert the fragments into living cells. Since cells have the natural mechanisms to copy DNA, we can harness the power of the cell to make more copies of the *DRA* fragment.

After following a protocol to fuse *DRA* fragments en masse into TOPO vectors, the fragments were ready to by taken up by cells, which in this experiment were specifically *E. Coli* bacteria. The process of inserting the TOPO Vectors + fragments into *E. Coli* is called transformation.

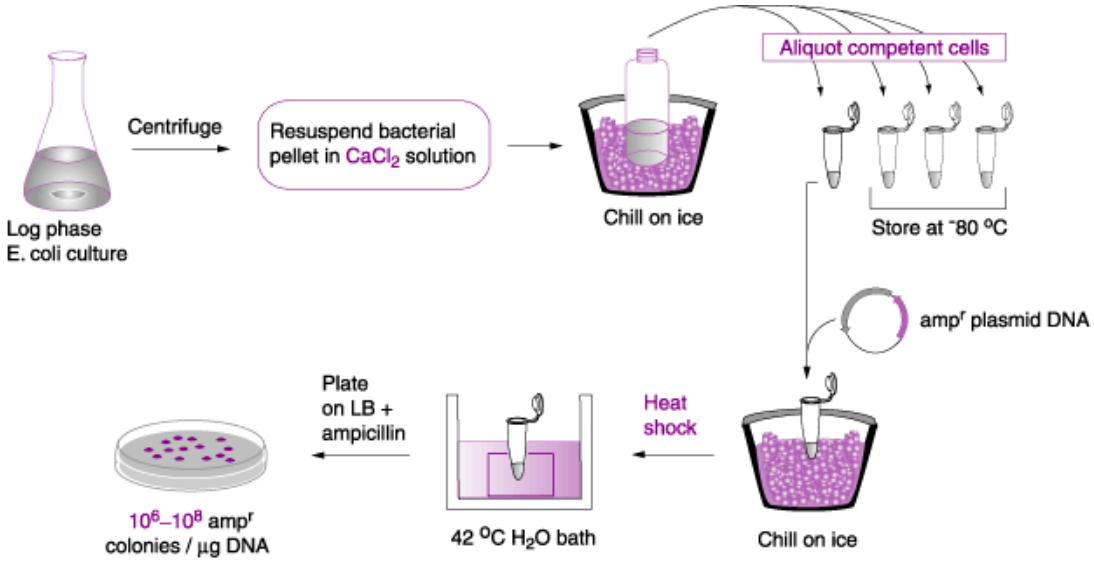


Figure 4: A diagram for the steps of transformation.

E. Coli cells were kept in a vial at a cool temperature. When the mixture containing the TOPO Vectors + fragments was placed into the vials, the vial was quickly transferred to tub of hot water, before being put on ice again. This process is called a heat shock, and the shock is designed to

Pouring an Agarose Gel

get *E. Coli* to accept the TOPO vectors + fragments.

To encourage the *E. Coli* to grow, the transformed *E. Coli* was dropped into a flask with nutritious media. The flask is placed in a shaking machine overnight, so as to give ample time for the *E. Coli* to multiply.

4.5 Preparing for Yeast Two-Hybrid Testing: Plasmid Extractions, LR reactions and Transforming into Yeast.

The flasks containing the *E. Coli* were removed from the shaking machine and the *E. Coli* is filtered out suing a protocol. A plasmid extraction protocol is used to remove the TOPO Vectors + fragments from the *E. Coli.*

1. Label small Eppendorf (EP) tubes
2. Shake each flask gently. Pipette 1400 µl of flask media into each tube.
3. Centrifuge EP tubes at 13,000 RPM for 1 minute. This allows the *E. Coli* to pellet at the bottom of the tube.
4. Pour out the top layer of fluid, leaving only the *E. Coli* pellet at the bottom. Pipette another 1400 µl of flask media. Make sure the tubes are filled from the same exact flask used in the first round.
5. Add 250 µl of Buffer P1 to each EP tube. Vortex each tube thoroughly.
6. Add 250 µl of Buffer P2. Invert each tube 6-8 times. This mixes the new buffer in the sample. Be gentle while mixing.
7. Add 350 µl of Buffer N3 to each tube. Mix immediately, but gently. This mixes the new buffer in the sample. Since the cells are starting to break apart, be extremely careful not to shake the tubes too hard.
8. Centrifuge 12,000 RPM for 10 minutes
9. Take out column tubes, and label each tube.
10. After centrifugation, slowly take the EP tubes out from the centrifuge. Pipette as much fluid as possible into each column tube. The centrifuge condensed all the cell debris on the bottom of the tube. Try not to disturb the pellet when pipetting out the fluid.
11. Centrifuge the column tubes for 1 minute. The column tubes have a filter in the middle of the tube. The TOPO Vectors + fragments should get caught in the filter, but the liquid material should flow through.
12. Dump the flow through. Add 650 µl of Buffer PW. This is simply to wash the DNA caught on the filter.
13. Centrifuge for 1 minute.
14. Dump the flow through. Centrifuge again for anther 2 minutes.

15. Dump any flow through and remove the bottom half the tube. Leave the top half of the tube out on the table and open their caps. Let the tubes dry for 10 minutes. This helps remove any remaining fluid from the tube.
16. Pipette around 50 µl of water into the tubes. Centrifuge 1 minute.

Together, transformations and plasmid extractions were very important parts of the project. The *DRA* fragments were integrated into a TOPO Vector for a greater part of the project. Transformations and extractions allowed *E. Coli* bacteria to copy and store these TOPO Vectors + fragments.

After the initial transformation and extraction, the TOPO Vectors + fragments were checked using Agarose gels. If the gel results fit, the next step was to begin LR Reactions and transformation into yeast.

LR Reactions are fairly straightforward. The purpose of an LR Reaction is to move the *DRA* fragments from TOPO Vectors to a new "destination" vector. Switching the fragments into a new vector is necessary before running the Y2H test at the end of the project.

LR Reaction Components

1µl Destination Vector
1µl TOPO Vector + fragment
1µl LR Clonase
2µl dd H_2O

Preparatory Steps

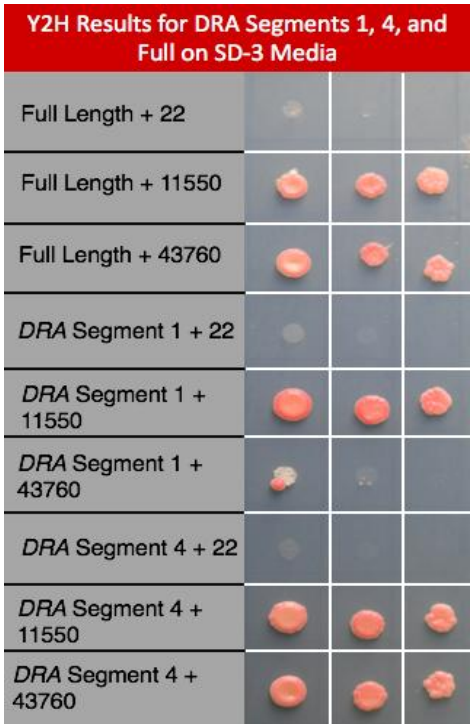
1. Centrifuge each tube for a moderate amount of time.
2. Place the tubes in a 22 degree Celsius fridge until 8 PM, regardless of start time. At 8 PM, the fridge with Yeast Cell is open.

After preparing the LR Reaction, a protocol was followed to chemically switch the fragments from one vector to the other.

The final step in the run up to the Y2H Test was to transform all the components into the sample yeast cells. The transformation process is similar to that seen in Figure 4 of Section 4.4, but diverges in the final steps. Using this specialized protocol, all the necessary fragments, Vectors, Binding domains, and Activating Domains were inserted into yeast cells. At that point, the Y2H test could be conducted.

5. Results

In the tables to the below, the pink disks are yeast colonies. The presence of a yeast colony indicates that the protein interactions in those yeast cells were successful. If little or no colonies are present, it signifies that protein interaction were abnormal. Therefore, the colonies with little or no growth are of particular significance.



Key:

Full Length: Uses the protein from the full length *DRA* gene.
***DRA* Segment 1:** *DRA* gene base pairs 1-210.
***DRA* Segment 2: :** *DRA* gene base pairs 1-420
***DRA* Segment 3: :** *DRA* gene base pairs 1-630. Not shown in tables, currently in testing.
***DRA* Segment 4: :** *DRA* gene base pairs 1-840.
***DRA* Segment 5: :** *DRA* gene base pairs 1-1050.
11550: *TCP* Protein Sample 1
43760: *TCP* Protein Sample 2
SD-2 and SD-3: Yeast colony media missing 2 and 3 essential amino acids respectively.

6. Analysis and Conclusions

At this point in time, 4 of the 5 original gene fragments have been evaluated. The final fragment, *DRA* segment 3 ("R3"), is currently undergoing testing. As the data for R3 becomes available, a better picture of the results will form.

Despite this fact, there are still intriguing conclusions that can be drawn from the data already available:

1. **Since *DRA* segment 2 ("R2") and *DRA* segment 4 ("R4") are both successful, the anomalous section of the gene is likely not anywhere in the first 420 base pairs of the gene.** Although R3 data is still unavailable, the success of the R4 fragment allows for inferences despite the lack of R3 data. Since the R4 fragments succeeded, the R2 fragment that cover the first part of the gene are confirmed to play no role in influencing the deformed rice anthers.

2. **When testing the same fragment with two different *TCP* gene proteins (11550 and 43760), the same fragment may fail one test while passing the other, suggesting different parts of the gene interact with different proteins**. This is an intriguing development on the role of the *DRA* gene. The *DRA* gene segment 2 data on SD-3 media (Page 14, top right table) demonstrates this conclusion. While the segment successfully interacted with one *TCP* gene protein (11550) it did not do so with the other (43760). *TCP* gene proteins are critical for plant development. Knowing that different parts of the *DRA* gene interact with different *TCP* proteins may change the defined role of the *DRA* gene in plant development. Therefore, each part of the *DRA* gene has its own role in influencing plant developments.

The data suggests that the successful development of rice plant anthers is heavily contingent on the *DRA* gene. Although some parts of the *DRA* gene have not been implicated in causing the deformed anthers, all parts of the *DRA* gene have the ability to shape development. The project reaffirms the need for further research on the *DRA* gene to completely illuminate all the biochemical pathways that influence the initial days of plant structure development. The proper growth of plant reproductive structures shapes the life of a plant, and influences its likelihood of propagation.

The significance of this project lies in the insights it tries to create on plant reproductive structures. Exploring the role of specific *DRA* gene segments fits into a bigger picture challenge of understanding failures in plant development. In the real world, plant reproductive issues have a significant role in influencing crop yields and overall agricultural productivity.

7. Reflection

For two months I was immersed in the Beijing bustle, and I found myself enjoying the faster pace of life. My residence in China was firmly planted in the middle of the metropolitan area, and though I was coming from a fairly large city in the US, I still had to adapt to a new way of life. Even in the slower summer months, Peking University was filled with students and tourists.

Occasionally, on the walk between the lab and my dorm, I would be approached by a lost tourist wandering the campus. The would come up to me and ask something in Chinese, but unfortunately, I only understood a few words of the language. As I was approached more and more every week, I started to pick up on some of the words they used. Almost all questions would start off with the phrase "Beida Ximen". I asked Dr. Kang about these tourists, and he laughed when he heard about my amusing situation. She explained that "Beida" was a nickname for the Peking University and "Ximen" meant "West Gate". Beida Ximen was Peking University's famed west gate—a land mark I happened to pass every day for lunch and dinner. After that, whenever a tourist asked me a question, I always listened for that phrase. If I heard it,

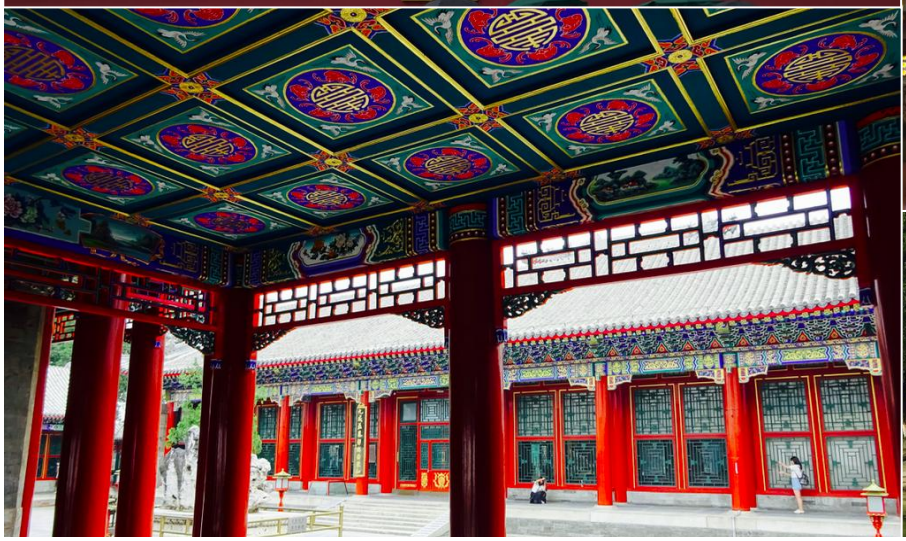
I just had to point west!

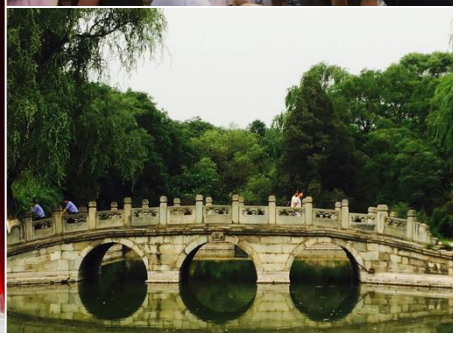
Above: Beida Ximen, the liveliest spot on campus.

As an Indian-American, I stood out practically everywhere I went. Frequently, students in my dorm would ask me where I was from, and in one memorable instance a young girl asked to have a photo with me when I visited the Summer Palace. Before coming to China, I thought being an outsider would make me uncomfortable. Instead, it became a great way to start a conversation, meet new people, or learn something new about China.

Sundays were reserved for exploration and adventure. Accompanied by my mentor and lab members, I would venture past the campus walls and truly discover Beijing. Sometimes the experiences were enjoyably simple, like buying local tea, and sometimes the experiences were ones I will treasure forever, like standing atop the famed Badaling section of the Great Wall of China.

8. Gallery



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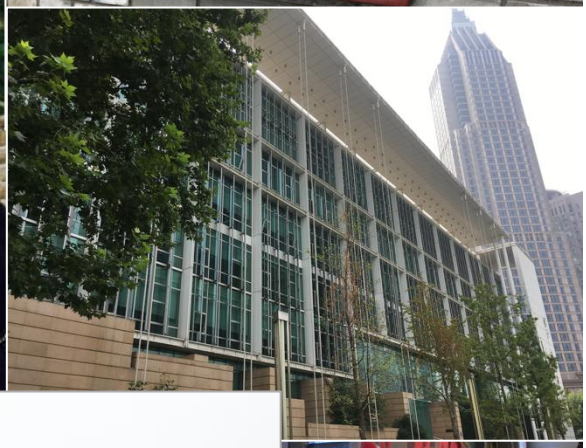

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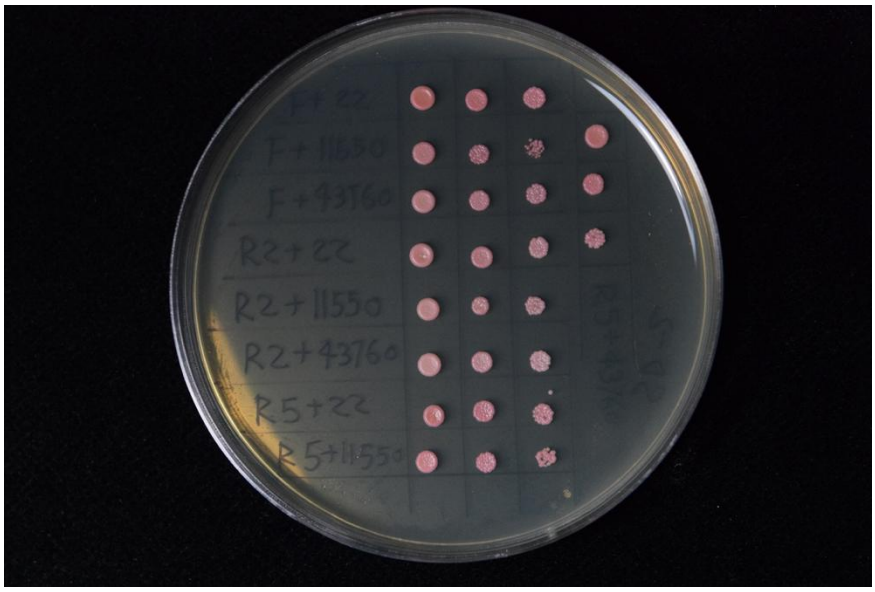
9. Appendix

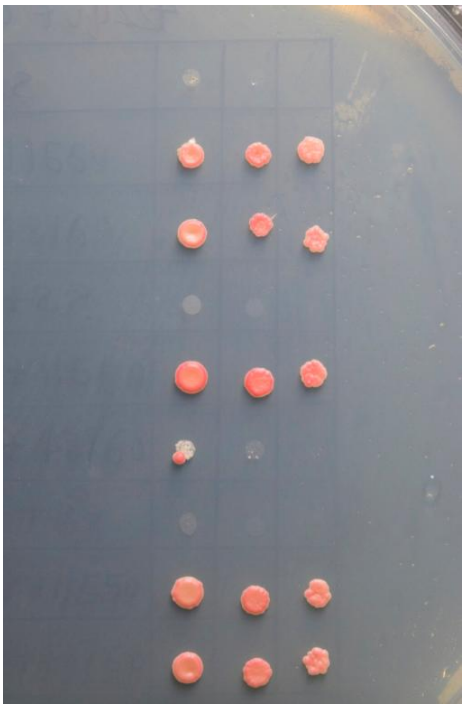
Table 1. *Arabidopsis* and rice TCP gene nomenclature

Identifier (*Arabidopsis*)	Name[a]	Alternative name	Refs (alternative name)	Type	Identifier (rice)	Name[b]	Alternative name	Refs (alternative name)	Type
At1g67260	*AtTCP1*			CYC/TB1	Os04g11830	*PCF1*	*OsTCP15*	[5]	PCF
At4g18390	*AtTCP2*			CIN	Os08g43160	*PCF2*	*OsTCP23*	[5]	PCF
At1g53230	*AtTCP3*			CIN	Os11g07460	*PCF3*	*OsTCP19, OsTCP26*	[5,14]	PCF
At3g15030	*AtTCP4*	*MEE35*	[83]	CIN	Os01g11550	*PCF5*	*OsTCP1*	[5,14]	CIN
At5g60970	*AtTCP5*			CIN	Os03g57190	*PCF6*	*OsTCP8, OsTCP14*	[5,14]	CIN
At5g41030	*AtTCP6*			PCF	Os01g55100	*PCF7*	*OsTCP4*	[5]	CIN
At5g23280	*AtTCP7*			PCF	Os12g42190	*PCF8*	*OsTCP22, OsTCP29*	[5,14]	CIN
At1g58100	*AtTCP8*			PCF	Os03g49880	*OsTB1*	*OsTCP13*	[5]	CYC/TB1
At2g45680	*AtTCP9*			PCF	Os01g41130	*OsTCP3*			PCF
At2g31070	*AtTCP10*			CIN	Os01g55750	*OsTCP5*	*OsTCP2*	[14]	CIN
At2g37000	*AtTCP11*			PCF	Os01g69980	*OsTCP6*	*OsTCP3*	[14]	PCF
At1g68800	*AtTCP12*	*BRC2*	[32]	CYC/TB1	Os02g42380	*OsTCP7*	*OsTCP4*	[14]	PCF
At3g02150	*AtTCP13*	*PTF1, TCP10*	[67,69]	CIN	Os02g51280	*OsTCP9*	*OsTCP5*	[14]	PCF
At3g47620	*AtTCP14*			PCF	Os02g51310	*OsTCP10*	*OsTCP6*	[14]	CIN
At1g69690	*AtTCP15*			PCF	Os02g58180	*OsTCP11*			PCF
At3g45150	*AtTCP16*			PCF	Os03g30880	*OsTCP12*			PCF
At5g08070	*AtTCP17*			CIN	Os04g44440	*OsTCP17*	*OsTCP10*	[14]	PCF
At3g18550	*AtTCP18*	*BRC1, TBL1*	[32,33]	CYC/TB1	Os05g43760	*OsTCP18*	*OsTCP11*	[14]	CIN
At5g51910	*AtTCP19*			PCF	Os06g12230	*OsTCP19*	*OsTCP12*	[14]	PCF
At3g27010	*AtTCP20*			PCF	Os07g04510	*OsTCP20*	*OsTCP13*	[14]	CIN
At5g08330	*AtTCP21*	*CHE*	[64]	PCF	Os07g05720	*OsTCP21*	*OsTCP14*	[14]	CIN
At1g72010	*AtTCP22*			PCF	Os08g33530	*OsTCP22*	*OsTCP15*	[14]	CYC/TB1
At1g35560	*AtTCP23*			PCF	Os09g24480	*OsTCP24*	*OsTCP17, REP1*	[14,30]	CYC/TB1
At1g30210	*AtTCP24*			CIN	Os09g34950	*OsTCP25*	*OsTCP18*	[14]	PCF
					Os12g02090	*OsTCP27*	*OsTCP20*	[14]	CIN
					Os12g07480	*OsTCP28*	*OsTCP21*	[14]	PCF

The right half of Table 1 lists all the TCP genes found in rice. Items highlighted in blue are the TCP genes used in testing *DRA* fragments for this project.



Raw results from Y2H Testing on full Length *DRA*, R2 and



Raw results from Y2H Testing on full Length *DRA*, R1 and R4.