

ATSAF - CGIAR++ Junior Scientists Program Final Report

Name of the student: Namratha Pavuluri

University: Christian-Albrechts-Universität zu Kiel

Supervisor at University: Prof. Dr. Christian Jung

International Agricultural Research Center: International Maize and Wheat Improvement Centre (CIMMYT)

Country: Mexico

Supervisor at IARC: Dr. Susanne Dreisigacker

Start and end date of stay at IARC: 03 May 2023 to 10 Sept 2023

Title: Validation of QTLs for stem rust resistance in durum wheat

Funded by the German Federal Ministry for Economic Cooperation and Development (BMZ)







A long time ago in a galaxy far, far away....

At the danger of sounding like a cliche, let me tell you, it was my dream to work at CIMMYT, ever since I read about it in one of my undergrad classes in India. The ATSAF program provided me with the initial step towards realizing this dream. After arriving in Germany for my master's studies, I actively sought internship opportunities. With CIMMYT at the forefront of my mind, I wrote an email to Dr Ravi Singh (about whom I read being the most influential Indian in Wheat breeding) asking about an internship at CIMMYT. He informed me that CIMMYT wasn't allowing visiting students due to the COVID-19 pandemic. He forwarded my résumé to his colleagues and one of them contacted me asking if I was interested in doing a virtual internship. That person was Dr Susanne Dreisigacker, Head of Wheat Molecular Breeding Lab, who would guide me through the next 18 months and who would later become my master thesis supervisor. The internship was on the topic of "Evaluation of gene-based molecular markers in CIMMYT spring bread wheat germplasm".



During the 5 months of my internship, I came to know about the foundation of CIMMYT and how it works in collaboration with scientists from all around the world. After I finished my internship, I started a thesis at my university. I couldn't cope with the pressure and had to drop my thesis. Now, I was back to square one again and started searching for thesis projects at various research institutions. I got back in touch with Dr Susanne and asked if she had any thesis topics. But I was still worried about my funding if I had to move to another country. After a month, Susanne told me she had two topics we could work on (Fine mapping of stem rust resistance genes in durum wheat and validation of genomic regions associated with rust resistance in wheat applying a QTL atlas) and about the ATSAF fellowship. Now, I began writing the research proposal, it went back and forth many times between Susanne and me, that it

became the meme ("Final".doc). I know it's a bit exaggerated, but I have never written something like that before. It was very kind of Susanne to push me to revise the document several times, giving me the necessary input and correcting the document till it was up to the standard.

Now came the shocker that the JSP program was ending, and I became sceptical again. I wrote to Sabine Baumgartner, who encouraged me to apply for the fellowship. After arranging my visa, I was finally going to Mexico.

In the Land of Aztecs

My first impression of Mexico was how similar it looked to India, I felt at home. Dr Susanne arranged CIMMYT transport to pick me up at the airport. After travelling for almost 18 hours, it was a relief to finally reach my destination. The next morning, with a severe jetlag, I managed to drag myself from the bed and get



ready for the day. I met Karen (Dr Susanne's assistant) first and she gave me small tour of CIMMYT, all the various facilities etc. Then I met Dr Susanne, and she is one of the kindest souls I have ever met. We started off with catching up on my journey and then we came to the topic of what I am to do in my thesis project, and she sent me some datasets to begin with. By 2pm, I was already tired and that was how my first day at CIMMYT ended.

CIMMYT has seminars every Monday and Susanne invited me to the first seminar and introduced me to other scientists and to the programme director. Now, the seminar starts. And let me tell you, I have never felt so stupid in my life as I felt that day. All these scientists and other students, they understood what was being said and raising questions and here I was feeling dumb. I feel everyone has experienced this at least once in their life, am I the stupid one or all these other people geniuses. After the seminar ended, I went to speak with Dr Susanne. I asked her if she understood everything when she was my age. She just smiled and said "I know how you feel and no, I did not know everything in my masters. You will gain knowledge and understanding little by little, don't worry about such things." It gave me an immense boost to my self-confidence.

The lab: where the magic happens (or not)

After gathering literature and designing my KASP markers, it was time to put the knowledge I gained to practice. This is the place where I met my second mentor at CIMMYT, Yoloxochitl (lab technician in WMB group). Coming from India, I didn't have much lab experience. She taught me how to use various instruments and how to interpret the results. I have never worked with a multi-pipette before or with such large sample volumes before. It was a learning experience. The only issue was communication as she doesn't speak much

English and although I knew a bit of Spanish (thanks to Enrique Iglesias) before coming to Germany. But only now I was thinking in German rather than Spanish. With my limited Spanish and her limited English, we agreed upon the scientific methodologies. It is quite challenging as science is all about small details and precision. This taught me another important lesson; science doesn't have linguistic boundaries. I gained a lot of knowledge from all the lab-technicians in WMB lab -Yolo, Claudia, Adrianna, Alma, Aldo, Silverio, Fernando and Maria.



Fig: WMB team at CIMMYT



Preliminary Results

My thesis project includes two chapters: using KASP markers on a biparental population and on a multiparent AMPAN population.

I validated the stem rust resistance QTLs which were discovered previously in durum wheat biparental population using KASP markers. Three QTLs on chromosomes 3B, 4B, and 7B were discovered, out of which, two are minor



QTLs and 3B QTL is a major one. They were monitored in 4 different locations - 2 Kenyan environments and 2 Ethiopian environments. All the QTLs were found to be consistent and significant across environments. Then, I used rTASSEL (R package) to perform a GWAS (genome-wide association studies) and to develop BLUPs from the phenotypic data which gives us the estimates for selecting the lines. Finally, I used IciMapping (tool from CIMMYT) to construct a linkage map which fine maps the QTLs. I calculated the additive effects of all 3 QTLs and determined if they are useful to be introgressed or not.



Consecutively, I used the KASP markers in AMPAN (Association Panel)/elite CIMMYT germplasm and found that only 4B QTL is present while others were absent. As the 3B QTL is a major QTL, it should be introgressed into the CIMMYT durum wheat germplasm. Using the GBS data from the previous studies, I fitted a mixed linear model (MLM) on the data using rGAPIT (and FarmCPU method) for visualising the significant QTLs. I also compared various methods -GLM, MLM, FarmCPU to see which one is more efficient.

GAPIT is mainly useful for performing GWAS for multi-parent association panel studies whereas, TASSEL is useful for biparental population.



Hanging out with world-class scientists

CIMMYT has collaborations with scientists from around the world. This is where I met Dr Nadia Kamal, a visiting scientist/bioinformatician from Helmholtz Centre Munich. She gave a direction to my future to become a bioinformatician. The long talks with her gave me an understanding of how genomics will shape the future and how I can combine biology with big data. She offered me an internship with her team, which has fortified my desire for a career in bioinformatics. Another person who has influenced me is Dr Zerihun Tadesse, a wheat researcher from Ethiopia. He taught me much about how to use different R packages – rGAPIT, rTASSEL, rMVP, etc., how to use my data for maximum effect, how to visualise and interpret my data, and how to use bioinformatics to supplement my lab findings.



Fig: Becoming a Panda

I was a black bear, now I am working towards becoming a Panda!

Multi-faceted CIMMYT

GeneBank

CIMMYT genebank was started in 1996 but it has collections from 80 years ago. Why we need centres of genetic resources? Because we need to protect Maize & Wheat from heat stress, climate change, to conserve the culture as well. Genebank is a present solution for the future problems. Basic functions include – Collection & observation, diffusion or distribution, regeneration, molecular review & genetic fingerprinting,



and testing for GMOs. CIMMYT Genebank has a collection of 28000 Maize accessions mainly

of 28000 Maize accessions mainly from – Mexico, Brazil & Peru. It also has 150000 Wheat accessions mainly from – Iran, Mexico, Syria, Turkey, EUA, & 80 other countries. CIMMYT conserves the seeds in two cold chambers at -4°C and -18°C. It is said that Mexico has a vitamin called vitamin T – Tacos, tortillas, tlacoyos, tamales, tlacotes, tostadas etc. Mexico has 900 different dishes made from Maize.

Fig: In the cold chamber with the delegates from Germany, Peru, and Columbia.



Smart Mechanisation

In the mechanisation, CIMMYT provides tools, scale appropriate solutions, machines that are tailored for the operation size of the farmers that look into increasing the efficiency of input application – efficient in time, fuel, fertiliser application etc.

CIMMYT engineers try to include elements that make it easy for the farmers to adopt conventional practices. They take big machines from different countries and scale them to suit a marginal land/small farmer. CIMMYT has a whole structure around training the machine suppliers/manufacturers and also for making them available, so they have machine points in Mexico where farmers can rent them, learn how to work with them and the engineers can understand the actual demand for the machines. Commercial industry doesn't build these machines in Americas/Global south as the farmers here have less purchasing power and are not connected to the spare parts system which is the income for many of the companies.

Fare well, my friend

Karen said to me a few days prior to my departure that the students who have come to CIMMYT under Susanne's guidance are like her - kind and affable. To me, this is one of the greatest compliments I could receive. Mexicans are one of the most kindest and the warmest people I have met. I felt welcomed and accepted in this country.



Fig: Farewell party with WMB team



Cheers to the crew who made this dream a reality

I would like to thank first and foremost Dr Susanne Dreisigacker for hosting me and giving me this opportunity. None of this would've been possible without her support and encouragement. Next, I would like to thank the ATSAF organisation and Sabine Baumgartner for guiding and encouraging me. To my CIMMYT crew - Dongdong (visiting PhD), Nuri (student intern), Michi and Lukas (visiting master students), Pedro (Maize researcher), and Nadia (visiting scientist) - thank you for making my stay memorable. May our paths cross again. Here's to hoping to swap some crazy science stories. Finally, to Karen and her family - thank you from the bottom of my heart for accommodating me and leading me on some crazy adventures. Hasta pronto!



Fig: On Mount Tlaloc with Biosciences team