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The Director

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UMSAEP REPORT: 2022-2023 UMSAEP Award:

Dear Prof. Uphoff,

This serves as the final report of the activities associated with the award received me from the University of Missouri South African Education Program (UMSAEP) for research visits undertaken by myself in September 2022 and by my PhD student Miss. Lee-Ann Niekerk in May 2023 – July 2023.

Preamble of collaboration:

In 2015, the University of the Western Cape (UWC) hosted a joint Plant Science symposium with members of the Interdisciplinary Plant Group (IPG) from the University of Missouri-Columbia (MU). A total of 12 participants from MU, including 2 graduate students and 1 postdoc, attended the symposium. At the symposium, I met Prof. Antje Heese (Department of Biochemistry) and Prof. David Mendoza-Cozatl (Division of Plant Sciences) who had shared interest in gaining insight into the underlying mechanisms of metal uptake in plants. We worked on a joint application for UMSAEP funding which was successful, and I travelled to MU in 2016 to spend 3 months in the laboratories of Prof's. Heese and Mendoza-Cozatl. Ever since, we have formed a strong collaboration and I have visited MU (Columbia) on various occasions on UMSAEP funding, and to attend and present at the annual IPG symposium.

2022 – 2023: Project background:

In 2021, I applied for an UMSAEP grant to advance the collaboration with Prof. Mendoza-Cozatl on iron sensing in plants. The research visit was originally planned to span over 3 months from 7 September 2022 until 8 December 2022. I visited the Mendoza-Cozatl lab from 7 September 2022 until 7 October 2022, as per the original plan but Mr. Ruomou Wu (PhD candidate) couldn't visit the Mendoza-Cozatl lab from 7 September 2022 until 8 December 2022 due to visa complications. We then decided to replace Mr. Wu with Miss. Lee-Ann Niekerk (PhD candidate). Miss. Niekerk is co-supervised by Prof. Mendoza-Cozatl and previously travelled to MU to spend 8 months in the lab of Prof. Heese. We planned Miss. Niekerk's trip for May 2023 which coincided with the 2023 IPG symposium where she presented.

2022 – 2023: Research visit objectives:

1. To strengthen the ongoing collaboration between Prof. Keyster and Prof. Mendoza-Cozatl.

Outcome:

My visit was focused on networking with Prof. Mendoza-Cozatl and peer-similar researchers from MU and from other states. On 17 September 2022, Prof. Mendoza-Cozatl and I travelled to Arkansas State University to visit Prof. Argelia Lorence's laboratory in the Arkansas Biosciences Institute. On this visit, we obtained *Marchantia* plants from Prof. Lorence for use in our studies on iron sensing in plants. *Marchantia* plants are prehistoric organisms with much simpler lifestyles and metabolism than higher land plants.

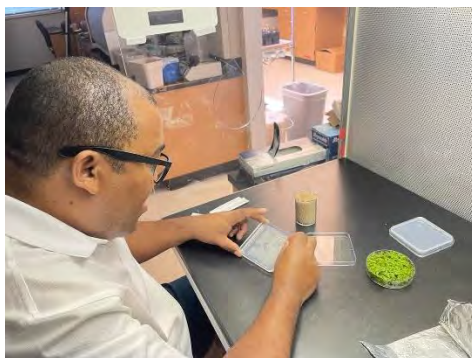


Figure 1. Me growing *Marchantia* back at MU in the Mendoza-Cozatl laboratory.

The aim of this networking travel was also to initiate work on *Marchantia* in my laboratory at UWC. In 2023, I recruited 3 BSc (Hons) students to work on various *Marchantia* projects such as “*Heavy metal stress effects on Marchantia*”, “*Bacterial infection studies on Marchantia*”, and “*Fungal infection studies on Marchantia*”.



Figure 2. *Marchantia* growing on MS media-agar in my laboratory at UWC (Picture courtesy of Chelsey Smith).

2. Three research articles have been published since the start of the collaboration and therefore we would like to increase our joint research output.

Outcome:

During my visit in 2022 we have submitted 1 other paper to the Journal Metallomics (Impact factor 3.4) on metal-binding proteins and metabolites in *Brassica napus*.

We are also working on other articles from Miss. Niekerk's PhD thesis as well as on the results obtained from her MU visit in 2023.

- 3. We are supervising one PhD student together and therefore we would like to use this visit to talk about current and future student supervision.**

Outcome:

During my return visit to MU in 2023 (from own leverage funds), Prof. Mendoza-Cozatl and I worked on the completion of Mr. Kudzanayi Charidza's MSc dissertation. Mr. Charidza graduated from the MSc degree at UWC with a distinction in the September 2023 graduation. His work focused on endophytic bacteria and iron deficiency alleviation in plants.

We also worked on Miss. Niekerk's PhD dissertation which was passed by external examiners as well as senate and she will graduate in December 2023.

- 4. We have trained four students in each of the two laboratories – two students from Prof. Keyster visited Prof. Mendoza-Cozatl's lab and two students from Prof. Mendoza-Cozatl's lab visited Prof. Keyster's lab. Therefore, Mr. Wu will be the fifth student that will work on the joint bilateral project.**

Outcome:

Due to complications with Mr. Ruomou Wu's visa, he couldn't travel to MU in 2022 as planned. However, Mr. Wu travelled to MU in September 2023 to spend 3 months in the laboratory of Prof. Mendoza-Cozatl. In addition, Mr. Gerhard Basson (PhD candidate) also travelled to MU in September 2023 to spend 3 months in the laboratory of Prof. Mendoza-Cozatl. Mr's Wu and Basson is advancing the work of Miss. Niekerk, and this will hopefully lead to high impact research outputs.

- 5. Broaden our understanding of iron sensing and signalling in plants.**

Outcome:

Miss. Lee-Ann's visit focused on the evaluation of the spatiotemporal distribution of IRON-REGULATED TRANSPORTER 1 under iron limiting conditions. Previous research conducted between the three laboratories of Prof's Heese, Mendoza-Cozatl and mine have determined that IRT1 was abundantly present at the plasma membrane, as well as detected in the microsomal fractions. However, the results only provided a stoic measurement of IRT1, meaning from this previous analysis we could only decipher 1) the abundance of IRT1 in a specific cell fraction and 2) at a specific time point. Hence, Miss. Niekerk attempted to utilize a fluorescent protein approach to track IRT1 within plant cells, in order to overcome the limitations of our previous research.

Seven out of the 10 initial constructs have been successfully transformed and screened for growth capabilities under two varying iron concentrations. The constructs have been successfully cloned into other vectors. IRT1 V# and the IRT1-# have been successfully cloned and transformed into competent cells. The #-IRT1 construct was the only construct seeming to present difficulty in the infusion of the fluorescent tag in the IRT1 gene. All constructs were successfully validated by molecular sequencing.

Ongoing work:

Mr's. Wu and Basson are continuing to build the constructs at MU until we get 10 out of 10 of the IRT1 genes into the constructs and into competent cells (September – December 2023). They will use state-of-the art microscopy techniques to track IRT1 in plant cells. Furthermore, they will also work on various DNA-RNA-Protein interactions in order to identify the molecular interactors of IRT1. This will advance our understanding of iron sensing and signalling in plants.

At UWC, the 3 BSc (Hons) students are working on Marchantia and have progressed extremely well. This work will hopefully lead to the identification of novel signalling events of Marchantia under abiotic (heavy metal stress) and biotic stresses (bacterial and fungal infections).

Acknowledgements:

Lee-Ann and I would like to thank the UMSAEP selection committees at both MU and UWC for accepting our proposal. We would like to thank Prof. Uphoff and his MU team for financial and logistics support throughout both of our travels to Missouri (USA). We would also like to thank the academic and research support from our host Prof. David Mendoza-Cozatl and the members of his laboratory for their contribution to the research outlined in this report.

Yours sincerely,

A handwritten signature in black ink, appearing to be 'MK' or similar initials, written in a cursive style.

Marshall Keyster (Ph.D.)