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06 June 2025

The Director
University of Missouri
South African Education Program (UMSAEP)
213 Hulston Hall
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RE: UMSAEP REPORT: 2024 UMSAEP Award:

Dear Prof. Uphoff,

I am pleased to submit this report detailing the research activities conducted by Miss. Adelé Mariska Barker during her visit to the University of Missouri under the University of Missouri South Africa Education Programme (UMSAEP) 2024. As the applicant, I would like to extend our deepest gratitude to UMSAEP for their invaluable support and funding, which made this transformative academic exchange possible.

Miss. Barker's research at the University of Missouri, under the mentorship of Prof. David Mendoza Cozatl, focused on advanced molecular biology techniques, including gene cloning, plant transformation, and promoter analysis. Her work significantly contributed to our collaborative project on understanding plant molecular mechanisms under nutrient deficiency and metal toxicity. The skills and knowledge she gained during this period will undoubtedly enhance her future research journey and further strengthen the partnership between our institutions.

Beyond the laboratory, Miss. Barker's experience in Missouri was enriched by cultural and academic exchanges, which broadened her perspective and fostered personal growth. This report highlights both her scientific achievements and the memorable experiences she encountered during her stay.

We sincerely appreciate UMSAEP's commitment to fostering international collaboration and academic excellence. This opportunity has not only advanced Miss. Barker's research but also reinforced the ties between our universities. Should you require any further information, please do not hesitate to contact me.

Thank you once again for your generous support.

Yours sincerely,

Marshall Keyster (Ph.D.)

Report on Research Activities Conducted at the University of Missouri

During my research visit to the University of Missouri, I participated in a series of molecular biology experiments focused on transcriptional regulation, reporter gene expression, and promoter analysis in plants. The work involved gene cloning, bacterial and plant transformation, and the use of imaging and microscopy to monitor gene expression.

Experiment 1: Transcription Factor–Promoter Interaction Analysis

The aim of this experiment was to determine whether selected *Arabidopsis thaliana* transcription factors could activate the *OPT3* promoter using a luciferase reporter assay in *Nicotiana benthamiana*.

Procedures:

- Transcription factors were amplified from *Arabidopsis* cDNA using gene-specific primers.
- PCR products were ligated into the pGreen binary vector and transformed into *E. coli*.
- Positive clones were confirmed by colony PCR and sequencing.
- Verified plasmids were transformed into *Agrobacterium tumefaciens* by electroporation.
- *Nicotiana benthamiana* leaves were co-infiltrated with *Agrobacterium* carrying both the transcription factor construct and a luciferase gene under control of the *OPT3* promoter.
- Luciferase activity was monitored using the IVIS Lumina S5 imaging system to assess promoter activation.

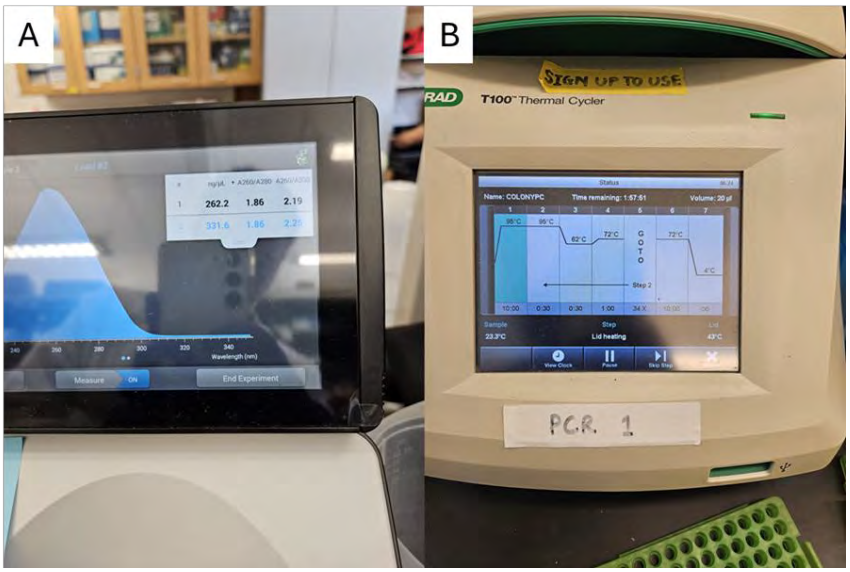


Figure 1. Instruments used during molecular cloning experiments. (**A**) Spectrophotometer used to determine DNA concentration. (**B**) Thermal cycler (PCR machine) used to amplify the region of interest.

Figure 2. *Nicotiana benthamiana* plants grown under controlled conditions in preparation for co-infiltration with *Agrobacterium* strains carrying transcription factor and luciferase reporter constructs.

Figure 3. Electroporation system used for introducing plasmid constructs into *Agrobacterium tumefaciens*.

Figure 4. IVIS Lumina S5 imaging system used to detect bioluminescence in transformed *Nicotiana benthamiana* leaves following co-transformation with transcription factor and luciferase reporter constructs.

Experiment 2: Neon Green Fluorescent Reporter Assay

This experiment was designed to visualize protein expression and track fluorescent protein dynamics using a neon green reporter gene.

Procedures:

- The neon green gene was PCR-amplified from an existing plasmid and cloned into the pGreen vector.
- The construct was confirmed by sequencing and transformed into *Agrobacterium* via electroporation.
- Transient expression in *Nicotiana benthamiana* was achieved by leaf infiltration.
- Fluorescence was monitored daily under a microscope for one week to study expression and localization.

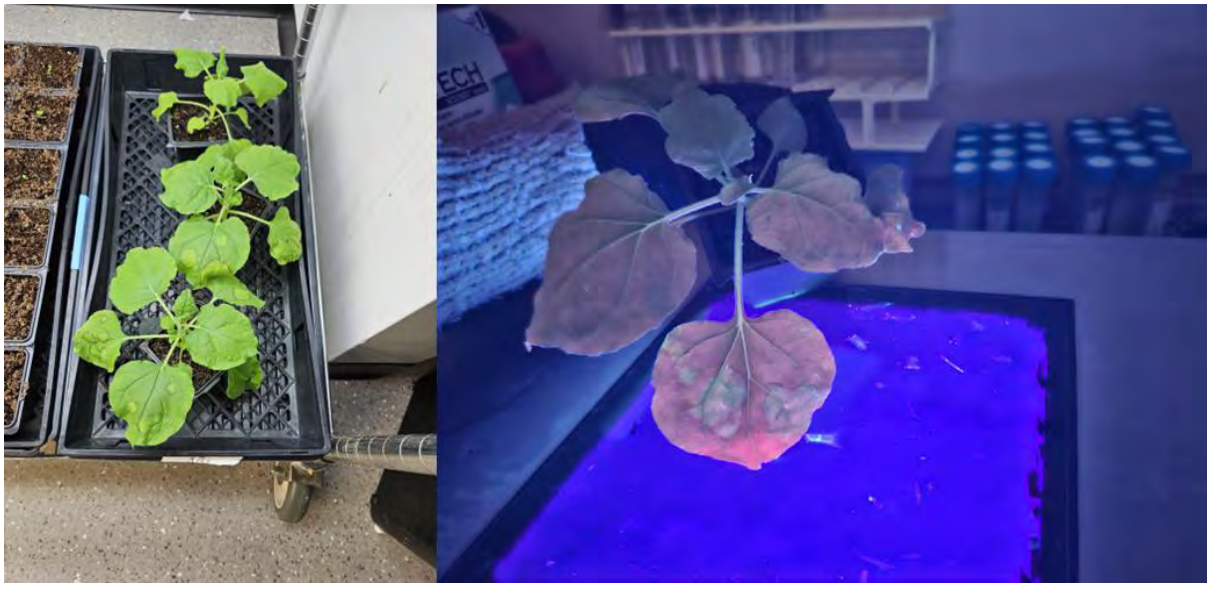


Figure 5. Visualization of *Nicotiana benthamiana* leaves after transformation with neon green construct. (Left) Transformed tobacco plants several days post-infiltration. (Right) Fluorescence observed under UV light, indicating expression of the neon green fluorescent protein.

Figure 6. Fluorescence microscopy setup and visualization of neon green expression in Nicotiana benthamiana. (Left) Experimental setup showing the positioning of infiltrated plants under the fluorescence microscope. (Right) Captured image displaying fluorescence signal from neon green protein in leaf tissue.

Experiment 3: Promoter Region Cloning

In this experiment, the promoter regions of two selected genes were amplified to study their regulatory elements.

Procedures:

- Promoter regions of two target genes were amplified via PCR using genomic DNA as the template.
- The resulting amplicons were ligated into plasmid vectors.
- The constructs were transformed into *E. coli*, and colony PCR was used to verify successful insertion.
- Positive clones were stored for downstream applications, such as reporter assays or further promoter activity studies.

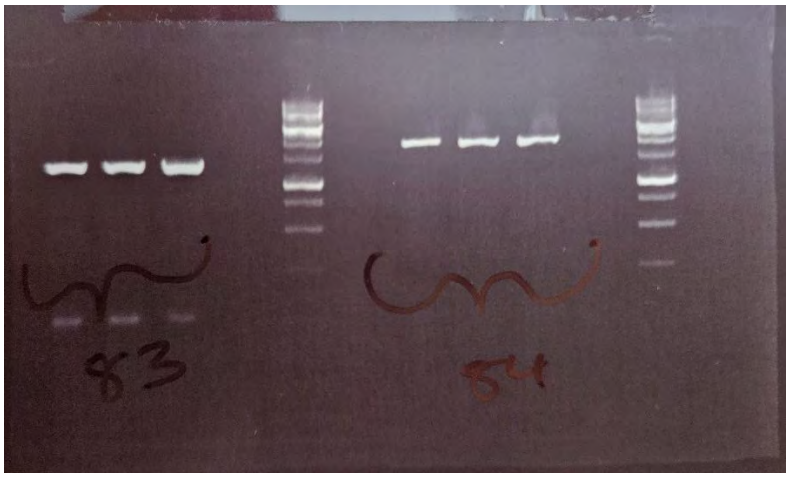
- 

  Figure 7. Gel electrophoresis showing PCR amplification of two promoter regions.Lanes labeled “83” and “84” correspond to the successfully amplified promoter fragments from two different genes. DNA ladders in the center and far-right lanes were used as molecular size markers to estimate fragment lengths.

These activities provided hands-on experience in cloning techniques, bacterial and plant transformation, fluorescent imaging, and promoter analysis, significantly contributing to my technical skills in molecular plant biology.

Beyond the laboratory, my time in Missouri was equally enriching and filled with many memorable firsts. I experienced snowfall for the very first time—a magical moment that truly captured the beauty of winter. I had the opportunity to explore the vibrant city of St. Louis, where I visited the iconic Gateway Arch and enjoyed breathtaking views from the top. I immersed myself in the local culture by attending a live music concert, watching a play, and exploring the festive atmosphere of a Christmas market. I also spent quiet moments browsing through cozy local bookshops and enjoying the warmth of Missouri’s coffee shops. One highlight was attending my first-ever basketball game, an exciting and high-energy experience. I embraced American traditions by celebrating Halloween through pumpkin carving, and I also spent time outdoors visiting the scenic Stephens Lake Park waterfall. A particularly meaningful moment was seeing the original tombstone of Thomas Jefferson—an unexpected but

memorable encounter with American history. Altogether, these experiences made my stay in Missouri deeply fulfilling, both professionally and personally.

Figure 8. Experiencing snowfall for the first time during my stay in Missouri — a joyful and unforgettable moment.

Figure 9. Visit to the Gateway Arch in St. Louis, Missouri. Top: Festive holiday scene near the Old Courthouse and Gateway Arch during a Christmas market. Bottom left: Daytime view of the Gateway Arch. Bottom right: Inside the Gateway Arch Museum and Visitor Center

Figure 10. Seasonal celebrations during my stay in Missouri. (Left) Halloween pumpkin carvings displayed on the porch. (Right) Snowman built during my first snowfall experience.

Figure 11. Attending my first basketball game at Mizzou Arena, University of Missouri, an exciting introduction to American college sports culture.