2021 Summer Research & Creative Achievements Forum

Developing Cleaved Amplified Polymorphic Sequence (CAPS) analysis to identify point-mutation in Arabidopsis hormone exporter gene *PDR9*

Presenter: Gabriela Kauffman

Rising sophomore student of the Biological Sciences Department

MU Honors College - Stamps Scholars Program

Dr. Antje Heese's Laboratory – Biochemistry Department

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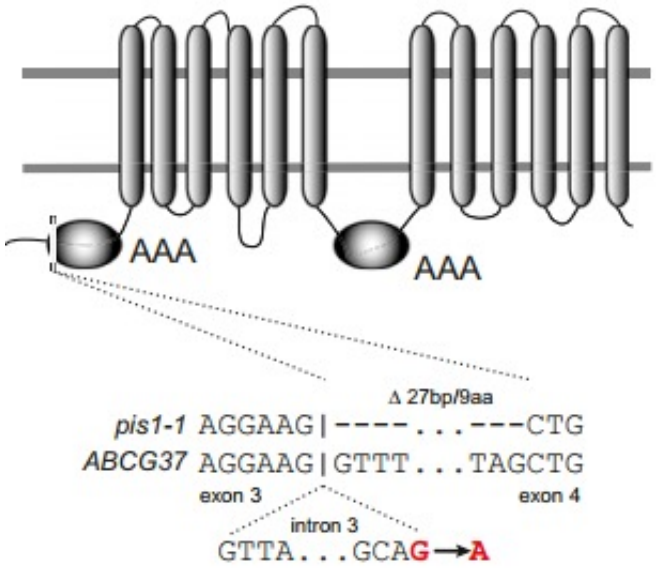
What is PDR9 and *pis1-1*?

- **PDR9** is an **IBA (indole-3-butyric acid) exporter** that localizes to the plasma membrane of the root cells
- ***pis1-1*** (*polar auxin transport inhibitor sensitive 1*)
  - **point mutation in *PDR9*** nucleotide sequence
  - leads to an altered splicing and deletion of 9 amino acids
  - results in PDR9 mis-localization



Ruzicka et al., PNAS (2010)

Currently, there is no genotyping method to distinguish between *PDR9* (wild-type gene) and *pis1-1* (point-mutation in *PDR9*)

Project part I: Establish CAPS analysis specific for PDR9

Step 1: Use Polymerase Chain Reaction (PCR) and specific primer (Fw 827/ Rv 781)
=> that can amplify both PDR9 wild-type copy and *pis1-1* mutated copy of the gene

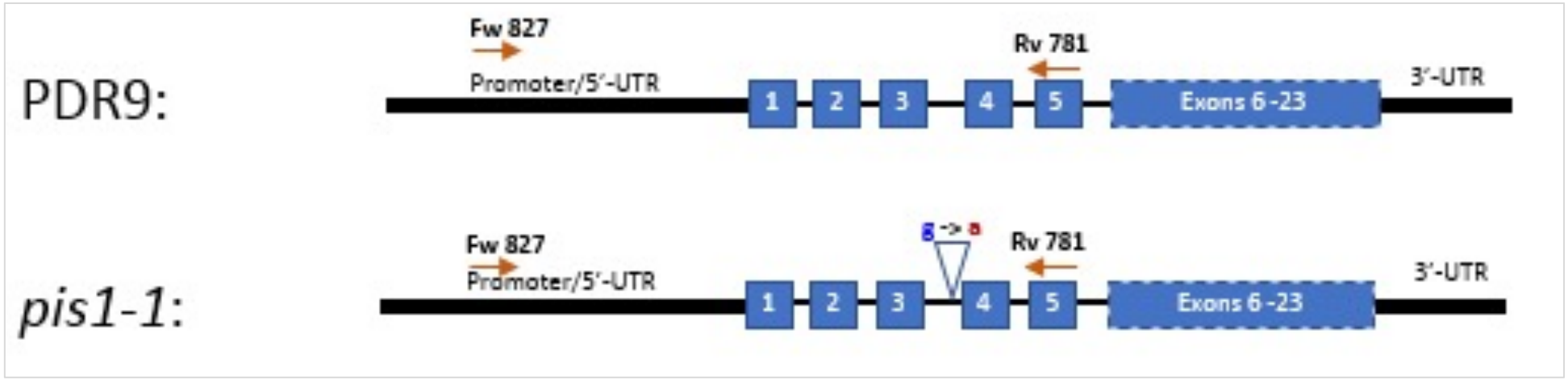
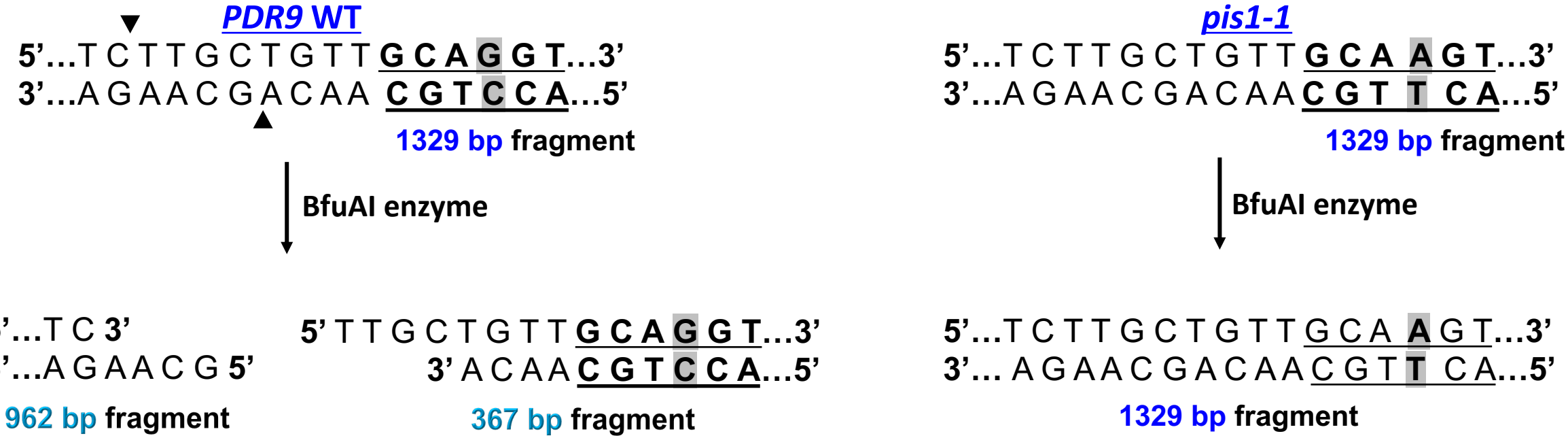


Illustration of step 2

Step 2: Restriction digestion with BfuAI enzyme

- Amplified *PDR9* WT fragment: cut into two DNA fragments
- Amplified *pis1-1* DNA fragment: intact / no cleavage



- **Bold-underlined** in PDR9-WT DNA fragment indicates BfuAI recognition site
- ▼ indicate restriction sites, the cut is asymmetric and outside of the recognition site
- **Highlighted indicates** nucleotide G changed to A in *pis1-1*

Illustration of step 3 & Results

Agarose gel electrophoresis to visualize the products after restriction enzyme digestion

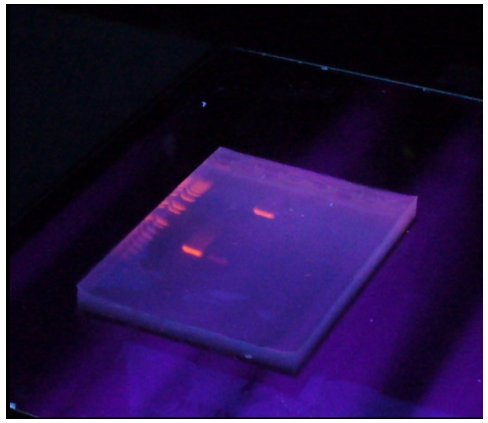
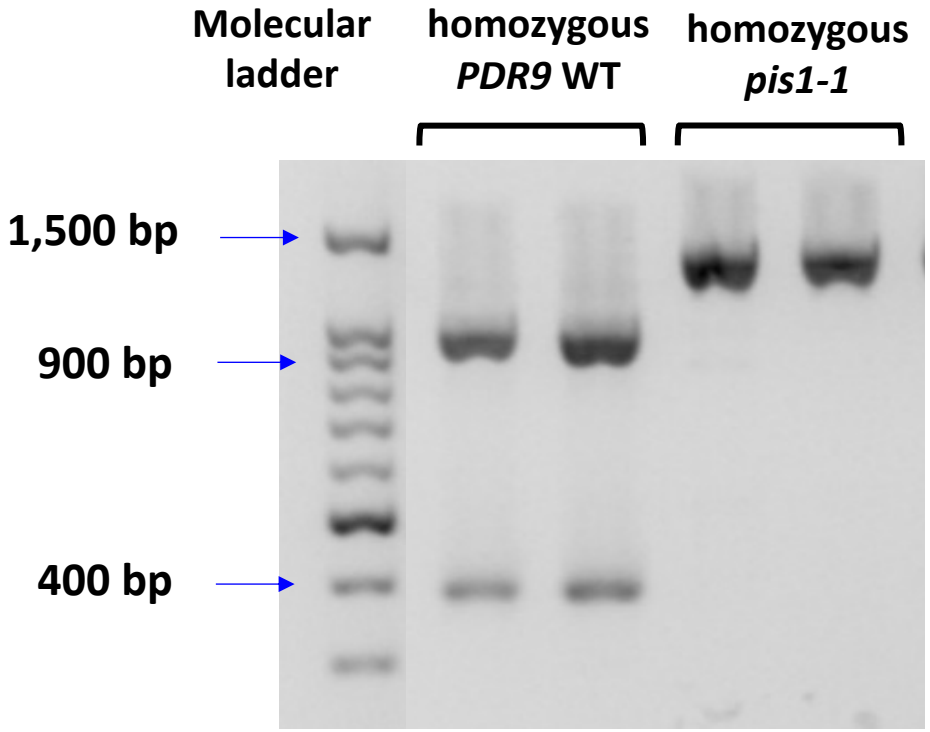


Illustration of EtBr binding to DNA molecules and emits orange light under the UV light (Image source: Wikipedia)



To visualize the DNA bands, the digested PCR products and a molecular DNA ladder were loaded into a 2.5% TAE agarose gel with Ethidium bromide (EtBr). Gel was run at 110 Voltage in 75 min for band-separation. The PCR products were then visualized under UV light.

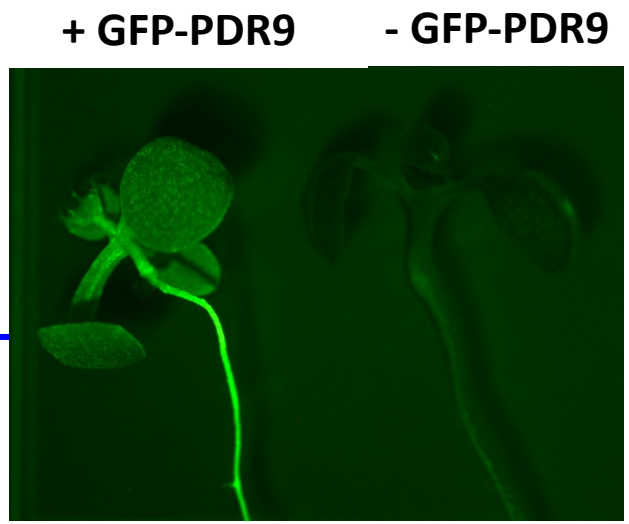
Conclusion

- Developed CAPS analysis to genotype and differentiate between PDR9 and *pis1-1*

Current & Future direction

- Cross *pis1-1* expressing **GPF-PDR9** with Arabidopsis mutant which lacks vesicular trafficking proteins (such as EPSIN1)
- => longer-term goal: to determine whether EPS1 helps PDR9-GFP to reach the plasma membrane, the site of PDR9 function

- Spoiler: I have already identified
  eps1-2 pis1-1* double mutant expressing *GFP-PDR9
  EPS1 pis1-1* single mutant expressing *GFP-PDR9



Imaging using Leica M205 at MU imaging core

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