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

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In plants, indole-3-butyric acid (IBA) is the storage form of the plant hormone auxin required for proper root growth and development. In the model plant *Arabidopsis thaliana*, PLEIOTROPIC DRUG RESISTANCE 9 (PDR9) protein, also called POLAR AUXIN TRANSPORT INHIBITOR SENSITIVE 1 (PIS1), is a critical IBA exporter that localizes to the plasma membrane (PM) for proper IBA export from root cells. To gain insight into the cellular roles of PDR9, several mutants in the *PDR9* gene have been identified, including the loss-of- function mutant *pis1-1*. Because *pis1-1* has a single nucleotide mutation in the DNA sequence of *PDR9*, standard genotyping methodology cannot distinguish between *PDR9* wild-type (WT) and *pis1-1* mutant gene copy.

For my project, I helped establish a novel genotyping procedure that used Cleaved Amplified Polymorphic Sequence (CAPS) analysis to differentiate wild-type *PDR9* from the *pis1- 1* point-mutation. First, I isolated genomic DNA from individual Arabidopsis WT, *pis1-1* mutant or plants with unknown PDR9/*pis1-1* gene copies. Next, I applied a combination of polymerase chain reaction (PCR) using *PDR9/PIS1* gene-specific primers, digestion of the amplified DNA fragment using a specific restriction enzyme, and separation of these DNA fragments by agarose gel electrophoresis. I provide evidence that this procedure allowed me to identify Arabidopsis plants that are homozygous for the *PDR9/PIS1* WT, homozygous for the *pis1-1* mutation, or heterozygous for the WT and *pis1-1* mutant gene. I have started to utilize this CAPS protocol with fluorescent microscopy to identify homozygous *pis1-1* mutants that express PDR9 protein tagged with green fluorescent protein (GFP-PDR9). In conclusion, we developed a cost-effective genotyping method using CAPS analysis to identify homozygous *pis1-1* mutant plants within a pool of Arabidopsis plants.