UW College of Agriculture and Natural Resources

Global Perspectives Grant Program

Developing a new delivery of CRISPR-Cas genome editing system for crop improvement

Period Awarded: Spring 2020

Principle Investigator: Eunsook Park

Department: Molecular Biology

Email: epark4@uwyo.edu

Amount spent: $8,000.00

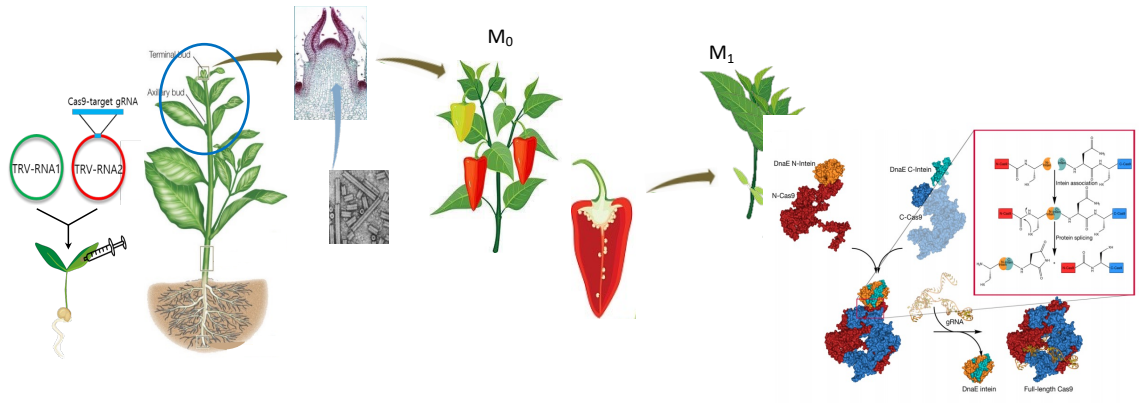
Non-technical summary

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a family of DNA sequences found in bacteria and archaea. Cas (CRISPR-associated protein) uses CRISPR sequences as a guide to recognize and cleave specific DNA sequences. This CRISPR/Cas system is significant to the gene-editing to envision effective gene therapy to cure human disease as well as improve crop security.

This project is to develop a simple delivery method of the CRISPR/Cas system into plant cells without the footprint of a foreign gene, as well as the regeneration process by laborious tissue culturing. This award allows us to establish an intimate collaborative workspace with a plant virus biotechnology lab in Valencia, Spain, to build an implemented research team for plant viral genome engineering and the molecular mechanism of plant-microbe interaction. We expect the synergistic effect of the collaboration to develop a convenient delivery system of the CRISPR/Cas system to engineer crops for crop sustainability.

REPORT

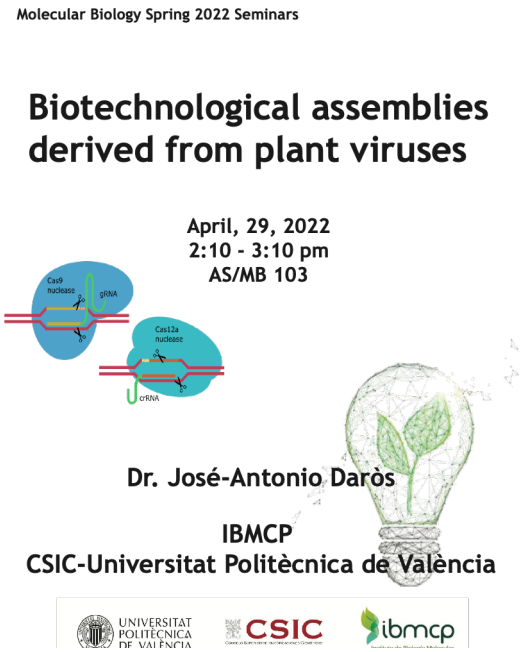
I. RESULTS OF ACTIVITIES PLANNED IN THE PROPOSAL



Overview of the research plan to develop a convenient method to deliver CRISPR/Cas system in crop plants

To avoid the plant genome integration, we selected plant virus to express the CRISPR/Cas system in parental plants expecting no foreign genes in the next generation. A few RNA viruses have been used to deliver foreign gene fragments into a model plant, Nicotiana benthamiana, for research purposes by several research groups, including Dr. José-Antonio Daròs lab from CSIC-Universitat Politècnica de València, Spain. Through this award, we could collaborate for this project with Dr. José-Antonio Daròs to develop the RNA virus-mediated CRISPR/Cas delivery system for the Solanaceae family plants, including a number of agronomically important crops such as tomato, pepper, and potato. In the longer term, we will expand the application of this method for economically valuable crops in Wyoming, such as dry bean and sugar beet.

Major activity I: Invite Dr. José-Antonio Daròs to the Molecular Biology department seminar



Left: the flyer of Dr. Daròs' seminar.
Right: Park lab hiking to Vedauwoo climbing area with Dr. Daròs. April, 30, 2022.

In Spring 2022, Dr. Daròs visited Laramie to present his plant virus biotechnology research works to the Molecular Biology department.
For successful gene editing, the Cas9 protein must be stably expressed in the somatic cells and/or meristematic cells for inheriting the edited genomic information to the next generation. Due to host specificity and infection activity of viruses, we will search optimal virus species, engineer them for stable Cas9 protein expression transiently, and also optimize Cas9 proteins to improve their movement to the somatic or meristematic cells. As proof of concept, we will use Nicotiana benthamiana, a model plant in the Solanaceae family.

Major activity II: Visit Dr. Daròs lab in Valencia, Spain

After the initial discussion with Dr. Daròs during his visit to Laramie, we were able to modify the experimental procedure to generate a mixture of two virus delivery systems to improve stability and deliver the protein via an optimized viral expression system, using Tobacco rattle virus (TRV), Potato virus X (PVX), Tobacco etch virus (TEV).

In November 2022. Dr. Park (PI) and Dr. Woo (A primary researcher of this project) visited the Universitat Politècnica de València to give a department seminar (Dr. Park) and to have a comprehensive meeting with Daròs lab to make a detailed plan to proceed with this project together.

Left: Dr. Park and Dr. Woo at the Science museum in Valencia, Spain
Right: Daròs lab lunch with Dr. Park and Dr. Woo after a long meeting to plan a successful collaboration.

II. IMPACT OF OUTCOMES AND FUTURE COLLABORATION PLANS

Dr. Daròs lab has studied various virus systems and has engineered several virus-mediated gene expression systems across multiple plant species. The Park lab has expertise in understanding the plant's immune responses at the cellular level. Therefore, we expect this collaboration would provide a synergistic effect on understanding the biological properties of virus-plant interaction to increase the capability for stable Cas9 protein expression via virus as well as to acquire optimal conditions for the target gene editing by the CRISPR-Cas9 system.
Upon completion of this project, the viral delivery system developed in this project will be widely utilized for crop improvement. Dr. Park and Dr. Daròs are seeking a collaborative grant from the National Science Foundation (NSF-PCRP), the Spanish research council, and EU funds to expand the application of the CRISPR-Cas system to other economically important crops.