

# Plant Pathology Seminar Series

## “Applications of CRISPR-Cas system in Plant Pathology”

Afsha Tabassum

Bacteria and yeast have been shown to carry out precise editing of DNA through the phenomenon called ‘Restriction and Modification (RM) system’, which ushered in the new era of using restriction enzymes and the subsequent birth of recombinant DNA technology (4). The discovery of the RM system and restriction enzymes coupled with advances in our understanding of DNA repair pathways in bacteria and yeast have contributed to increased efforts in developing strategies for targeted genomic engineering (3).

The CRISPR (Clustered Regularly Interspaced Palindromic Repeat) - Cas (CRISPR-associated) technology is at least hundreds of millions of years old. Bacteria originally used CRISPR as a survival mechanism to fight infection by viruses. It was not until the early 2000s, the CRISPR- Cas mechanism was understood (7). This adaptive immunity occurs in three stages: 1. Short sequence of invading DNA insertion as a spacer into CRISPR array; 2. Precursor crRNA (pre-crRNA) transcription to generate individual crRNAs; and 3. Cleavage of foreign nucleic acid using crRNA and cas proteins (3).

Two different groups are credited with having developed the CRISPR-Cas system: Jennifer Doudna, University of California, Berkeley together with Emmanuelle Charpentier (now at Max Planck Institute in Berlin), and Feng Zhang, at Broad Institute of Harvard and MIT. There is a litigation underway over the ownership of this technology (7).

CRISPR-Cas9 approach has already been applied to modify, regulate or mark genomic loci in a wide variety of cells and organisms from all three domains of life: Archaea, Bacteria and Eukarya (7). Dr. Yinong Yang, a plant pathologist at Pennsylvania State University, targeted one of the six-polyphenol oxidase enzymes in common white button mushrooms (*Agaricus bisporus*) that led to reduction of browning in mushroom by 30%. The USDA issued guidance that it will not regulate this CRISPR-engineered mushroom (9).

CRISPR- Cas9 system was used on different pathogen groups to a) target RXLR effector gene Avr 4/6 in *Phytophthora sojae*, which causes damping off of soybean seedlings (5), b) to identify *Xanthomonas oryzae* strain hosts for *Xanthomonas oryzae* phages Xop411, Xp10 (8), c) against Tomato yellow leaf curl virus which is potentially applicable to all plant DNA viruses (1) and d) eif4e non-transgenic cucumber mutants resistant to Cucumber vein yellowing virus, Zucchini yellow mosaic virus and Papaya ring spot virus-W (2).

Most recently, Dr. Luo from Clemson University has proposed a project to engineer Citrus greening/Huanglongbing (HLB) tolerant/resistant citrus using CRISPR genome editing technology (6).

Regulatory oversight is less clear in the application of CRISPR-Cas to food crops. Commercialization of CRISPR-based crop traits ultimately depends on regulatory oversight and consumer acceptance (7).

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Agricultural, Human,  
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WASHINGTON STATE UNIVERSITY

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