W-1

Present and future of CRISPR technology and genome editing for new plant breeding technology

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The history of crop breeding in the past ten thousand years is closely in line with the history of human civilization. Breeding methods such as selection breeding, mutation breeding, and crossing breeding were carried out in the absence or little knowledge of genetic information 'DNA or genome'. Whether natural or artificial breeding methods, the mutation was random and the farmer was able to select the seeds of the crops that showed good agricultural traits. Unlike the past, the breeding of the 21st century has made it possible to produce seeds with good agricultural traits as they design. The technologies that made this possible are transgenic technology (GM technology) and gene scissors (genome editing technology). In particular, the third-generation gene scissors, CRISPR-Cas9 technology, which has revolutionized current biotechnology and life sciences, has the advantages of being easy, fast, and economical compared to previous technologies and also experiencing rapid artificial evolution by the worldwide laboratories. This lecture looks over past and current status of gene scissors technology and predicts future development trends.

W-2

Global Genome Engineering Market
-including current progresses by MNC-

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Genome engineering is a relatively new technology that will improve agriculture research in the near future. The techniques which include CRISPR/Cas-9(Clustered regularly interspaced short palindromic repeats associated protein 9), TALEN(Transcription activator-like effector nuclease), and ZFN(Zinc finger nuclease) hold the promise of creating genetic variation and enabling both agricultural research and crop improvement. The size of global genome engineering market was evaluated about two billion dollars in 2015 and estimated from 10% to 12% CAGR for 2016-2020. CRISPR was the largest part of the genome engineering market during 2015, especially Cas-9 from *Streptococcus pyogenes* has become the largest and fasted growing segment. Researchers keep trying to find other novel CRISPR nucleases for more efficient method albeit major agricultural and pharmaceutical companies rely on branded products so far that help market growing. MNCs such as DuPont Pioneer, Monsanto, and Syngenta lead the application of CRISPR tool to develop customized agriculture solutions. Agribody Technologies' patented genome editing technology also delays plant senescence, while increasing resistance to diseases and sublethal stresses in many key crop plants. Benson Hill Biosystems has a large portfolio of validated novel CRISPR nucleases with diverse chemistries and unveils the Cms1 portfolio which has four unique nucleases validated in vivo. 2Blades and its collaborators are using advanced technologies to isolate rust resistance. Furthermore Biotechnology Regulatory Services (BRS) in USDA-APHIS clarified on this March 28th they does not regulate or have any plans to regulate genome editing in plants.

W-3

Precise Plant Genome Editing

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Genome editing tools have been developed to manipulate genomic DNA in living organisms; in particular, the recent genome editing tool, CRISPR/Cas9, allows us to induce targeted mutagenesis in several plant species. Currently, many researchers apply this genome editing technique to improve crop quality and quantity. In this talk, I will talk about how we can apply CRISPR/Cas9 system to induce precise nucleotide substitutions. Cas9 proteins fused with cytidine deaminase can induce C to T nucleotide substitutions at a specific site when directed by guide RNAs. We examined the substitution activity and the substitution range of two base-editing systems: APOBEC1-nCas9 and nCas9-PmCDA1 to each other in the protoplasts of *Nicotiana tabacum* and *Brassica napus*. We then converted the specific amino acid in the *acetolactate synthase* gene of *N. tabacum* to generate herbicide-resistant plants. This work provides guidelines on which a base editor to use and how to adjust the length of a guide RNA for nucleotide substitutions at the desired genomic position in plants.

W-4

Precision genome engineering through adenine base editing in plants

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CRISPR genome editing in plants holds promise as a revolutionary tool for basic research and biotechnology. Canonical CRISPR nucleases cleave DNA in a targeted manner resulting in small indels at target sites. In plant research, however, inducing point mutations rather than indels remains a challenge, although point mutagenesis is one of the key strategies to achieve crop improvement and to decode natural genomic variations. The recent development of adenine base editors (ABEs), composed of the Cas9 nickase and engineered tRNA adenosine deaminases, has enabled efficient programmable A/T-to-G/C base conversions in eukaryotic cells. For *in planta* ABE applications, we tested ABEs with several plant-specific expression systems. We found that a plant-compatible ABE system can be successfully applied to Arabidopsis plants via agrobacterium-mediated transformation to obtain organisms with desired phenotypes. For example, targeted precise A-to-G substitutions generated a single amino-acid change in the FT protein or mis-splicing of the *PDS3* RNA transcript with germline transmission of such edited alleles, and we could thereby obtain transgenic plants with late flowering and albino phenotypes, respectively. Taken together, we demonstrate 'proof-of-concept' *in planta* ABE applications that can lead to induced neo-functionalization or altered mRNA splicing, opening up new avenues for plant genome engineering and biotechnology.