



Webinar Series on Applications of Gene Editing in Sustainable Agriculture and Food Security in Asia-Pacific Region

Webinar 1: Genome Editing Tools and its Applications for Targeted Plant Breeding

Date: 21 July 2021; 10:30 AM ICT (Bangkok time)

ABSTRACTS

Gene-edited high GABA tomato for human health

Dr Hiroshi Ezura, Professor/Director, Tsukuba Plant Innovation Research Center, University of Tsukuba

Gene editing is a new breeding tool. We applied it to improve a nutritional trait, GABA (γ -aminobutyric acid) content, in tomato fruits as a case study. Here I would like to share our challenge and experience in development and implementation of the high GABA tomato. GABA is a non-proteinogenic amino acid with health-promoting functions for human. Although tomato fruits have a relatively high GABA content, the levels must be further increased to effectively confer the health-promoting functions such as lowering blood pressure. Glutamate decarboxylase (GAD) is a key enzyme in GABA biosynthesis in tomato; it has a C-terminal autoinhibitory domain that regulates enzymatic function, and deletion of this domain increases GAD activity. Then, to increase GABA content in tomato fruits, we deleted the autoinhibitory domain of GAD using CRISPR/Cas9 technology. Introducing a stop codon before the autoinhibitory domain in the GAD gene increased GABA accumulation in the fruits. A university-lunched venture company has developed a commercial cultivar using the gene editing technology, and has started providing the high GABA tomato cultivar to more than 4000 home-gardeners in May, 2021. We hope that our gene-edited tomato contributes to get public understanding of this new technology.

Advances in genome editing for crop improvement

Dr. Jose (Jimmy) Botella, Professor of Plant Biotechnology, School of Agriculture and Food Sciences, University of Queensland, Australia

Traditional breeding approaches for crop improvement rely heavily on 'natural' or artificially generated genetic variation. Once identified, introgression of new traits into elite varieties is a time-consuming, laborious and costly process that gets even more complicated in polyploid species. The development of genome-editing technologies brought the promise of more efficient breeding programs, but the technical complexity of the initial gene editing technologies discouraged many scientists from pursuing this approach. CRISPR has changed everything. CRISPR, derived from the bacterial immune system, is extremely easy to use, very accurate and has already been used in a large number of species. The original use of CRISPR



was to produce targeted genome mutations but has now been expanded into innovative applications. I will discuss the technical nature of CRISPR and describe a number of examples in which CRISPR has been used for ultrafast breeding.

Crispr-Cas9 genome editing to confirm gene function and confer salt tolerance in rice

Zeba I. Seraj^{1}, Sabrina H. Labonno¹, Aysha A. Laboni¹, Imran K. Hridoy¹ and Sabrina M. Elias^{1,2}*

¹Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh

²School of Environment and Life Sciences, Independent University, Bangladesh

**zebai@du.ac.bd*

The rice landrace Horkuch is endemic to the coastal south of Bangladesh, where there is high salinity, particularly in the dry season. RNAseq and quantitative PCR identified specific transporters which were down-regulated under salt stress in the tolerant Horkuch. These include the Potassium ion Channel gene, OsAKT1 and the ATP Binding Cassette Transporter of the B family, OsABCB7. Downregulation of these genes by homologous sgRNA using in planta transformation of sensitive IR29 rice conferred salt tolerance to the latter. This confirmed that one of the defence mechanisms used by Horkuch to fight salt stress is to downregulate these transporter genes. Among the upregulated genes when Horkuch was subjected to salt stress were the Vacuolar Two Pore K⁺ channel, OsTPKa and Hypothetical Protein of the High Affinity K⁺ family, OsHAK-like. The Crispr-Cas9 mediated loss in function using specific sgRNA and in planta transformation of Horkuch targeting these two transporters conferred salt sensitivity to the rice landrace. We hypothesize that OsTPKa and OsHak-like work in a coordinated manner to confer salt tolerance to the landrace Horkuch and therefore these genes can be upregulated in sensitive rice for conferring salt tolerance.

Dr T.R. Sharma, Deputy Director General (Crop Science), Indian Council of Agricultural Research, India

Rapid developments in gene editing technologies and their commercial potential have spurred extensive research and development efforts by public sector research institutions as well as industry in India. The Indian Council of Agricultural Research and the Department of Biotechnology have supported several projects to apply gene editing for enhancing stress tolerance and nutritional quality in a number of crops such as rice, wheat, mustard, banana etc. Several interesting leads have been developed and need to be tested in the field. An appropriate regulatory environment is required for harnessing the benefits of the technology particularly for small holder farmers. In addition, public private partnerships are required for use of protected technologies.



Dr. Donghern Kim, Vice President, Future Food Resources Forum, Korea

Status of Research initiatives/potential/challenges related to Gene editing in agriculture in Korea

Currently, R&D for genome edited crops are ongoing quite actively. The Rural Development Administration launched a national research program to develop and use of new breeding technologies in 2020. The program supports researchers and scientists who are developing GE crops for domestic as well as global use. Some of their activities will be introduced during the webinar.

Act on the transboundary movement of living modified organism is the central legislation in Korea to regulate LMO in every aspect of GMO from research to commercialization and to export/import. The MOTIE, the Ministry of Trade, Industry and Energy, which is the competent national authority, recently announced a plan to revise the act. According to the draft revision, developers of any new living modified organism including genome edited one submit the application for pre-review process to MOTIE. If such organism belongs to one of three categories, it will be exempted from the requirement of risk assessment and of approval for production/use/import. Korean science societies evaluate it positively since it is the first movement toward the risk management based on the potential risk of products. However, they are concerned the negative affect of it since the movement would act as a negative signal to researchers as well as industries due to government's stance on GE crops by categorizing them, especially SDN1 products, as LMOs. Detailed information on the draft revision will be presented in the webinar.

Dr. Chwan-Yang HONG, Professor, Department of Agricultural Chemistry, National Taiwan University, Taiwan

Status of research initiatives/potential/challenges related to Gene editing in agriculture in Malaysia

In recent years, researchers in Taiwan have invested in gene editing R&D on staple crops, fruits, vegetables and flowers and hope to improve their resilience to climate change by SDN-1 type gene-editing. Many countries have exempted SDN-1 type gene-editing from GMO regulation of crop plants. In Taiwan, we continue to communicate with stakeholders and collect relevant opinions. We would carefully regard the international trend on the regulatory and management issues on this precision breeding technology to closely in line with international consensus. Although the legal status of genome editing is still under discussion, in the last few years we have held several symposiums on the applications of Genome Editing in Crop Research, and also discuss the impact of gene-editing on the economy, society and management cost issues in order to establish appropriate management measures. We wish to develop innovative R&D promotion methods suitable for our economy, and to ensure that gene-edited organisms could be regulated under reasonable management.



Status of Research initiatives/potential/challenges related to Gene editing in agriculture in Malaysia

Professor Kok Gan Chan, Deputy Head of Department, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Modern biotechnology is the key technology for future agriculture in Malaysia and state-driven biotechnology policy is in place in Malaysia. Similar to other countries, Malaysia has policies, regulations and laws favouring development of crop biotechnology and thus creating an enabling environment to meet the needs of the future. This modern biotechnology includes, but not limited to, modern technology such as gene-editing, whole genome sequencing and OMICs technology. The rise of CRISPR-Cas9 gene editing and transcription activator-like effector nucleases (TALENs) are revolutionary gene-editing tools that are game changing technologies. CRISPR-Cas9 is the most widely used technology in Malaysia and the region though no products based on genome editing have reached the market in the Asia-Pacific region. More recently, an alternative genetic engineering system called Retron Library Recombineering (RLR), that works without cutting DNA and it may open up more opportunities to be applied to develop crops with novel traits. The gene/genome editing technology will certainly be important in crop biotechnology in Malaysia, but whole genome sequencing and gene/genome editing are more demanding that require good infrastructure, skilled personnel, and availability of state-of-the-art instruments. Consequently, this will post more challenging tasks for the novice biosafety regulator to regulate notably crops that are created using gene/genome editing.
