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Agriculture & Food Systems Institute

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Early Career and Innovative Start-ups Symposium

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Declaration of Interests

Agriculture & Food Systems Institute (AFSI) speakers have no actual or potential conflict of interest in relation to the presentations given as part of the ***Early Career and Innovative Start-Ups Symposium***. Invited speakers have been instructed to disclose financial interests related to the subject matter of their presentations. AFSI's activities related to this workshop are supported by a grant from the from the USDA FAS New Technologies and Production Methods Division.

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Overview

This one-day symposium shared new developments in agricultural biotechnologies and emphasized the role of youth in innovation. Through panel discussions, lightning talks, and a poster session, early career researchers presented on or learned of the impacts of policies on their work, empowering them as advocates for the development of trade- facilitating, science-based policies on agricultural biotechnology. In addition to highlighting early career researchers and start-ups from APEC member economies, this symposium provided an opportunity for researchers working in the field of agricultural biotechnology in the Asia Pacific region to exchange ideas. Industry representatives delivered presentations on enabling policy environments, current research and development, and career opportunities. As one of the activities on the margins of the High Level Policy Dialogue on Agricultural Biotechnology (HLPDAB) plenary, the symposium contributed to strengthening sustained information sharing related to agricultural biotechnologies between APEC member economies through highlighting innovations in the field.

Keynote Speaker

Lawrence Kent

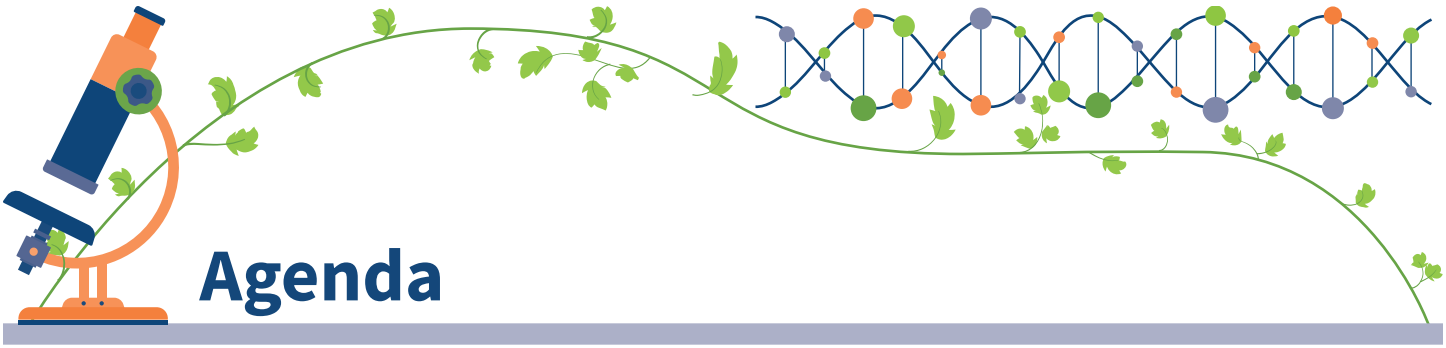
Bill & Melinda Gates Foundation

For the past 16 years, Lawrence Kent has been serving as a Senior Program Officer on the Agricultural Development team at the Bill & Melinda Gates Foundation. He is based in Seattle but travels frequently to Africa and Asia to support grantees implementing programs to develop seed systems, build regulatory capacities, and test, deregulate, and deploy new crop varieties enhanced through both biotechnology and conventional breeding. Lawrence currently manages grants advancing transgenic disease-resistant cassava, insect-resistant cowpea, nutritionally enhanced rice, and drought-tolerant and insect-resistant maize hybrids. He also manages support for the HarvestPlus program, which has reached over 20 million farm families with seeds of crops bred to include elevated levels of iron, zinc, and pro-Vitamin A.

Prior to joining the Gates Foundation, Lawrence served as director of international programs at the Danforth Plant Science Center in St. Louis, Missouri (2002-2007), where he developed and supported programs leveraging biotechnology to produce nutritionally enhanced and virus-resistant cassava and disease-resistant sweetpotato with African partner institutions. He also led capacity-building programs on biosafety.

From 1990 until 2006, Lawrence worked on agricultural development and policy reform programs in Africa and Asia funded by USAID, the World Bank, UNDP, and the Asian Development Bank. He lived and worked for four years in Egypt, two years in Chad, one year in Burkina Faso, one year in Bulgaria, and conducted short-term consultancies in 25 countries, mainly in Africa.

An economist by training, Lawrence earned his master's degree at Princeton University in New Jersey after two years working as a Peace Corps Volunteer in West Africa (1985-87).



Agenda

Time	Presentation/Activity	Speaker/Facilitator
9:00 am	*Welcome and Introduction to the Symposium*	*Dr. Jennifer Rowland* Science Advisor New Technologies & Production Methods Division Foreign Agricultural Service U.S. Department of Agriculture The United States of America
9:05 am	*Opening Remarks*	*Ms. Sanah Baig* Deputy Under Secretary for Research, Education, and Economics U.S. Department of Agriculture The United States of America
9:15 am	**Session 1 – Research and Innovation Lightning Presentations** *8 Lightning Talks* Invited early career researchers will each give a 4-minute presentation to highlight their research and encourage further interaction during the poster session. *Moderator: Dr. Bhavneet Bajaj, Manager - Scientific Programs, Agriculture & Food Systems Institute, The United States of America*	
	Engineering Plants with Nitrogen Fixation Capabilities	*Dr. Christina Gregg* Research Scientist Commonwealth Scientific and Industrial Research Organisation Australia
	Implementation of CRISPR-Based Gene-Editing Tools in Brassica napus Canola	*Mr. Neil Hickerson* Graduate Student Researcher University of Calgary Canada
	Tuning Glutathione Content through Multiplex Genome Editing for Studying Dehydration Tolerance in Rice	*Dr. Chin-Yu Wu* Postdoctoral Research Fellow National Taiwan University Chinese Taipei
	Developing the Fruits for the Future: Precision Breeding in Fruit Crops	*Dr. Bernardo Pollak* Chief Executive Officer Meristem Chile

Time	Presentation/Activity	Speaker/Facilitator
	Induction of Resistance to Sugarcane Mosaic Virus by RNA Interference Targeting Viral Coat Protein in Sugarcane	*Dr. Rikno Harmoko* Research Scientist National Research and Innovation Agency Indonesia
	Use of Genetic Engineering to Develop Resistance to Biotic Stress in Plants	*Dr. Tetsuya Yoshida* Researcher Crop Disease Research Group Institute of Agrobiological Sciences, NARO Japan
	CRISPR Tools-Mediated Pepper Genome Editing	*Dr. Hyeran Kim* Assistant Professor Kangwon University The Republic of Korea
	Offspring Production of a SRY-knock in Bull	*Dr. Alba Ledesma* Post-Doctoral Researcher University of California, Davis The United States of America
9:55 am	*Agricultural Biotechnology Innovations Leading Change Around the Globe* *Q&A Opportunity*	*Dr. John Sedbrook* Professor of Genetics College of Arts and Sciences Illinois State University
10:30 am	*Coffee/Tea Break*	
11:00 am	**Session 2 – Research and Innovation Lightning Presentations** *8 Lightning Talks* Invited early career researchers will each give a 4-minute presentation to highlight their research and encourage further interaction during the poster session. *Moderator: Dr. Bhavneet Bajaj*	
	Developing Recombinant Wheat Cultivars with Drought Tolerance Traits	*Dr. Xiaoqing Li* Research Scientist Commonwealth Scientific and Industrial Research Organisation Australia
	Tastier Pea Protein Through CRISPR/Cas9 Gene-Editing	*Dr. Connor Hodgins* Researcher University of Calgary Canada
	Development of Allergenicity and Toxicity Assessment Methods for Evaluating Transgenic Sugarcane	*Dr. Widhi Dyah Sawitri* Assistant Professor Gadjah Mada University Indonesia

Time	Presentation/Activity	Speaker/Facilitator
	RNAi-Mediated Protection Against Cucumber Mosaic Virus (CMV) in Rockmelon (Cucumis melo L.)	*Ms. Dharane Kethiravan* Graduate Student Researcher University of Malaya Malaysia
	Biofortification of Rice Grains Through Genome Editing: Addressing Zinc Deficiency in Asia-Pacific Region	*Mr. Erwin Arcillas* Assistant Scientist International Rice Research Institute The Republic of the Philippines
	Microbial-Based Technology as an Alternative to Address Overflow of Agricultural Residues: Production of Vanillin from Lignin Derivatives Using the Recombinant Cell	*Dr. Panaya Kotchaplai* Researcher Institute of Biotechnology & Genetic Engineering Chulalongkorn University Thailand
	Improving Pennycress Seed Size and Glucosinolate Domestication Traits	*Ms. Liza Gautam* Research Scholar Illinois State University The United States of America
	Improved Bacterial Leaf Blight Disease Resistance in Major Elite TBR225 Rice Cultivar Using CRISPR/Cas9 System	*Dr. Nguyen Duy Phuong* Head of Molecular Pathology Department Agricultural Genetics Institute Viet Nam
11:40 am	**Poster Session** Early career researchers will present at a poster session, during which symposium participants can further engage with them about their research.	
12:40 pm	**Keynote Presentation** *Lunch Served* *Agricultural Biotechnology in the Developing World: Opportunities and the Role of the Gates Foundation*	*Mr. Lawrence Kent* Senior Program Officer Agricultural Development Bill & Melinda Gates Foundation The United States of America
	Panel Discussions	
1:50 pm	*Panel Discussion 1: Finding your Market, Traits and Targets, Regulatory Considerations, and Beyond* *Moderator: Dr. Jennifer Rowland*	*Mr. Dan Jenkins* Vice President of Regulatory and Government Affairs Pairwise The United States of America
		Mr. Shimpei Takeshita President Sanatech Seed Co., Ltd. Japan

Time	Presentation/Activity	Speaker/Facilitator
		Dr. M. Tahir Director of Research & Regulatory Affairs Okanagan Specialty Fruits Canada *Ms. Rebecca Catlett* Director of Marketing & Communication Okanagan Specialty Fruits Canada
3:20 pm	*Panel Discussion 2: Encouraging an Enabling Environment for Agricultural Biotechnology* *Moderator: Dr. Stuart Smyth, Associate Professor, University of Saskatchewan, Canada*	*Ms. Chantal March* Director of Quality & Regulatory Compliance AquaBounty Canada, Inc. Canada
		Mr. Martin Mariani Ventura Global Seeds & Traits Manager Bioceres Crop Solutions Argentina
		Mr. Paul Spencer Global Trade Policy Advocacy Leader External Affairs Corteva Agriscience™ The United States of America
		Dr. Chee Hark Harn Director, Seed R&BD Headquarters ToolGen, Inc. The Republic of Korea
4:20 pm	*Closing Remarks*	*Dr. Jennifer Rowland*
4:30 pm	*Early Career Researchers: Group Photo*	
4:45 pm	**Meet and Greet with Early Career Researchers** *Soft Drinks and Snacks Served*	
5:45 pm	*Symposium Ends for All*	

Early Career Researchers: Abstracts and Bios

Engineering Nitrogen-Fixing Plants: Reducing the Need for Nitrogen Fertilizer

Dr. Christina Gregg, Research Scientist, Commonwealth Scientific and Industrial Research Organisation, Australia

Currently, the nitrogen requirements of most crops are met by supplying synthetic nitrogen fertilizer. While nitrogen fertilizer is critical to agriculture and has been the main driver of the world's population expansion in the last century, it also comes with great economic and ecological costs. Here, we show our progress towards engineering plants that can fix nitrogen to reduce the need for nitrogen fertilizer.

About the Presenter

Christina Gregg is a biochemist and currently works on engineering plants with nitrogen fixation capabilities. She studied Chemistry at the University of Erlangen-Nuremberg, Germany, and completed her Ph.D. at the Humboldt-University of Berlin, where she worked on the biosynthesis pathway of enzymes containing metal clusters. Christina Gregg joined the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Australia) as a Postdoctoral Fellow in 2017 and is now a Team Leader. She works on engineering plants that can fix their own nitrogen by directly transferring genes of nitrogenase, the enzyme that catalyzes biological nitrogen fixation, into plants. At CSIRO, Christina established an anaerobic biochemistry facility, and is currently focusing on assessing the function of individual nitrogenase components in order to build the complete nitrogenase biosynthetic pathway.

Implementation of CRISPR-Based Gene-Editing Tools in *Brassica napus* Canola

Mr. Neil Hickerson, Graduate Student Researcher, University of Calgary, Canada

Nearly 30 million tonnes of canola oil are produced globally each year, making canola among the top oil-seed crops worldwide. Canola has primarily been grown for its high-quality cooking oil. However, additional uses include industrial oil products for lubricants, adhesives, and biodiesel production, as well as a high-protein seed meal for plant-based protein products and animal feed stocks. Canola seeds feature a low-impurity oil with very low saturated fat content and high oleic acid (omega-9 fatty acid), which have been shown to improve cardiovascular health and contribute to its relatively high smoke point. Genetic transformation techniques have been in place for canola species since the mid-1990's and have been used to successfully create genetically modified (GM) canola varieties readily grown in North America. Targeted genome engineering via CRISPR-Cas systems provides a highly selective approach for molecular breeding of canola varieties and could potentially result in relaxed regulation of novel varieties compared to GM technologies among global trading partners. Owing to its unique genetic makeup, well-annotated genome, and genetic similarity to the model plant *Arabidopsis thaliana, Brassica napus* has been selected for extensive study and manipulation by gene-editing. We have been successful in the implementation of CRISPR-Cas9 gene editing systems via *Agrobacterium*-mediated genetic transformation of *B. napus* followed by subsequent segregation of transgenic material, resulting in stable, transgene-free, gene-edited plants. Gene knockouts have been the most reliable approach to achieving desired phenotypes by gene-editing, but additional variants and enzyme fusions also make it possible to achieve modified genes previously identified by chemical mutagen screens. Our work focuses on the manipulation of key regulatory pathways governing plant yield (e.g., stem architecture and reproductive potential) in order to maximize canola yield and improve breeding technologies. Continued expansion of the CRISPR-Cas9 toolset will allow for further optimizations to *in planta* editing efficiency and have made accessible nearly all annotated genes of the *Brassica napus* genome.

About the Presenter

Neil began his graduate studies in 2017, passing his Ph.D. candidacy exam in March 2020. This was immediately followed by a guest lecture series on CRISPR-Cas9 topics in plant biotechnology. His graduate research focused on the hormonal and post-translational regulation of seedling development, and he became involved in the design and implementation of the CRISPR-Cas9 system in the Samuel Lab for *Arabidopsis thaliana*, *Cicer arietinum* (chickpea), *Pisum sativum* (pea), *Glycine max* (soybean), and *Brassica napus* (canola) for the targeted mutation of various genes. This involves careful analysis of genomic DNA, gene expression, and precise selection of potential target positions within the gene of interest to reduce the risk of off-target effects and enable proper Cas9 function. Neil has expanded this work and applied it to several research projects within the Samuel Lab, with the goal of uncovering mechanisms for agricultural trait improvement focused on optimizing crop yield.

Tuning GSH Content by Multiplex Genome Editing Affects Drought Tolerance in Rice

Dr. Chin-Yu Wu, Postdoctoral Research Fellow, National Taiwan University, Chinese Taipei

Glutathione (GSH), a tripeptide, is involved in many physiological processes and plays a role in the response to stresses in plants. In addition to acting as a non-enzymatic antioxidant, GSH modulates physiological functions through S-glutathionylation or redox change in plant cells. Water stress is a major negative factor in agricultural production. Our recent study showed that exogenous GSH reduces dehydration tolerance in rice. To further investigate the role of GSH in dehydration tolerance, we studied the T-DNA insertion mutant of the key GSH synthesis gene, OsGSH1-1. Unfortunately, the loss of GSH1 caused a lethal phenotype. To obtain rice plants with varying levels of GSH, we performed multiplex genome editing on the promoter of the OsGSH1-1. Additionally, we generated rice plants with elevated GSH levels using an estrogen-inducible system. The genome-edited lines exhibited a decrease in GSH content by 27% - 49% compared to the WT, while GSH was increased by 20% in the estrogen-inducible line. The attenuation of GSH content resulted in a dehydration-tolerant phenotype compared to the WT. Conversely, elevated GSH increased sensitivity to dehydration stress in rice. These results demonstrate that GSH could act as a negative regulator for dehydration tolerance in rice.

About the Presenter

Chin-Yu Wu is a dedicated postdoctoral fellow in the field of crop functional genomics at National Taiwan University. His primary research revolves around the intricate interplay between two essential plant compounds, glutathione and abscisic acid (ABA), with a specific focus on rice. Chin-Yu's work delves into the fascinating realm of seed germination and the plant's response to dehydration stress. By investigating the interaction of glutathione and ABA in these processes, he aims to unlock crucial insights into the molecular mechanisms governing plant growth and adaptation to environmental challenges. With a passion for scientific discovery and a commitment to sustainable agriculture, Chin-Yu's research has the potential to pave the way for innovative strategies to enhance crop productivity and resilience in the face of changing climatic conditions. As a promising young scientist, Chin-Yu Wu's contributions to the field of crop functional genomics hold great promise for the future of agriculture and food security.

Developing the Fruits for the Future: Precision Breeding in Fruit Crops

Dr. Bernardo Pollak, Chief Executive Officer, Meristem, Chile

Meristem is a biotech startup that aims for the future. Our approach involves integrating innovative *in vitro* culture techniques, gene editing, and regeneration to develop a streamlined workflow for trait improvement in elite fruit cultivars. The experiment design for our *In vitro* Organogenesis Pipeline Platform involves strategies for experimentation and process optimization that allow testing a wide range of conditions with just two or three variables, facilitating the formulation of culture media. The use of this approach allows limiting the number of experiments and at the same time, obtaining enough data to carry out statistically powerful analyses. The culture media are prepared according to the experimental design, considering the tissue that we want to finally obtain–callus, shoots, or roots. Different organs are propagated *in vitro* (e.g., stem, leaves, roots), and they are used as a starting material for our transformation platform. The responses of each tissue are analyzed based on a wide range of variables, allowing us to determine the best *in vitro* condition for each desired process.

About the Presenter

Dr. Bernardo Pollak graduated in Biochemistry from the Pontifical Catholic University of Chile and earned a Ph.D. in Plant Sciences from the University of Cambridge, specializing in Plant Synthetic Biology. Subsequently, he conducted postdoctoral research at the J. Craig Venter Institute in La Jolla, California, where through a Moore Foundation grant, he developed foundational tools for diatom genetic engineering. He returned to Chile with the aim of developing technology in the fruit industry, and he founded Meristem, a biotech startup focused on developing novel fruit varieties using gene editing, in 2020.

Induction of Resistance to Sugarcane Mosaic Virus by RNA Interference Targeting Viral Coat Protein in Sugarcane

Dr. Rikno Harmoko, Research Scientist, National Research and Innovation Agency, Indonesia

RNA interference (RNAi) inhibits gene expression through RNA-mediated sequence-specific interactions and is considered an effective approach to control viral infection in plants. In this study, the *SCMVCp* gene encoding the coat protein (CP) was inserted into the pGreen0179 plasmid in both sense and antisense orientations. The 35SCaMV and ZmUbi promoters were selected to drive the transcription of the RNAi constructs, called HpSCMVCp-CaMV and HpSCMVCp-Ubi, respectively. Transgenic sugarcane expressing these constructs was generated through *Agrobacterium*-mediated transformation. Southern blotting revealed a single stable insertion of the DNA target in the genome of transgenic sugarcane lines. After artificial virus infection, lines that developed mosaic symptoms were classified as susceptible, whereas those that remained green without symptoms were classified as resistant at 42 days post-inoculation. Immunoblotting revealed CP expression at 37 kDa in susceptible and non-transgenic sugarcane, but not in resistant lines. RT-PCR analysis confirmed viral *Cp* and *Nib* gene expression in susceptible lines and their absence in resistant lines. We concluded that RNAi is effective for inducing resistance against SCMV and that the Ubi promoter is an effective promoter for producing transgenic sugarcane.

About the Presenter

Rikno Harmoko is a Researcher in the Research Center for Genetic Engineering, National Research and Innovation Agency, Indonesia. Previously, he was a researcher at the Indonesian Institute of Sciences (LIPI) for two years. He holds a bachelor's in agronomy from Jember University, Indonesia. Harmoko completed his master's and Ph.D. at Gyeongsang National University, Republic of Korea, where he also did the post-doctoral program for two years. He is very interested in molecular biology/biotechnology and has actively collaborated with researchers in plant science, particularly in plant stress response, plant hormone signaling, and glycobiology. Focusing his research on monocot crops, Dr. Harmoko develops sugarcane resistant to mosaic disease using molecular biology approaches such as RNA interference and genome editing. In rice, he investigates the contribution of the N-glycan structure of the protein to plant growth, phytohormone regulation, and stress response. Dr. Harmoko has published his research in several reputable journals in plant science.

Use of Genetic Engineering to Develop Resistance to Biotic Stress in Plants

Dr. Tetsuya Yoshida, Researcher, Crop Disease Research Group, Institute of Agrobiological Sciences, NARO, Japan

Crop production equivalent to feeding 800 million people is lost due to crop diseases. Disease resistance breeding is a powerful strategy for controlling plant diseases. Disease resistance can be developed by genetic engineering, such as gene editing. Knocking out a host factor involved in plant-pathogen interaction by conventional plant gene editing technology can confer disease resistance. However, this has some bottlenecks. For example, it is time- and labor-intensive, removing transgene by segregation is required, and the expression level of gene-editing components is not always high. Plant gene editing using virus vector is advantageous because it can be simple and easy, bypassing the use of transgene and have high expression level of gene editing components.

About the Presenter

Dr. Yoshida is a researcher at the Crop Disease Research Group, Division of Plant Molecular Regulation Research, Institute of Agrobiological Sciences at the National Agriculture and Food Research Organization (NARO) in Japan. He received his bachelor's degree and Ph.D. in agriculture from the University of Tokyo in 2013 and 2019, respectively. His research focuses on the interactions between plants and viruses and the development of strategies to control plant viruses. He has studied the molecular mechanisms underlying the replication of plant positive-sense RNA viruses, including viruses that cause significant crop loss, and the functions of host proteins that affect viral accumulation. He is also currently working on the development of plant gene editing technologies using virus vectors.

CRISPR Tools-Mediated Pepper Genome Editing

Dr. Hyeran Kim, Assistant Professor, Kangwon University, The Republic of Korea

Targeted crop improvement is critical for achieving global food security and improving human nutrition. Traditional breeding programs and modern molecular breeding techniques have increased crop yield and quality. However, conventional plant breeding procedures have been time and resource constrained. A newly improved crop takes a long time to reach the market, and genetic sources from wild species are not always available for crops of interest. CRISPR-Cas9, a distinguished tool in the field of genome editing, has gained prominence due to its remarkable speed, simplicity, and cost-effectiveness, surpassing previous methods like zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN). The utilization of CRISPR tools has significantly accelerated fundamental and applied crop science research. Several genome-edited (GE) products, such as high oleic soybean, powdery mildew-resistant wheat, and brown-free mushrooms, have reached the global market, indicating their readiness for commercialization. We explored DNA-free genome editing techniques utilizing CRISPR-Cas9 ribonucleoprotein (RNP) and CRISPR-Cpf1 RNP for precise crop editing in *Brassicaceae*, *Solanaceae*, soybean, and other plant species. Despite the availability of cutting-edge genome editing tools for crops, the commercialization of precise editing applications in recalcitrant species for plant regeneration remains a challenge. Here, we present significant achievements, limitations, and recent advancements in the molecular breeding of pepper through precise genome editing.

About the Presenter

Hyeran Kim obtained a Ph.D. at the POSTECH in the Republic of Korea in 2007 and did a Postdoc at the MPIPZ in Cologne for four and half years. Her earlier work involved plant cellular protein trafficking and plant-microbe (fungal) interactions for seven years. She joined the Institute for Basic Science (IBS) to study Plant Genome Editing in 2014. Since September 2017, as an associate professor, she started her own group for various research interests; vesicle trafficking, environmental stresses, plant genome editing, and crop improvement. Recently, her group has been focusing on pepper genome editing.

Analysis of XX, SRY Positive Offspring of a SRY-knock in Bull

Dr. Alba Ledesma, Post-Doctoral Researcher, University of California–Davis, The United States of America

In mammals, the sex-determining region of the Y chromosome (SRY) expresses a protein in early embryogenesis that initiates male sexual differentiation and inhibits formation of the female gonad. Previously, a targeted knock-in of SRY:GFP into the safe-harbor H11 locus of chromosome 17 was accomplished using CRISPR-Cas9 genome editing in bovine zygotes to produce a XY bull calf, Cosmo (Fig. 1A). Sequencing revealed a compound heterozygote biallelic edit at the target location on chromosome 17, comprised of a complex 38 kb knock-in allele with seven concatenated copies of the SRY:GFP template and a single copy of the donor plasmid backbone on one chromosome (Fig. 1C), and a random 26 base pair insertion on the other (Owen et al., 2021). It was predicted that the offspring of this SRY knock-in bull would be 75% male (50% XY males, and 25% XX infertile phenotypic male individuals), and 25% fertile XX females (Fig. 2). Additionally, 50 % of blastocysts resulting from fertilization with Cosmo's semen would be expected to exhibit green fluorescence due to the inheritance of SRY:GFP on CHR17. The objective of this experiment was to test the hypothesis that inheritance of SRY on chromosome 17 by the offspring of Cosmo would result in XX infertile individuals with a male phenotype.

About the Presenter

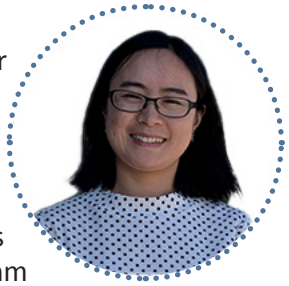
Alba Ledesma is a postdoctoral researcher at the Laboratory of Alison Van Eenennaam at the University of California, Davis. Currently, she is investigating the application of stem cell technologies and genome editing in mammals. She obtained her D.V.M. from Central Buenos Aires University, an M.S. in Animal Science, and a Ph.D. in Agronomy Science from Mar del Plata National University Argentina, where she specialized in gametes collection, evaluation, cryopreservation, and embryo production. She received the Next Gen Leadership Award for Advances in Genome Biology and Technology in 2022. Dr. Ledesma's interests are aimed at the application of technology to increase livestock productivity and the promotion of activities for scientific awareness. In 2022, she organized the "Inspiring Women and Femmes in STEM Symposium."

Developing Recombinant Wheat Cultivars with Drought Tolerance Traits

Dr. Xiaoqing Li, Commonwealth Scientific and Industrial Research Organisation, Australia

Drought is a major constraint for agricultural production around the world, and with climate change, the severity of drought and its frequency will increase in many regions. Studies have shown that more than 40% of inter-annual wheat production variability is mainly due to heat waves and drought conditions throughout the world. Under drought, roots are the first organ exposed to the drying soil and the origin of the signals that coordinate the plant's response. Optimization of the root system is critical for developing crops that are better adapted to a drying climate. In this work, we used recombinant inbred lines (RILs) generated from two parental lines with contrasting root traits to phenotype for various root and shoot traits and to identify quantitative trait loci (QTLs) for key root traits. The results from this work provide opportunities to breed cultivars for particular environments, such as those susceptible to drought.

About the Presenter



Dr. Xiaoqing Li is an early career researcher. Her research experience ranges from plant molecular biology to plant physiology and morphology. She started her research career at China Agriculture University (CAU, China), where she studied the formation of cluster root in white lupin. Xiaoqing pursued her Ph.D. at Lancaster University in the United Kingdom to investigate plant root development and hormone signaling during soil drying. Her postdoctoral project in CSIRO Agriculture and Food (2016-2020) aimed to boost crop yields by delivering energy-efficient roots through phenotyping in the major crop, wheat. Xiaoqing then joined the Cotton Fibre Quality Team in CSIRO as a Research Scientist to develop novel cotton fibres through genetic engineering. Xiaoqing is now developing various capabilities, including gene editing technologies to support the Cotton Breeding Program, while also leading the development of Traceable Cotton Fibres. She received the Science and Innovation Awards for Young People in Agriculture, Fisheries, and Forestry in 2022.

Tastier Pea Protein Through CRISPR/Cas9 Gene-Editing

Dr. Connor Hodgins, Researcher, University of Calgary, Canada

Pea protein is a vital component of sustainable agriculture systems. The problem is that saponins cause bitter off-flavors in peas. Mutation of BAS can prevent their biosynthesis in peas. In our research, gRNAs 2, 3, and 4 were selected based on *in vitro* testing. The CaMV35S and AtU6-26 promoter were tested for gRNA expression. A multi-gRNA expression system was optimized in pea hairy roots, and two homozygous mutant lines were identified with a >99% reduction in saponin.

About the Presenter

Dr. Connor Hodgins is currently a post-doctoral fellow working on a collaborative project between the University of Calgary and a start-up company, AgGene. His Ph.D. training utilized CRISPR/Cas9 gene-editing to study the biosynthesis of specialized chemicals in lettuce and peas. Of particular interest to his current position was his development of novel traits in peas related to improved flavor. This was accomplished through the editing of a biosynthetic pathway in peas, which produced chemicals called saponins. Saponins are purified with pea protein and give it bitter and astringent off-flavors. The trait he developed in peas prevents them from producing saponins, thereby improving the flavor of the pea's protein. The skills he developed that allowed him to improve pea flavor are useful for almost any trait. His work at AgGene is based around utilizing these same skills to further develop improved pea varieties.

Development of Allergenicity and Toxicity Assessment Methods for Evaluating Transgenic Sugarcane

Dr. Widhi Dyah Sawitri, Assistant Professor, Gadjah Mada University, Indonesia

Sugarcane is considered an industrial crop that produces sugar. The number of transgenic sugarcane on the market is currently increasing. Therefore, investigation of the potential allergens and toxins in transgenic sugarcane is necessary since there is less information regarding food safety for human consumption. Bioinformatics and experimental analysis were used for the validation of the allergenic potential of transgenic sugarcane, such as analysis of amino acid sequences using the AllergenOnline software; *in vitro* assessment method using heat stability, simulated gastric fluid (SGF), simulated intestine fluid (SIF); and *in vivo* assessment method using ELISA analysis for IgE measurement in rats. An acute oral toxicity assay was performed by oral gavage of transgenic sugarcane juice in mice. In this study, we propose the development of a method for allergenicity and toxicity assessment in transgenic sugarcane.

About the Presenter

Dr. Widhi Dyah Sawitri is an Assistant Professor of Plant Genetic Engineering at the Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada (UGM), Indonesia. Her research interests focus on agricultural biotechnology and the biochemical study of enzymes in plants, particularly sucrose phosphate synthase from C4 plants. Previously, she worked at the Center for Development of Advanced Science and Technology (CDAST), University of Jember for three years (2016-2019). At that time, she was involved in the development of genetically engineered sugarcane through overexpression of sucrose phosphate synthase and coat protein of sugarcane mosaic virus. In addition, her work on protein engineering through site-directed mutagenesis technique is undergoing research to support functional studies of certain enzymes.

RNAi-Mediated Protection Against Cucumber Mosaic Virus (CMV) in Rockmelon (*Cucumis melo* L.)

Ms. Dharane Kethiravan, Graduate Student Researcher, University of Malaya, Malaysia

Rockmelon is an important tropical fruit with a wide range of health benefits and high nutritional value. However, Cucumber Mosaic Virus (CMV), an aphid-transmitted virus, causes severe damage to its production. The existing control strategy to control viral infection using chemical insecticides are minimally effective and causes deleterious effects on the environment and human health. RNAi is a powerful biotechnological tool that can be used to combat viral infection in plants. RNAi can be triggered in plants by inserting dsRNA of viral genes into the plant genome or by exogenously applying dsRNA on the surface of the plants. In the current study, the protective effect using dsRNA of viral genes by exogenous application was tested in rockmelon. The effectiveness of RNAi protection was measured by the disease severity index (DSI) and compound enzyme-linked immunosorbent assay (Compound ELISA). Based on the DSI and ELISA, rockmelon treated with dsRNA to trigger RNAi in rockmelon showed a reduction in viral symptoms (4.31-fold lower) and titer (4.91-fold lower) compared to rockmelon plants that were not treated with dsRNA. These results indicate that exogenous treatment of dsRNA is an interesting approach as a biopesticide to combat the spread of CMV in rockmelon crops safely and effectively.

About the Presenter

Dharane Kethiravan graduated with a Bachelor of Science in Biology from the University of Malaysia Terengganu. She received an award for being the best student academically during her degree. With her knowledge and passion in biological sciences, she is pursuing a Ph.D. She is currently a Ph.D. candidate at CEBAR, University of Malaya, Malaysia. Her Ph.D. project is on protecting crops by using RNAi, a powerful biotechnology tool that can knockdown viral proteins to protect plants against virus infection. Her passion and interest is to make a meaningful contribution to addressing the global food demand while mitigating environmental risks.

Biofortification of Rice Grains Through Genome Editing: Addressing Zinc Deficiency in Asia-Pacific Region

Mr. Erwin Arcillas, Assistant Scientist, International Rice Research Institute, The Republic of the Philippines

Zinc deficiency has a high prevalence in the Asia-Pacific region, with the indicator of stunting in children under 5yo recorded at 77.2 million in 2018 (FAO, UNICEF, WFP, and WHO 2019). Diversification of diet, supplementation, and commercial food fortification are practiced but are inaccessible to marginalized populations. Traditional biofortification through breeding is limited by gene pool restrictions and linkage drag. Genome-editing using Site-directed Nucleases (SDN) is employed to exploit the rice nicotianamine synthase 2 (*OsNAS2*) gene, which encodes an enzyme responsible for synthesizing the zinc chelator nicotianamine. Promoter modification (SDN-1), promoter replacement (SDN-2), and targeted insertion (SDN-3) using CRISPR-Cas9, and TALENs are performed. Zinc concentrations in seeds of edited and non-edited plants are measured.

About the Presenter

Erwin Arcillas is a molecular plant biologist with a passion for translational crop research. He received his B.Sc. in Agriculture and M.Sc. in Genetics from the University of the Philippines Los Baños. His research focus is on the development of new rice varieties using plant biotechnology, with a particular interest in varieties that are more nutritious to alleviate micronutrient deficiencies. In addition to his research, Erwin is also passionate about graphic design. He enjoys designing posters and slide decks, as well as creating illustrations for book chapters, journal articles, and review articles. He believes that graphic design can help people understand complex scientific concepts in a clear and engaging manner.

Microbial-Based Technology: Their Roles in Sustainable Agriculture

Dr. Panaya Kotchaplai, Chulalongkorn University, Thailand

Microorganisms play many important roles in promoting sustainable agriculture. For example, plant growth-promoting microorganisms can enhance plant tolerance to stresses, ultimately increasing crop yield and quality. Certain microorganisms can produce allelochemicals, which reduce the need for agricultural chemicals and promote environmentally friendly practices. Some microorganisms aid in the degradation of contaminants and the restoration of degraded land, contributing to environmental conservation. One issue resulting from the increasing agro-industrial activities is the accumulation of lignocellulosic by-products and waste, which can have negative environmental consequences if not managed properly. To address this concern, researchers are exploring the biovalorization of lignocellulosic biomass. Lignin, a complex structure comprising heterogeneous aromatic compound, has been proposed as a renewable aromatic source for valuable compound production. Our study focused on the conversion of ferulic acid, an abundant lignin derivative, to vanillin, a highly demanded compound in the food and fragrance industries. In well-studied strains such as *Pseudomonas*, *Amycolatopsis* and *Streptomyces*, ferulic acid is typically converted to vanillin via the CoA-dependent pathway. However, in certain *Bacillus* and yeast strains, phenolic acid decarboxylase (encoded by *padC*) catalyzes the rapid conversion of ferulic acid to 4-vinylguaicol, a highly toxic compound. Interestingly, the decreasing 4-vinylguaiacol was found to be concurrent with the increasing amount of vanillin. We then explored *Bacillus* enzymes involved in vanillin production and found that CYP102A2, a *Bacillus* cytochrome P450, may be a potential enzyme. However, developing PadC-CYP102A cascade proved challenging due to low vanillin yield and cell stress caused by cytochrome P450 overexpression. Recently, aromatic dioxygenase (Ado) has been reported for its ability to catalyze the coenzyme-free oxidation of 4-vinylguaiacol to vanillin. Resting cells of *Escherichia coli* BL21(DE3) overexpressing *Bacillus* PadC and codon-optimized Ado demonstrated rapid conversion of ferulic acid to vanillin. This CoA-independent enzyme cascade presents an efficient biocatalyst for vanillin production and holds potential for further development as a cell-free system, making it a promising approach for lignin biovalorization

About the Presenter

Panaya Kotchaplai got a B.Sc. in Biochemistry and a Ph.D. in Environmental Management from Chulalongkorn University, Thailand. During her Ph.D., she focused on how bacteria respond and adapt to stressors, and how these adaptive changes affect their phenotypes and activity. Following her Ph.D., she took on a role as an industrial postdoctoral researcher at the Department of Biochemistry, Faculty of Science, Chulalongkorn University, where she developed an interest in the biovalorization of agro-industrial waste and byproducts. She focused

on the development of a microbial biocatalyst for bioproduction of vanillin from lignin derivative(s). Currently, she is a researcher at the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, where she primarily focuses on harnessing microbial-based technology to promote a sustainable future. Her work involves developing biocatalysts to valorize agro-industrial residues, providing an alternative approach to agricultural waste management. Additionally, she also focuses on bioremediation and the restoration of degraded land.

Pennycress (*Thlaspi arvense*) Seed Size Mutants Affect Oil Accumulation Differently

Ms. Liza Gautam, Research Scholar, Illinois State University, The United States of America

Domesticated pennycress varieties (CoverCress™) have been developed having reduced seed coat fiber content, low erucic acid seed oil content, and which produce over 1,500 pounds of seed per acre, yielding 65 gallons of oil and 1,200 pounds of meal per acre. To improve this oilseed cash cover crop further, we are exploring ways to increase seed size and oil content. Three genes in which we have generated mutations are *DA1*, *DA1-RELATED* (*DAR1*), and *UBIQUITIN PROTEIN LIGASE3* (*UPL3*). In Arabidopsis, *DA1* and the functional homologue, *DAR1*, encode ubiquitin receptors thought to set final seed and organ size by restricting the period of cell proliferation in the seed integuments. UPL3 was shown in Arabidopsis to mediate proteasomal degradation of, among other targets, the transcription factor LEC2. LEC2 is known to activate expression of seed maturation and seed lipid accumulation genes. We found that pennycress *da1dar1* double mutants produced seeds that were 44 percent larger and 50 percent heavier than wild type; *upl3* mutant seeds were 17 percent larger and 16 percent heavier than wild type. Surprisingly, pennycress *upl3* mutant seeds had less oil per seed than wild type, even though the *upl3* mutant seeds were 17 percent larger. By contrast, *da1dar1* double mutant seeds had nearly 41 percent more oil per seed. Our results indicate that mutations in *DA1* and *DAR1* may be attractive targets for increasing seed size in conjunction with increasing seed oil content in domesticated pennycress and other Brassica oilseed crops.

About the Presenter

Liza Gautam is a Ph.D. candidate from Kathmandu, Nepal. Currently, she is working at Prof. John C. Sedbrook's laboratory in the Department of Biological Science at Illinois State University (ISU). She completed her Bachelor of Technology in Biotechnology from Kathmandu University (KU), Nepal, and her Master of Science in Biotechnology from the Norwegian University of Science and Technology (NTNU), Norway. After completing her master's degree, she worked as an Assistant Research Fellow in the Nepal Academy of Science and Technology (NAST), a leading research station and governmental organization in Nepal. As a molecular geneticist, Ms. Gautam is currently working on domesticating an oilseed cash cover crop-Pennycress (*Thlaspi arvense L.*). Her primary focus is implementing CRISPR-Cas9 genome editing technology to target multiple genes at different loci to increase seed size, reduce glucosinolate content, and increase drought tolerance in pennycress, and she would like to pursue a career in research and development.

Improved Bacterial Leaf Blight Disease Resistance in Major Elite TBR225 Rice Cultivar Using CRISPR/Cas9 System

Dr. Nguyen Duy Phuong, Head of Molecular Pathology Department, Agricultural Genetics Institute, Viet Nam

Bacterial leaf blight disease (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a significant rice disease in Viet Nam. Most Vietnamese commercial rice varieties, including TBR225, are susceptible to BLB. The virulence of *Xoo* depends on the transcriptional activation of specific host disease-susceptibility (S) genes by transcription activator-like effectors (TALEs). TALEs bind to specific host nuclear gene promoter sequences termed Effector-Binding Elements (EBEs) and induce target gene expression to benefit the pathogen. Three S genes, *OsSWEET11*, *OsSWEET13*, and *OsSWEET14*, coding for transmembrane sugar exporter proteins are known to be targeted by several unrelated TALEs of all Xoo strains in nature. The clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 nuclease (CRISPR/Cas9) system is a simple and efficient gene-editing tool developed in the past few years. This project focuses on improving the BLB resistance of the TBR225 cultivar through identifying and editing the transcriptional target of Vietnamese *Xoo* (VXO) in the TBR225 genome by using CRISPR/Cas9 tool.

About the Presenter

Dr. Nguyen Duy Phuong is a senior researcher at the Agricultural Genetics Institute in Viet Nam. He is also a visiting lecturer at the Viet Nam National University of Agriculture and University of Engineering and Technology, Viet Nam National University, Hanoi, Viet Nam. He completed a bachelor's, master's, and Ph.D. in Biochemistry at Hanoi University of Sciences – Viet Nam National University, Hanoi, Viet Nam. During his Ph.D., he did an internship and completed his doctoral thesis on identifying the transcription factor encoding the gene related to drought tolerance in rice at the International Centre for Genetic and Engineering Biotechnology, New Delhi, India. He has published over 50 publications in scientific journals of repute and three monographs. His current research looks at plant pathology, abiotic stress response, developing plant pathogen detection kits, and applying new technology in crop breeding.

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