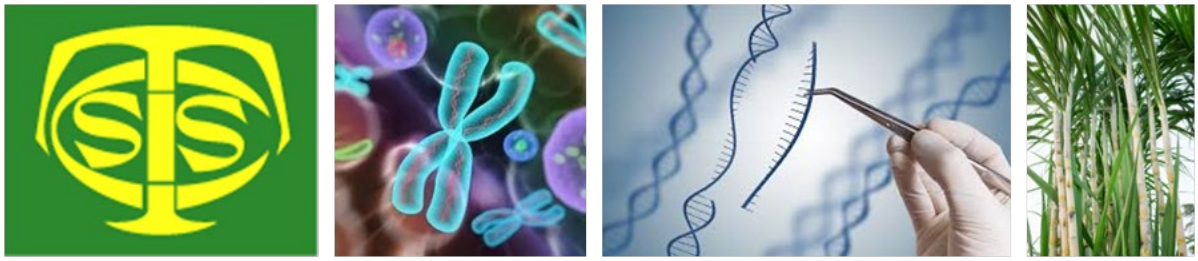

ISSCT MOLECULAR BIOLOGY WEBINAR

Tuesday 2 August 2022

11 a.m. GMT

“Genome Editing of Sugarcane"

As part of the series of Webinars that will be organized by the ISSCT in 2022 for its different disciplines, the **Molecular Biology** Webinar of the Biology Commission will be held on Tuesday 2 August 2022 at 11 a.m. GMT.

Programme
Introduction by María Francisca Perera, from the ISSCT Molecular Biology Section.

Main topics

Technology, applications and regulation

- **Editing the Complex Sugarcane Genome: Technology Overview, Challenges and Perspectives by Prof. Fredy Altpeter (University of Florida)**

Sugarcane is a prime feedstock for commercial production of biofuel and table sugar. Genome editing tools such as CRISPR/Cas9 and TALEN have been employed in several crops including sugarcane. They enable precise targeting and introduction of double stranded DNA breaks *in vivo*. Subsequent cellular repair mechanisms, predominantly non-homologous end joining (NHEJ), act as critical steps to endogenous gene editing. However, there is very limited control over these mechanisms, which generate an abundance of random insertions and deletions (indels). Frameshift mutations associated with these indels of unspecified size and sequence might result in loss of function phenotypes of agronomic importance. Gain of function mutations, on the other hand, generally requires precise nucleotide substitutions in the target locus. This can be accomplished with the aid of a homologous repair template by involving the cellular homology directed repair (HDR) mechanism, or alternative approaches including base editing and prime editing. The progress with both targeted mutagenesis and gene targeting since our first report of successful editing of the complex sugarcane genome in 2016 will be reviewed. Challenges posed by highly polyploid genomes and synthetic biology perspectives for fueling the emerging bioeconomy will be discussed.

- **International perspectives on the (de)regulation of genome-edited crops by Dr. Hennie Groenewald (Biosafety South Africa; ISBR Board member)**

Genome editing (GEd) holds huge potential benefits as it allows the relatively quick, efficient, accurate and cost-effective modification of valuable genetic traits in crops, livestock and micro-organisms. However, to realize these benefits, products

developed using GEd must be subject to fit-for-purpose, science-based safety regulations that satisfactorily manage national priorities, while allowing meaningful international integration. Discussions regarding the governance of GEd invariably raise questions whether the organisms resulting from its application should be considered ‘‘genetically modified’’ (GM) or not - and as a result be regulated as such or not. Depending on the regulatory approach taken in each country or region, the commercialization of these crops and their products may or may not require approval from the respective regulatory authorities. GEd crop developers, therefore, need to be aware of the current mosaic of regulatory schemes that affect these products. This presentation will provide an overview of the varied approaches to GEd regulation taken by several jurisdictions around the world and discuss the underlying principles on which these approaches are based.

Country case studies

The projects of four research centers in sugarcane genome editing will be presented by:

- Viktoriya Coneva from Centro de Tecnologia Canavieira (CTC), Brazil

Progress towards the development of efficient transgene-free sugarcane genome editing tools at CTC

Genome editing approaches enable targeted gene knock-out and templated modifications, thereby allowing for the generation of plants with precise genome alterations. Remarkably, such changes can be accomplished without T-DNA integration (transgene-free), which is of prime relevance to the ability to directly edit elite sugarcane germplasm. Scientists at CTC Genomics, a subsidiary of CTC located in St. Louis, MO, USA, are working to adapt and optimize genome editing tools for using in sugarcane. Current progress will be discussed with an emphasis on producing GM-free knock-outs in the context of the main challenges presented by sugarcane – its polyploid genome with limited genomic resources and the vegetative propagation of elite germplasm.

- Germán Serino from Chacra Experimental Agrícola Santa Rosa, Argentina

Sugarcane editing research in Chacra Experimental Agrícola Santa Rosa

Genome editing was tested in sugarcane using ALS herbicide tolerance as a model. Three vectors, each targeting specific segments of the sugarcane ALS gene and also three DNA templates, were designed. Combinations of the editing vectors, DNA templates and a transformation vector bearing selective marker NPTII were co-transformed into the cultivar NA05-860 and resistant calli selected in culture medium using standard protocols currently used at Chacra. Plants derived from independently transformed calli were transferred to the biosafety greenhouse, DNA extracted from leaf samples and analyzed by RT-PCR and sequencing. Sequencing confirmed that editing occurred in some of the analyzed plants. Editing resulted in the deletion of the target fragment but not in the desired codon modification. Efforts continue to optimize editing conditions that result in the desired modification.

- Jershon Lopez from Centro de Investigación de la Caña de Azúcar (CENICAÑA), Colombia

Gene editing of sugarcane: a new genetic improvement tool to obtain sugarcane varieties with higher productivity

Since 2018, The Colombian Sugarcane Research Center, Cenicaña, has been working on genome editing, first as a proof of concept to demonstrate that the Crispr Cas9 system could be used in sugarcane. Cenicaña is carrying out research on obtaining a DNA-free protocol using ribonucleoproteins that will allow the generation of non-transgenic edited plants that would be considered as conventional sugarcane varieties under Colombian Agricultural Institute resolution 29299 of 2018. This protocol has been developed through the edition of the sugarcane PDS gene (phytoene desaturase) that will allow the edited plants to be distinguished by their albino phenotype produced after knocking out the gene. Additionally, work is being done on editing the ALS gene associated with herbicide resistance, which would allow easy discrimination of edited plants. Cenicaña is also working on an appropriate in vitro culture system that will allow gene editing with greater efficiency. It is expected that with the implementation of this genome editing methodology, Cenicaña will have new sugarcane varieties with higher sucrose and biomass contents, and with better response to water and nitrogen efficiency, making the Colombian sugar sector more sustainable and competitive.

- Yogesh Parmessur from Mauritius Sugarcane Industry Research Institute (MSIRI), Mauritius

Application of Gene editing in the knocking out of flowering genes in sugarcane

Profuse flowering in some commercial sugarcane cultivars poses a major problem in Mauritius due to its association with pith formation and sucrose loss. One approach to limit flowering is to suppress the activity of gene(s) in the sugarcane flowering pathway. This may be achieved by knocking out selected flowering genes using the CRISPR/Cas9 gene editing (GE) tool. Initial experiences of the strategy adopted for implementing the technology to sugarcane are described.

The facilitators are: Dr. Karen Aitken from CSIRO, Australia, Dr. Mike Butterfield from CTC, Brazil and Dr. Yogesh Pasmessur from MSIRI, Mauritius.

Discussion

Q&A Session

Conclusion

Language
The Webinar will be conducted in English.

Registration
The Registration Form for the Webinar may be accessed through the following link:
https://us06web.zoom.us/meeting/register/tZIqf--prDIsG9dlKKbvBLE1ZTiQVtWkONel

The Webinar is only open to compliant members of ISSCT. When you register for the webinar, your registration will be checked against ISSCT membership and payment records, and you will receive an e-mail with the meeting details (Username and Password to access the Webinar). If you have outstanding membership dues, you will be asked to pay these online before your registration is approved. The Individual Membership dues are USD 140 and may be settled through the following link:
http://members.issct.org/appform/issct_india_2022.aspx

Further information
For further information, please contact Francisca Perera, franciscaperera@yahoo.com.ar or the ISSCT Secretariat at issct@intnet.mu

Please click here for short biodata on the presenters