BioCubagro2025

From biotechnological research to agricultural products

June, 8th-13th

Varadero, Cuba

ABSTRACT BOOK

The most recent and novelties results in biotechnology applied to Agriculture. The proactive relationship of Agriculture-biotechnological research between basic science and applied research.

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CIGB CENTRO DE INGENIERÍA GENÉTICA Y BIOTECNOLOGÍA

PROGRAMA / PROGRAM

Lunes 09 / Monday 09

Sala Melia Internacional I / *Room Melia Internacional I*
Sesión: Mejoramiento de plantas mediante ingeniería genética y edición de genomas/
Session: Improvements of plants by genetic engineering and genome editing tools

08:30 - 09:30 Conferencia plenaria / *Plenary lecture*
The use of Genetic Engineering to Address Future Food Security and Sustainability
Mario Pablo Estrada García
CIGB, Cuba

09:30 - 10:30 **Conferencia plenaria / *Plenary lecture***
The experience of Brazil and EMBRAPA in editing plant genomes
Francisco Aragao
EMBRAPA, Brasil

10:30 - 11:00 RNAi-based Technologies for Management of Plant Viral Diseases
Basavaprabhu Patil
ICAR-Indian Institute of Horticultural Research, India

11:00 - 11:30 Merienda / *Coffee break*

11:30 - 12:00 Twenty years (2005-2025) of experience in the development of sugarcane transgenic events at ITANOA (EEAOC-CONICET)
Atilio Pedro Castagnaro
ITANOA, Argentina

12:00 - 12:30 Platform to improve maize and sugarcane using biotechnology and traditional methods
Pilar Tellez
CIGB, Cuba

12:30 - 13:00 Comprehensive approach to the soybean genetic improvement program by biotechnological methods at the CIGB
Gil Enríquez
CIGB, Cuba

13:00 - 13:30 Fronteras biotecnológicas en la producción de vainilla: avances en conservación, mejoramiento genético y procesamiento para una producción sostenible
Miguel Angel Esquivel Pérez
Instituto de Investigaciones de Ingeniería Agrícola, Cuba

13:30 - 15:00 Almuerzo / *Lunch*

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Uso de las plantas como Biorreactores para la Producción de fármacos/ *Session*: Use of plants as biorreactors for the pharmaceuticals production.

15:00 - 15:30 Developing Mutant-Resistant Monoclonal Antibodies Effective Against All SARS-CoV-2 Variants Using Plant Biotechnology
Qiang "Shawn" Chen
Arizona State University, USA

15:30 - 16:00 Bridging Synthetic Biology and Molecular Farming: a copper sensor and geminivirus-based Processors for engineering gene circuits in plants
Elena García Pérez
Earlham Institute, United Kingdom (online)

16:00 - 16:30 Update on the approach to plant molecular farming at the CIGB
Abel Hernández
CIGB, Cuba

16:30 - 17:00 Updated Critical Analysis of the Potential of Transgenic Plants and Plant Cells for the Production of Monoclonal Antibodies and Recombinant Proteins.
Rodolfo Valdes Veliz
CIGB, Cuba

17:00 - 17:30 GBS-based genotyping and morphoagronomical characterization of cacao accessions from Cuban gene bank reveal their potential for breeding and production
Angel Rafael Ramírez Ramírez
Universidad de Guantanamo, Cuba

Martes 10 / Tuesday 10

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Modelos de interacción planta-ambiente y uso de herramientas bioinformáticas avanzadas / *Session: Plant-environment interaction models and the use of advanced bioinformatics tools.*

08:30 - 09:30 Conferencia plenaria / *Plenary lecture*
Effect of Environmental conditions on Virus Infections, Gene Silencing and their implications on Virus Induced Gene Silencing (VIGS)
Basavaprabhu Patil
ICAR-Indian Institute of Horticultural Research, India

09:30 - 10:00 Rhizosphere bacteria for growth stimulation and increasing stress resistance of plants of the family Solanaceae
Ananyeva Iryna
Institute of Microbiology NAS, Belarus

10:00 - 10:30 Endophytic bacteria for increasing stress resistance of alfalfa Medicago sativa
Fedorenchik A.A.
Institute of Microbiology NAS, Belarus

10:30 - 11:00 Mechanism of copper accumulation in the marine alga *Ulva compressa* (Chlorophyta)
Alejandra Moenne
Universidad de Santiago de Chile, Chile

11:00 - 11:30 Merienda / *Coffee break*

11:30 - 11:50 Metabolization of polycyclic and linear hydrocarbons in the marine macroalga *Ulva lactuca* (Chlorophyta)
Alberto Gonzalez
Universidad de Santiago de Chile, Chile

11:50 - 12:10 Construcción de un modelo metabólico a escala genómica del hongo Leucoagaricus gongylophorus LEU18496 para el análisis de sus capacidades metabólicas a partir de datos ómicos.
Freddy Castillo Alfonso
Universidad Autónoma Metropolitana, México

12:10 - 12:30 Study of the secondary metabolite profile in Trichoderma spp. strains with antifungal activity using LC-MS technique
Beatriz Fernández Millares
Universidad de la Habana, Cuba

12:30 - 12:50 Epidemiology-based management strategies for citrus Huanglongbing
Renato B. Bassanezi
Fundecitrus, Araraquara SP, Brazil

12:50 - 13:10 Tracing microRNA targets in tomato plants infected with begomoviruses
Alejandro Fuentes Martínez
CIGB, Cuba

13:10 - 13:30 MicroRNA Analysis of Transgenic Tomato Plants Under Different Environments
Ricardo Bringas
CIGB, Cuba

13:30 - 15:00 Almuerzo / *Lunch*

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Diagnóstico de enfermedades en plantas / *Session: Diagnosis of diseases in plants*.

15:00 - 16:00 Conferencia plenaria / Plenary lecture
A century of diagnostic improvements in a sugarcane pathology changing context: from symptom observation to molecular assays
Philippe Charles Emile Rott
CIRAD, France

16:00 - 16:30 Metagenomic Revolution: The Contribution of the VANA Approach and High-Throughput Sequencing Technologies (Illumina and Nanopore) for the Detection of Emerging Plant Viral Diseases
Denis Filloux
CIRAD, France

16:30 - 17:00 The development of next generation sequencing and specific e-probes for pathogen diagnosis in sugarcane
Claudia Jill Kaye
Sugar USA, USA

17:00 - 17:30 Non-invasive imaging of salicylic and jasmonic acid activities in planta
Anastasia V Balakireva
Planta LLC, Russia

17:30 - 18:00 Loop-mediated isothermal amplification (LAMP) reaction as viable PCR substitute for diagnostic applications in agriculture
Lianet Rodríguez Cabrera
CIGB, Cuba

Miércoles 11 / Wednesday 11

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Actualización tecnológica en la producción de semillas de maíz y soja. Desarrollo de Agronegocios / *Session: Technological update in the production of corn and soybean seeds. Agribusiness Development.*

08:30 - 09:30 Conferencia plenaria / *Plenary lecture*
Hito e impactos del mejoramiento genético, la biotecnología y las buenas prácticas en la producción sostenible de soja.
Rodolfo Rossi
ACSOJA, Argentina

09:30 - 10:00 Estrategia y modelo de negocios de la industria semillera del maíz en el sur - sureste de México
Humberto Castro
Reycoll Seeds, México

10:00 - 10:30 Application and Development of Innovation in Grain Seed Production. Business Model Propose.
Lincidio Pérez Sánchez
CIGB, Cuba

10:30 - 11:00 Shandong Lukang Heber Biotech Cuban-Chinese Joint Venture, a collaborative experience in agrobiotechnology
Marisela Suarez Pedroso
Heber Biotec, Cuba (online)

11:00 - 11:30 Merienda / Coffee break

11:30 - 12:00 Comportamiento de las variedades transgénicas de soya en el Occidente de Cuba
Rodobaldo Ortíz Pérez
INCA, Cuba

12:00 - 12:30 Contribution of CENATOX to the estimation of environmental risk of genetically modified crops.
Odette Cecilia Beiro Castro
CENATOX, Cuba

12:30 - 13:00 Regulación de las Nuevas Técnicas de Mejoramiento (NBTs)
Martin Lema
Universidad de Quilmes, Argentina

13:30 - 15:00 Almuerzo / Lunch

Sala Melia Internacional I / Room Melia Internacional I

13:30 – 18:00 Posters Discussion

Jueves 12 / Thursday 12

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Uso de Bioproductos en la agricultura/ *Session: Use of Bioproducts in agriculture*.

08:30 - 09:30 Conferencia plenaria / *Plenary lecture*
Potential of the National Academy of Sciences of Belarus in the field of chemical sciences for bioagrotechnologies
Aleksei Trukhanov
National Academy of Sciences of Belarus

09:30 - 10:00 Pesticide Effects of Highly Stable Green Synthesized Silver Nanocomposites to be Used in Organic Tomato Crops
Luis E. Trujillo
Universidad de las Fuerzas Armadas ESPE, Ecuador

10:00 - 10:30 A project for the validation of the efficiency of a nanobiopesticide for commercial use in agriculture
Carlos María Noceda
Universidad de las Fuerzas Armadas ESPE, Ecuador

10:30 - 11:00 Desarrollo de Bioinsumos- Caso AGROSAVIA -COLOMBIA
Martha Isabel Gómez Alvarez
Agrosavia, Colombia

11:00 - 11:30 Merienda / *Coffee break*

11:30 - 11:55 HeberNem: characteristics and uses.
Jesus Mena Campos
CIGB, Cuba.

11:55 - 12:20 Biostimulation of Pseudoxanthomonas indica H32 on chard, chinesse cabbage and cucumber
Ileana Sánchez Ortiz
CIGB Camaguey, Cuba

12:20 - 12:45 Estudio de evaluación de efectividad biológica de un promotor de la brotación en tubérculos de papa Fianna
Edgar López
México

12:45 - 13:10 Bioestimulantes de oligosacarinas en la activación del crecimiento, la nutrición, el rendimiento y la protección antiestrés en soya.
Alejandro B. Falcón-Rodríguez
INCA, Cuba.

13:10 - 13:35 Valorization of agro-industrial waste: Use of citrus peels for agricultural pest control
José Manuel Pais Chanfrau
Universidad Técnica del Norte, Ibarra, Ecuador

13:35 - 15:00 Almuerzo / *Lunch*

Sala Melia Internacional I / Room Melia Internacional I
Sesión: Enzimas con uso en la Agro-industria/ Session: Enzymes used in Agro-industry.

15:00 - 15:30 KestoZyme: basis of an integral solution to the main constraints of the current fungal-based technology for short-chain FOS production from sucrose
Carmen Menendez
CIGB, Cuba

15:30 - 16:00 Kestozyme, an opportunity for the production of fructooligosaccharides in a circular economy in the environment of a sugarcane biorefinery
Enrique Pérez
CIGB SS, Cuba

16:00 - 16:30 Purification of fructooligosaccharides obtained from sucrose by a recombinant yeast expressing a mutated levansucrase.
Duniesky Martínez García
CIGB SS, Cuba

16:30 - 17:00 Removal capacity and enzyme stability, two variables influencing recombinant dextranase enzyme performance
Arianne Rubio Sánchez
ICIDCA, Cuba

17:00 - 17:30 Silage as Nature's Factory: Innovations in Food Production, Bioenergy, and Sustainable Solutions
Andrea Martínez
Dundalk Institute of Technology, Panama

Viernes 13 / Friday 13

Sala Melia Internacional I / Room Melia Internacional I

09:00 - 10:00 Conclusiones / *Conclusions*

Carteles/Posters

1. Avances en la obtención de cultivares mejorados de frijol común (*Phaseolus vulgaris L.*) para cosecha mecanizada. Víctor Daniel Gil Díaz y cols., Cuba.
2. Development of a platform for the evaluation *in vitro* of the activity of biological compounds on the growth of *Sporisorium scitamineum*. Javier Lezcano Laguna *et al.*, Cuba.
3. Efficient *Agrobacterium*-mediated transformation of soybean meristematic explants by phosphomannose isomerase/mannose selection. Alejandro Morales Basulto *et al.*, Cuba
4. Establishment of an efficient sugarcane transformation protocol using *Agrobacterium tumefaciens*. Ivis Moran Bertot *et al.*, Cuba.
5. Evaluation of the drought resistance potential of glyphosate-resistant transgenic soybeans. Raidell Saenz Padrón *et al.*, Cuba.
6. Expression of the artificial microRNA156 in soybean leaves using a simple and efficient agroinfiltration protocol. Natacha Soto Perez *et al.*, Cuba
7. Histidine-tagged defensin fusion construct for integration in tobacco plants as model. Kenia Tiel González *et al.*, Cuba.
8. *In vitro* propagation of Citrus spp. a tool for plant production and genetic improvement. María Ileana Oloriz Ortega *et al.*, Cuba.
9. Increasing genetic diversity in a seed bank of transgenic corn by traditional techniques. Davel Espinosa Martin *et al.*, Cuba.
10. Molecular characterization by RAPD markers of different plant varieties obtained by mutations. Ingrid Hernandez *et al.*, Cuba
11. Obtaining bean plants resistant to the herbicide LifeLine. Yadira Sánchez Guerra *et al.*, Cuba.
12. RNA interference-based resistance in transgenic tomato plants against geminiviruses. Natália Faustino Cury *et al.*, Brasil.
13. B3 transcription factors from Nicotiana spp. activate the β-phaseolin promoter *in N. benthamiana* vegetative tissue. Liliam de los Ángeles Rodríguez Deyá *et al.*, Cuba.
14. New products with a dermo-regenative effect derived from sericin hydrolysate. Yuliet Herrera Aguila e*t al.*, Cuba
15. Production of recombinant hemagglutinin from avian influenza in plants. Carlos Enrique González González *et al.*, Cuba.
16. Rapid production of bovine FSH protein in *Nicotiana benthamiana* leaves. Yanaysi Ceballo Cámara *et al.*, Cuba.
17. Characterization of a new isolate of Rhynchosia golden mosaic Yucatan virus (RhGMYuV) identified in soybean crop in Havana. Natacha Carlos Victoria *et al.*, Cuba.
18. Clustering and differential expression analysis of small RNAs; an expression profiling of microRNAs in greenhouse and in field cultivated transgenic tomato plants. Julio Enrique Duque Vizcaíno *et al.*, Cuba
19. Evaluación de genotipos de tabaco frente a *Peronospora tabacina* D. B Adam. Identificación del fragmento BRM1 asociado a la resistencia del moho azul. Verónica Toledo Sampedro *et al.*, Cuba
20. Fusarioids pathogens Causing Wilting of chilli Peppers (Capsicum spp.) in Mexico. Frank Angel Díaz Leyva *et al.*, Mexico.
21. Artificial parthenogenesis: a strategy for obtaining transgenic lines in *Bombyx mori* l. (lepidoptera, bombycidae). Adileidy Ruiz Barcenas *et al.*, Cuba.
22. Design of a molecular method for the diagnosis of *Phyllosticta citricarpa*, based on the amplification of MAT genes. Ana Margarita Manzano León *et al.*, Cuba.

23. Expression, purification and characterization of the capsid protein of the PMWaV-2 viral variant for the development of an immunodetection method associated with the pineapple wilt in Cuba. Ana María Guillén Garcia *et al.*, Cuba.
24. Noninvasive diapause disruption by corona discharge: applications in in *Bombyx mori* L., transgenesis. Daily Hernández Hernández *et al*., Cuba.
25. Obtaining biological reagents for the development of a method for diagnosing sugarcane leaf scald. Angélica Romero Martínez *et al*., Cuba.
26. Obtaining positive controls for the detection of two polymorphic regions in Cuban strains of 'Candidatus Liberibacter asiaticus'. Camilo Paredes Tomás *et al.*, Cuba.
27. Biological tools to enhance Cuban coffee production. Eduardo Canales López *et al.*, Cuba
28. Changes in the Sensory Profile of Robusta Coffee Through Controlled Fermentations with Yeasts and the Use of Natural Additives. Rafael Marcos Pimentel Pérez *et al.*, Cuba.
29. Chemical macroelements in soils of protected cultivation houses treated with Pseudoxanthomonas indica H32. Raúl González Riis *et al.*, Cuba.
30. Effect of HeberNem-S Application on Papaya Cultivation Under Open-Field Conditions. Yanara de la Victoria Portell *et al.*, Cuba.
31. Effect of HeberNem-S application on papaya seedlings. Sandra María Arias López *et al.*, Cuba.
32. Effect of Pseudoxanthomonas indica H32 on the growth of Beta vulgaris var cicla in organoponic. Dulemy Carrazana Granado *et al.*, Cuba
33. Effectiveness of Cuban bioproducts on the acclimatization of plantain and sweet potato cultivars propagated by *in vitro* culture. Yoel Beovides García *et al.*, Cuba.
34. Effects of HeberNem-S on tobacco germination in seedbeds. Matilde Sotomayor Pérez *et al*., Cuba.
35. Efficacy of Pseudoxanthomonas indica H32 for root-knot nematode (Meloidogyne spp.) control and growth promotion in protected horticultural crops. Ramón Franco Rodríguez *et al.*, Cuba.
36. Ensayos *in vitro* del efecto antagónico de diferentes cepas bacterianas frente a dos cepas de *Phytophtora parasítica* Breda de Haan. Rayza M. González R y cols., Cuba.
37. Good practices to stablish agricultural bioassays. Yunior López *et al*., Cuba
38. Evidence of the safety of the HeberNem-S bioproduct for its national and international commercialization. Licette León Barreras *et al*., Cuba.
39. Hebernem /Aikexian Product Technology Transfer. Introduction to China's Agricultural Production. Marisela Suarez Pedroso *et al.*, Cuba.
40. New strains with antagonistic activity against phytopathogens with potential for protection against abiotic stress. Danalay Somontes Sánchez *et al.*, Cuba.
41. Phylogenetic comparison of serine proteases from *Pseudoxanthomonas indica* H32 with those from other nematicidal microorganisms. Kimberly Aguilar Rodríguez *et al.*, Cuba.
42. Predictive models to optimize coffee sensory profiles through metabolic interactions in bean fermentation with microbial consortia. Yailen Valdes Ruiz *et al.*, Cuba
43. Recent progress in the synthesis of silk fibroin nanoparticles: a novel delivery system for bioactive molecules. Osmani Chacón Chacón *et al.*, Cuba.
44. Secondary metabolites and extracellular proteases contribute to the antagonistic action of indigenous Trichoderma strains against *Botrytis cinereal*. Annia Hernández Rodríguez *et al.*, Cuba.
45. Alternativas para el control del almidón en la industria azucarera. Héctor Luis Ramírez Pérez y cols., Cuba.
46. Bi-enzymatic transformation of sucrose for the diversified production of fructooligosaccharides. Odet Céspedes Hernández *et al*., Cuba.

47. Determinación de las propiedades estructurales y operacionales del biocatalizador Quitina-Quitosana-Invertasa. Leissy Gómez Brizuela y cols., Cuba.
48. Estabilidad de β-mananasas en el extracto enzimático producido por *Bacillus subtilis* E44. Madyu Matos Trujillo y cols., Cuba.
49. Evaluation of competitive inhibitors on chimeric dextransucrase enzyme DSR-F-ΔSP-ΔGBD-CBM2a produced in *E. coli* JM109. Camila Rojas Cos *et al.*, Cuba.
50. Switching regioselectivity in the fructooligosaccharides synthesis by *Gluconacetobacter diazotrophicus* levansucrase. Ana Gabriela Martínez Peña *et al*., Cuba.
51. Analysis of the inorganic components that affect the quality of irrigation water in the experimental plot. Dalgys Ercia Rodriguez-Mena *et al*., Cuba.
52. Determination of heavy metals in original seed of transgenic corn and soybeans. Licette León Barreras *et al.*, Cuba.
53. Production and increase of liquid inoculant based on *Bradyrhizobium japonicum* to obtain 300 hectares of certified soybean seed. Camilo Ferrero García *et al*., Cuba.
54. Protection of soil resources on the CIGB experimental plot. Leonardo González Herrera *et al.*, Cuba
55. Strategy for increasing soybean crop productivity. Claudia de la Caridad Viel Portuondo *et al*., Cuba.
56. Biophysical-chemical monitoring of the soil from post-sowing to post-harvest of areas planted with hybrid corn H-Ame 15. Ana Cristina Noa Rodríguez *et al.*, Cuba
57. Considerations for non-regulation of transgenic soybeans carrying the GTS 40-3-2 transformation event in Cuba. Leyenis García Santos *et al.*, Cuba
58. Detección e identificación de Organismos Genéticamente Modificados: Un pilar para la seguridad alimentaria. Madeline Blanco de Armas y cols., Cuba.
59. Installation of soil-based techniques for environmental monitoring of genetically modified crops. Vivian Prevot Cazón *et al.*, Cuba.
60. International Standards and Regulatory Environment of Agricultural Products of the CIGB. Jesus Mena Campos *et al.*, Cuba.
61. Managing biosafety related to genetically modified crops through agricultural extension. Leyenis García Santos *et al*., Cuba.
62. Monitoring and Surveillance System for Adverse Effects of Genetically Modified Organisms in Cuba. Marvis Esther Suárez Romero *et al*, Cuba.
63. Sistema de Gestión y Acreditación de la PCR en tiempo real para la Detección e Identificación de Organismos Genéticamente Modificados en Soya para Alimento Humano y Animal. Arsenio Betancourt Bravo y cols., Cuba.
64. Surveys for monitoring the adverse effects of genetically modified crops. Yordanka Domínguez Linares *et al.*, Cuba
65. Toxicological profile of hybrid corn with the transgenic events MIR162 and TC1507. Licette León Barreras *et al*., Cuba.
66. El impacto y uso de la inteligencia artificial: desafíos científicos-biotecnológicos para el logro de una agricultura sostenible. Oscar J. Villar Barroso y cols., Cuba
67. Enfoque ciencia-tecnología-sociedad en el proceso de investigación agrobiotecnológico: un desafío permanente. Yurama Cardet Chaveco y cols., Cuba.
68. Integrated management system with it platform for the agricultural research area at the Center for Genetic Engineering and Biotechnology. Ivon Menendez Valdés *et al.*, Cuba.
69. Key trends for the global agribusiness and biotechnology sector in business management and regulatory affairs. Adrian Griñan Cardet, Cuba.
70. Citrus disease management using plant elicitors. Meilyn Rodríguez Hernández *et al.*, Cuba.

Oral Presentations

The use of Genetic Engineering to Address Future Food Security and Sustainability

Mario Pablo Estrada Garcia
Agricultural Biotechnology Research, CIGB, Cuba
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Global population growth and climate change pose unprecedented challenges to food security and environmental sustainability. Agricultural biotechnology, particularly genetic engineering, emerges as a critical solution to meet these demands while minimizing ecological impact. This presentation highlights the role of genetic seed improvement as a transformative innovation in agricultural productivity, enabling the production of high-quality, nutrient-rich crops at scale.

Central to this discussion is the integration of bioproducts and industrial enzymes to replace chemical inputs, thereby reducing contamination and enhancing sustainable practices. Additionally, advancements in functional foods—including prebiotics, probiotics, and safer food production systems—will be explored as key strategies to address the nutritional needs of future generations.

Drawing on 30 years of pioneering research by the Center for Genetic Engineering and Biotechnology (CIGB), this presentation underscores the institution's commitment to developing scalable, environmentally responsible agricultural technologies. Case studies and outcomes from CIGB-led projects will demonstrate how biotechnology can align productivity with planetary health, offering actionable insights for policymakers, researchers, and stakeholders invested in securing a food-resilient future.

This work reinforces the imperative for nations to prioritize biotechnology investments, ensuring both food sovereignty and the preservation of ecosystems for generations to come

Oral Presentations

RNAi-based Technologies for Management of Plant Viral Diseases

Basavaprabhu L. Patil

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Plant pests and pathogens cause severe crop losses across the globe, especially in the developing world where there are no robust seed certification and pest control strategies in place. Developing disease resistant crop cultivars through biotechnological interventions is the most effective and economical way to control plant diseases. Gene silencing is one of the most important mechanisms of gene regulation in plants, which is mediated by 19-24 nt sized small RNAs. In plants, the small RNAs can be grouped into differentclasses, microRNAs (miRNAs), small interfering RNAs (siRNAs), trans-acting siRNAs (tasiRNAs) etc. Transgenically induced RNA interference (RNAi), has been employed to control diverse plant viruses with both DNA and RNA genomes. This method will be dealt in greater detail with special reference to the control of cassava infecting viruses in East Africa (Patil *et al.*, 2011, *Mol. Plant Pathol.* doi: 10.1111/j.1364-3703.2010.00650.x.). Further, the tasiRNAs are recently identified class of small RNAs, which are derived from *TAS* gene-derived transcripts after being acted upon by a miRNA. We have recently employed the miRNA-Induced Gene Silencing (MIGS) strategy to simultaneously silence multiple genes by fusing multiple MIGS modules (miR173 target site plus sequence of interest) to generate a single MIGS construct, which subsequently can be cloned into a binary vector for plant transformation. This fusion MIGS construct is capable of simultaneously silencing different genes from different target pests or pathogens with same efficiency (Hada *et al*., 2021, *Pest Management Science*, doi: 10.1002/ps.6384; Karthik *et al., Planta*. 2022, 257(1):20. doi: 10.1007/s00425-022-04055-2). I will also brief about our research on topical application of dsRNA for control of plant viral diseases (Voloudakis AE, Kaldis A, Patil BL. *Annu Rev Virol.* 2022 9(1):521-548. doi: 10.1146/annurev-virology-091919-073708).

Oral Presentations

Platform to improve maize and sugarcane using biotechnology a traditional method

Pilar Téllez Rodríguez [1], Ivis Morán Bertot[1], Javier Lezcano Laguna[1], Davel Espinoza Delgado[1].

1. Corn and Sugarcane Biotechnology group, Agriculture Research, Center for Genetic Engineering and Biotechnology (CIGB).

The main goal of this work is the development of a platform to improve maize and sugarcane in the laboratory. Both crops have strategic importance in the economy, for the production of food and feed in Cuba. In the case of maize the objective is to obtaining new varieties with characters for insects and herbicide resistance, while for sugarcane fungal and herbicide resistance are the characters with remarkable interest. These objects were complemented using transgenic and traditional methods. The transgenic procedures for the two crops were established via *Agrobacterium tumefaciens*. Several topics of the methodology for maize and sugarcane were optimized to increase the number of transgenic events. The traditional methodology was established for maize. The first step was the crossing between a variety that carrier a transgenic commercial event and a conventional variety with well adaptation to the agriculture conditions in Cuba. The following steps were the development of inbred lines and finally the generation of single hybrids.

Oral Presentations

Comprehensive approach to the soybean genetic improvement program by biotechnological methods at the CIGB

Gil A. Enriquez*, Natacha Soto, Alejandro Morales, Kenia Tiel, Camilo Ferrero, Claudia Viel, Raidell Saenz, Rodobaldo Ortiz[2], Leyenis Garcia, Mario P. Estrada & Abel Hernandez

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Soybean biotechnology has revolutionized agricultural practices, offering innovative solutions to improve crop yield, disease resistance, and environmental sustainability. Among the major problems with soybeans is their high rate of damage from a wide variety of fungal and weeds infections. Therefore, soybean improvements are always needed with respect to both agronomic performance and the quality of end products. For the climatic condition in Cuba, improvements in agronomic performance, the managements of these constraints, particularly the weeds, would lead always to increase the quality and productivity. This study was conducted to obtain different soybean lines by the expressing antifungal and cp4epsps genes, and to evaluate their resistance to fungi and herbicides, both *in vitro* and under field conditions. Thus, embryonic axes of soybean varieties were transformed by *Agrobacterium tumefaciens*. A group of transgenic plants resistant to the herbicide glyphosate were selected, which were characterized biological and molecularly. It was demonstrated in the field trial that many of the transgenic lines had a significant decrease of fungal growth of the selected lines for these new events. Another approach related to the introgression of the GTS40-3-2 event allowed for the evaluation of resistance to the herbicide glyphosate and other productivity and abiotic factor resistance traits in the selected varieties. Essential elements of improvement that could contribute to the soybean production program in Cuba are discussed.

Oral Presentations

Fronteras biotecnológicas en la producción de vainilla: avances en conservación, mejoramiento genético y procesamiento para una producción sostenible

Miguel Angel Esquivel Pérez[1/], Thaisbel Esquivel Gallardo[2/], Edgar López Herrera[3/]

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La vainilla (*Vanilla planifolia*) representa un cultivo de alto valor comercial cuya producción enfrenta importantes desafíos biológicos y agronómicos. Las herramientas biotecnológicas ofrecen soluciones innovadoras que abarcan todo el ciclo productivo, desde la propagación hasta el procesamiento post-cosecha. Esta presentación analiza los avances biotecnológicos en el cultivo de vainilla, comenzando por la micropropagación y conservación *in vitro*, que permiten obtener material vegetal certificado libre de patógenos como hongos, bacterias, nematodos y virus. Se destacan técnicas como la germinación asimbiótica *in vitro* de semillas de especies de *Vanilla* para desarrollar resistencia genética a patógenos. El rescate de embriones emerge como herramienta crucial en programas de mejoramiento, facilitando cruzamientos interespecíficos entre *V. planifolia* y especies resistentes como *V. pompona*, particularmente para enfrentar a *Fusarium oxysporum* f. sp. *vanillae*. Se abordan los avances en el estudio de microorganismos rizosféricos beneficiosos, incluyendo bacterias solubilizadoras de fosfato y bacterias fijadoras de nitrógeno con potencial como biofertilizantes. La caracterización bioquímica de estos microorganismos ha revelado mecanismos específicos de promoción del crecimiento vegetal y defensa contra patógenos. Finalmente, se exploran innovaciones en la producción de vainillina mediante biología sintética, como el desarrollo de levaduras modificadas genéticamente capaces de producir este valioso compuesto. Esta visión integral de las aplicaciones biotecnológicas proporciona herramientas para superar limitaciones productivas, mejorando la sostenibilidad del cultivo frente a desafíos como el cambio climático y la creciente presión de enfermedades.
Palabras clave: micropropagación in vitro, resistencia a Fusarium, biofertilizantes rizosféricos, hibridación interespecífica, producción biotecnológica de vainillina

Oral Presentations

Developing Mutant-Resistant Monoclonal Antibodies Effective Against All SARS-CoV-2 Variants Using Plant Biotechnology

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The COVID-19 pandemic, caused by SARS-CoV-2, has resulted in over 770 million confirmed cases and nearly 7 million deaths worldwide as of 2024. Despite the deployment of several therapeutic monoclonal antibodies (mAbs) under the U.S. Emergency Use Authorization (EUA) program—including bamlanivimab, casirivimab/imdevimab, tixagevimab/cilgavimab, and sotrovimab—many of these agents have lost efficacy due to viral evolution. Their high production costs and limited breadth of neutralization further constrain their utility, particularly in the face of ongoing variant emergence and immune escape. To address these challenges, we leveraged plant-based biotechnology to develop next-generation mAbs that are resistant to viral escape. Our dual strategy involves: (1) identifying broadly neutralizing class 4 mAbs that target a conserved region of the receptor-binding domain (RBD) less prone to mutation, and (2) engineering high-affinity binding antibodies with enhanced antibody-dependent cellular cytotoxicity (ADCC) activity, rendering them effective independently of RBD sequence variability and direct virus neutralization. This presentation will describe the successful development of these two categories of mAbs, including in vitro neutralization against all known SARS-CoV-2 variants and in vivo protection data. Our findings demonstrate the potential of plant-based platforms for rapid, scalable, and cost-effective development of next-generation, mutation-resistant antibody therapeutics.

Oral Presentations

Bridging Synthetic Biology and Molecular Farming: a copper sensor and geminivirus-based Processors for engineering gene circuits in plants

Elena García Pérez

Plant Synthetic Biology offers powerful tools for enhancing plant-based biomanufacturing, yet challenges remain in achieving precise and inducible gene regulation and optimizing production yields. In this work, we developed copper-inducible synthetic gene circuits in *Nicotiana benthamiana* to enable controlled and high gene expression for biotechnological applications such as Molecular Farming. We engineered a copper sensor that activates transcription in response to copper sulfate (CuSO), providing fine-tuned control over a CRISPR/dCas9-based transcriptional activator. This system offers a reliable platform for precise gene regulation with minimal background expression. Additionally, we developed CuBe, a copper-regulated geminivirus-based circuit that integrates inducible gene expression with DNA amplification, enabling scalable production of recombinant proteins, including pharmaceutical products such as antibodies. Our findings highlight the potential of synthetic gene circuits for Molecular Farming, offering new strategies for controlled gene expression in both transient and stable plant systems. These modular tools pave the way for more efficient and scalable plant-based bioproduction

Oral Presentations

Update on the approach to plant molecular farming at CIGB

Abel Hernández

Plants are a promising approach for cost effective and scalable biopharmaceuticals proteins production. Some of the available technologies utilize stable plant transformation, while others focus on transient production through agroinfiltration without gene integration to plant genome. In recent years, CIGB research has focused on the transient production of vaccine candidates against infectious diseases in *N. benthamiana* leaves, using specially designed vectors guided by the 35S promoter, meanwhile other studies have taken advantages of stable accumulation in tobacco seeds to produce monoclonal antibodies or vaccine candidates, in these cases regulated by seed-specific promoter phasoeolin from *Phaseolus vulgaris*. Although, expression with seed-specific promoters is a finely regulated process confined to seed tissue, under certain circumstances such as the production of PvAlf transcriptional factor and increased ABA concentration, it can activate the activity of this promoter in vegetative tissue. The usefulness of expressing PvAlf or its orthologs isolated from *N. tabacum* or *N. rustic* to turn on phaseolin promoter activity in vegetative tissues, as a way to increase the accumulation of pharmaceutical proteins, will be discussed.

Oral Presentations

Updated Critical Analysis of the Potential of Transgenic Plants and Plant Cells for the Production of Monoclonal Antibodies and Recombinant Proteins

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Monoclonal antibody (mAb) and recombinant protein expression in transgenic plants and plant cells was sponsored, decades ago, as a cost-effective heterologous protein production platform, based on a potential high production capacity, low-cost, similar protein posttranslational modifications and not biosafety risks for the human being. However, the heterologous protein market continues ruled by microbes and mammalian cell production systems, so far. It is, likely, a consequence of the low target protein yield achieved in plants and plant cells, great batch-to-batch inconsistence and also of many obstacles faced in large-scale downstream processing. A proper example of this assertion is linked to a hepatitis B surface antigen specific mAb production, one decade ago, in tobacco plant leaves and seeds, where a lower cost production was verified, in comparison to the hybridoma technology, but countless technological and regulatory obstacles for obtaining the mAb with high purity and productivity at large-scale were faced, so industry finally opted for continuing working with the conventional technology. Nevertheless, there are some others heterologous proteins produced in plants that have reached commercial stage, demonstrating that plants can be considered still as a suitable alternative for very specific application-heterologous protein production at very large-scale, widely their application spectra from the highly regulated biopharmaceutical industry to the non-therapeutic protein production industries such as the less regulated animal feeding, diagnosis and cosmetic industries. Therefore, the aim of this work is making an updated critical analysis of the potential of transgenic plants and plant cells to produce mAb and recombinant proteins.

Oral Presentations

GBS based genotyping and morphological characterization of cacao accessions from Cuban gene bank reveals its potential for breeding and production

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The poor use of Theobroma cacao diversity in productive areas worldwide threatens cacao production sustainability. In Cuba, most cacao clones currently use in breeding and production belong to the Trinitario type and were introduced to the country in the 50's last century. A breeding program based on morphological descriptors was launched in 1990's aimed to develop improved varieties. Accessions derived from the program along other prospected plants are conserved in the Cuban national cacao gene bank (CG). Unfortunately, genetic diversity of CG has been poorly described, limiting the exploitation of local cacao genetic resources in breeding and production. To assess the population structure of CG, a new ddRADseq protocol for cacao was applied to genotype CG plants. DNA sequences from 238 CG plants were obtained and used to build a large dataset containing 11,425 SNPs. SNPs spread unevenly throughout the 10 cacao chromosomes and mainly laid in non-coding regions of the genome. Population structure analysis based on this SNPs dataset detected seven significant different genetic groups among CG plants (p=0). Amelonado (49.22 %) and Criollo (16.82 %) were the more abundant cacao ancestries in CG. Based on population structure data, 89 clones were selected for characterization with 36 morphoagronomical descriptors from fruit and seed. A wide morphological variability was detected among the characterized clones, which was congruent with their cacao ancestry diversity. Seven clones showed values of seed fresh weight per fruit and fresh weight of one hundred seeds similar to the cacao clones currently used for breeding and production. Six clones had cotyledons with light colors, which has been associated to a better sensorial quality of cacao. Cacao plants carrying attractive traits constitute local genetic resources with potential for the breeding and production of cacao and could contribute to the development of sustainable production systems.

Oral Presentations

Effect of Environmental conditions on Virus Infections, Gene Silencing and their implications on Virus Induced Gene Silencing (VIGS)

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RNA silencing is a sequence-specific post-transcriptional gene inactivation mechanism that operates in diverse organisms and which can extend beyond its site of initiation, owing to the movement of the silencing signal, called as non-autonomous gene silencing. Previous studies have shown that several factors manifest the movement of silencing signal, such as, the size of secondary siRNA produced, or the steady-state concentration of siRNAs and their cognate mRNA, or due to a change in sink-source status of plant parts affecting the phloem flow. Our studies show that, both light intensity and temperature have significant impact on the type of silencing phenotypes obtained in transient agro-infiltration studies. At high light intensities (>450 $\mu Em^{-2}s^{-1}$) and high temperature (>30°C), the silencing was localized without any systemic spread and the virus infected plants recovered from the symptoms. Whereas at low light intensities (<300 $\mu Em^{-2}s^{-1}$) and at a temperature of 25°C, there was strong systemic movement of silencing signal and also there was reduced recovery from virus infections. Accumulation of siRNA was reduced at higher temperature due to reduction in the accumulation of transcript on transient agro-infiltrations, mostly because of poor T-DNA transfer activity of agrobacterium. In contrast the virus infections at higher temperature produced increased amount of virus specific siRNA, eventually leading to reduced viral transcript and enhanced symptom recovery. However reduced systemic silencing and the reduced viral symptom severity at higher light intensities were due to the change in sink-source status within the plant, ultimately affecting the phloem translocation. There are several potential applications of these important findings in the field of functional genomics and virus control using gene silencing as a method of choice. These studies will significantly help in carrying out successful functional genomics studies using VIGS (VIGS: Virus induced gene silencing).

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Oral Presentations

Rhizosphere bacteria for growth stimulation and increasing stress resistance of plants of the family solanaceae

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114 rhizospheric bacterial isolates were isolated from the rhizosphere of tomatoes, potatoes and tobacco, of which 35 synthesize indole-3-acetic acid, 12 possess a complex of agronomically valuable properties (nitrogen fixation, phosphate mobilization) and stimulate the growth and development of Solanaceae plants at the early stages of their ontogenesis under exposure to drought, salinity, high temperature. Active growth promoters were identified by a complex of physiological, biochemical and molecular genetic properties. Sequencing of the amplified fragment of the 16S rRNA gene of bacterial isolates RNT7, RNT9, ST1, NT5 using the eubacterial universal primer 926r enabled to isolate nucleotide sequences of 813–848 bp, allowing to establish affiliation of isolate RNT7 to the species *Rhodococcus wratislaviensis*, isolate RNT9 – to the species *Agrobacterium* sp., isolate ST1 – to the species *Pseudarthrobacter oxydans*. The results of the analysis of the nucleotide sequences of the 16S rRNA gene and the gyrB gene indicate the most likely position of isolate NT5 to the species *Stutzerimonas stutzeri*. The identified strains do not have an antagonistic effect on each other, are not pathogenic, toxic or toxigenic. A binary consortium was formulated based on the nitrogen-fixing strain *Rhodococcus wratislaviensis* RNT7 and the phosphate-mobilizing strain *Stutzerimonas stutzeri* NT5. The nutrient medium was optimized and the conditions for separate submerged cultures of both strains were determined. It was shown in laboratory experiments that pre-sowing inoculation of tomato seeds with the developed consortium improved the growth and development of plants exposed to drought, salinity and high temperatures. The bacterial consortium *Rh. wratislaviensis* RNT7 + *St. stutzeri* NT5 can be used as a basis for designing microbial preparations to increase the stress resistance of plants of the Solanaceae family growing under exposure to adverse abiotic factors.

Oral Presentations

Endophytic bacteria increasing stress resistance of *Medicago sativa*

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280 endophytic bacteria were isolated from alfalfa tissues, of which 33 isolates were able to fix nitrogen, solubilize phosphates, produced the phytohormone indole-3-acetic acid (IAA), 4 isolates possessed nifH gene in the genome, 4 isolates synthesized sideraphores, 6 isolates produced enzyme ACC deaminase. Most isolates were resistant to lead and cadmium ions (10 mM and 1 mM, respectively).
Identification of the selected 9 endophyte isolates with a complex of agronomically valuable properties was carried out using Burker Daltonik MALDI Biotyper. Among the isolates representatives of genus *Pseudomonas* dominated, as well as *Sinorhizobium meliloti* and *Agrobacterium tumefaciens* were identified.
DNA sequencing of the alfalfa root nodule bacterial strain *S. meliloti* S3 with subsequent bioinformatics processing of the reads allowed to obtain a full-genome nucleotide sequence, further deposited in GenBank database under designations CP123003-CP123006.
The genome of the *S. meliloti* S3 strain contains 7,243,810 nucleotide base pairs and is represented by a ring chromosome of 3,781,018 b.p. and three circular plasmids: pSme442-SymA, pSme442-SymB and pSme442-208. The strain is characterized by the presence of genetic determinants that encode resistance to abiotic stresses. Resistance to osmotic stress is provided by the presence of betaine aldehyde dehydrogenase catalyzing the last, irreversible stage of synthesis of the osmoprotector glycine betaine from choline. Resistance of heavy metals is provided by metallopeptidases of families 17, M20, 48, metallohydrolases of M20/M25/M40 family.
The bacterial strain *A. tumefaciens* S5 is capable of synthesizing IAA and has grown-stimulating activity. Full-genome nucleotide sequence was obtained, deposited in the GenBank database under the numbers CP072745.1- CP072747.1. Genetic apparatus of *A. tumefaciens* S5 bacterium is represented by three replicons: linear and circular chromosomes and pAt-B441 plasmid.
A. tumefaciens S5 strain shows multiple resistance to heavy metals, owing to the presence of a protein responsible for resistance to cobalt-zinc-cadmium.

Oral Presentations

Mechanism of copper accumulation in the marine alga *Ulva compressa* (Chlorophyta)

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In terrestrial plants, heavy metals such as copper are accumulated through the binding to reduced glutathione (GSH) and/ or phytochelatins (PCs), that are condensed units of GSH, and transported to the vacuole. In addition, heavy metals can bind metallothioneins (MTs), that are small proteins enriched in cysteines residues located in the cytoplasm. In the case of GSH, PCs and MTs, heavy metals are coordinated with sulfhydryl groups of cysteines residues. The marine green alga *Ulva compressa* is a cosmopolitan alga found in copper-contaminated coastal sites of northern Chile that can accumulates copper. It was initially hypothesized copper was accumulated through the binding with GSH, PCs or UcMTs. It is important to mention that six UcMTs genes have been identified in the algal genome. It was initially shown that the alga cultivated with 10 mM of copper displayed an increase in GSH levels, the synthesis of PCs, mainly PC2 and PC4, and an increased expression of five UcMTs. In addition, *U. compressa* collected in copper-polluted sites of northern Chile was analyzed by Transmission Electron Microscopy (TEM) coupled to Emission Dispersive X-ray Spectroscopy (EDXS) showing electrondense particles in the chloroplast that contained copper and sulfur, but not N, suggesting that copper is accumulated as copper sulfide. Recently, it was determined that increasing concentrations of a sulfide donor (NaHS) enhanced copper accumulation in the alga, and a sulfide acceptor (hypotaurine) decreased copper accumulation. Moreover, the alga treated with copper and 200 µM of NaHS analyzed by TEM-EDXS showed an increased accumulation of electrondense particles containing copper and sulfur in the chloroplasts, but also in the cytoplasm, compared with the alga treated only with copper. Thus, copper is accumulated as copper sulfide, and not bound to GSH, PCS or UcMTs, in the marine alga *U. compressa*.
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Oral Presentations

Metabolization of polycyclic and linear hydrocarbons in the marine macroalga *Ulva lactuca* (Chlorophyta)

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In the recent decade, there has been an alarming rate of fuel spillages in coastal sites of central Chile, were most of the industrial and residential cities are located. Those spillages are detrimental to the marine environment, fisheries, tourism, and human health. Among the most toxic components of crude oil are the polycyclic aromatic hydrocarbons (PAHs) that are very stable molecules, that are accumulated in sediments, and they are also biomagnified in the trophic chain. PAHs can cause damage to the skin, lungs, and nervous system and they can induce DNA damage and cancer. We found that the marine alga *U. lactuca* was able to tolerate high concentrations of two PAHs, anthracene and benzopyrene, and was chosen as a candidate for bioremediation. We performed *in vitro* experiments that were almost complete absorption of both PAHs was observed between 6 and 12 h of culture, and the absorbed molecules were decreasing in quantity inside the tissue. The antioxidant system of the alga was rapidly activated to control the cell damage and several oxidative enzymes were overexpressed and its activities increased. After a transcriptomic analysis, the metabolic routes were described for the alga to convert the PAHs into small molecules capable to be used in the carbon metabolism. Interestingly, enzymes for the metabolization of linear hydrocarbons were also found overexpressed and the latter lead us to study the absorption and metabolization of linear hydrocarbons of diesel. We found that the alga can rapidly absorb linear hydrocarbons from C7 to C16, and more slowly those from C17 to C28, and the absorbed hydrocarbons could be converted to carboxylic acids that can enter into carbon metabolism.
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Oral Presentations

Construcción de un modelo metabólico a escala genómica de *Leucoagaricus gongylophorus* para el análisis de su capacidad metabólica

Freddy Castillo Alfonso, Roberto Olivares Hernández, Juan Gabriel Vigueras Ramírez, Juan Carlos Sigala Alanìs

Se realizó un ensamble genómico de Leucoagaricus gongylophorus LEU18496, obteniéndose un genoma de aproximadamente 125 Mbp, diploide y con una distribución bimodal en el contenido de GC: una región R0 (75% del genoma) rica en AT, con baja densidad de genes y presencia de elementos transponibles, y una región R1 con mayor contenido GC y alta densidad genómica donde se concentra la mayoría de los genes predichos (6,748 en total, según herramientas como Augustus). El análisis funcional evidenció una abundancia de enzimas activas en carbohidratos (CAZymes) y enzimas oxidativas de la lignina (FOLymes), fundamentales para la degradación de sustratos complejos. Además, cultivos de L. gongylophorus en glucosa y celulosa durante fase exponencial y estacionaria del creciemiento permitieron la obtención de perfiles transcriptómicos, con un alto porcentaje de mapeo (91–93%) y un agrupamiento consistente de réplicas biológicas, demostrando que la fuente de carbono es el factor principal que modula la expresión génica. Aproximadamente el 70% de los genes identificadso en el genoma se expresaron, identificándose 2,031 genes con cambios significativos (1,088 upregulados y 943 downregulados) , destacandose categorías funcionales como CAZymes, FOLymes, transportadores y factores de transcripción. Se observó una alta expresión de enzimas implicadas en la degradación de polisacáridos complejos, especialmente en condiciones de celulosa (con sobreexpresión de genes de familias GH7 y GH6), mientras que en glucosa se mantuvo una expresión constitutiva de ciertos CAZymes, acompañada de una elevada actividad de transportadores; además, se identificó una regulación dinámica del regulador CreA, que modula la secreción de CAZymes. Utilizando estos datos genómicos, se construyó un modelo metabólico a escala genómica (iCF502) que integra 502 genes, 769 reacciones y 626 metabolitos, y cuya simulación mediante análisis de balance de flujos reprodujo las fases de crecimiento y la distribución de flujos metabólicos dependientes del sustrato, aportando un marco predictivo de gran relevancia para aplicaciones biotecnológicas en la transformación de diferentes sustratos.

Oral Presentations

Study of the secondary metabolite profile in Trichoderma spp. strains with antifungal activity using LC-MS technique

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In the last decade, research on Trichoderma secondary metabolites (SM) has experienced a major upswing driven by the advancement of analytical techniques and the growing demand for natural alternatives in agriculture and medicine. The metabolic versatility of these fungi includes the production of SM such as antibiotics, siderophores and volatile compounds, among others. The present work aims to identify secondary metabolites with antifungal activity in autochthonous strains of Trichoderma spp. by using LC-MS technique. Of the total strains evaluated, the strains previously identified as Trichoderma harzianum showed the most diverse metabolomic profiles. Compounds reported in the literature with important antifungal activity were found, such as harzianolide and derivatives, diketopiperazines and the compound 6-PAP, a member of the pyrone family. In the case of strain T14, high intensity signals were observed between minutes 7-10 that could not be identified by comparison with databases, so a more detailed study using techniques such as NMR and UV will be necessary for the identification of these compounds. The remaining tested strains identified as T. asperellum and T. asperelloide did not show metabolic profiles with antifungal compounds. The antifungal capacity of the metabolite extracts was evaluated and inhibition of pathogen growth as well as decreasing effect over time was observed. These studies reinforce the important role of Trichoderma secondary metabolites as part of their mechanisms of action for pest control and evidence the effectiveness of techniques such as LC-MS for the preliminary analysis of their metabolomic profiles.

Oral Presentations

Epidemiology-based management strategies for citrus Huanglongbing

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The rapid spread, difficulty in control, and severe fruit yield and quality loss make Huanglongbing (HLB), caused by the phloem-restricted bacterium '*Candidatus* Liberibacter asiaticus', the main citrus disease in the world. Due to the absence of resistant varieties and curative measures the disease control is based on exclusion, eradication, and protection measures. However, aspects of HLB epidemiology make its control very difficult. Citrus is a perennial crop exposed for many years to infections. The disease latent period (~14 days) much shorter than the incubation period (≥4 months) allows infected trees to serve as an inoculum source before they are detected what makes the disease eradication almost impossible. Inoculum sources outside commercial orchards and the long-distance dispersal of the vector, the Asian citrus psyllid *Diaphorina citri*, makes the disease primary spread as or more important than the secondary spread for the epidemic progress, resulting in almost no effect of removing diseased trees only within commercial orchards on reducing the disease incidence. In addition, diseased adult trees maintain residual production for a few years, leads growers to not immediately remove them and keep potential inoculum sources inside the orchard. Psyllids prefer new shoots to feed on, which requires a greater frequency of insecticide sprays to keep a protective barrier to prevent the bacteria transmission during the development of new leaf tissues. Secondary infections are almost fully controlled with fortnightly insecticide sprays that break the development of psyllid life cycle on infected trees and kill adult psyllids that acquired the bacteria on diseased trees before the latency period of the bacteria in the vector was concluded. In turn, primary infections by infective psyllids that developed in diseased trees and moved to commercial orchards are partially controlled even with weekly insecticide sprays during the vegetative flushing period (poor coverage, rain-wash, growth of unprotected new tissues). The decreasing gradient of the psyllid and diseased trees populations from the orchard edge to the center has been directed the inspections for the vector and diseased trees, as well as the application of measures that reduce the primary dispersion to the interior of the property, such as: planting density and direction, planting of varieties with different vigor, trap-crop, more intensive applications of insecticides and repellent kaolin. The seasonality of the psyllid population and the period of greater disease symptom expression have directed the periods of highest frequency of insecticide application and actions to detect symptomatic trees, respectively. However, all mentioned measures partially control the primary infections, and their efficacy is dependent on the amount of primary inoculum. Thus, for the effective HLB control, citrus growers must control the vector and reduce inoculum sources both inside and outside the orchards in a coordinated and joint way, which still difficult to achieve in practice due to their diverse profile. Good disease control was achieved where regional and integrated management was carried out, and this is the way until more effective and sustainable measures, such as resistant citrus cultivars, are available.
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Oral Presentations

Tracing microRNA targets in tomato plants infected with begomoviruses

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MicroRNAs (miRNAs) are small non-coding RNAs that play a critical role in regulating gene expression at the post-transcriptional level. Geminiviruses, particularly begomoviruses are devastating pathogens affecting tomato crops worldwide, inducing significant transcriptional and post-transcriptional changes in infected plants. Previous studies have shown that miRNAs are involved in plant defense responses, yet their precise role during begomovirus infections remains poorly understood.
In a previous study, RNA interference (RNAi)-based transgenic tomato plants resistant against Tomato yellow leaf curl virus (TYLCV) in a field trial became infected with Tomato latent virus (TLV), a recombinant derivative of TYLCV in which the RNAi target sequence was replaced with a respective sequence from an yet-unknown bipartite begomovirus to evade RNAi-mediated gene silencing, as demonstrated by Illumina sequencing analysis of virus-derived small interfering RNAs (Fuentes et al. 2016 doi: 10.1094/MPMI-08-15-0181-R). Currently, our project is focusing on plant microRNAs differentially expressed in response to infection with TLV, to characterize plant mRNAs targeted by those miRNAs as potential markers of the resistance or susceptibility to begomoviruses.
To this end, Illumina sRNA sequencing data from TLV-field-infected and non-infected transgenic tomato plants were analyzed using bioinformatics pipelines to identify differentially expressed miRNAs and to predict their potential targets. In addition to TLV, several other begomoviruses known to infect tomato in the local environment were employed to establish infectious models under controlled conditions for studies on plant gene regulation mediated by miRNAs.
Preliminary analyses indicate that TLV infection in the field significantly alters the expression of a subset of microRNAs. The gene targets of these miRNAs, as well as other previously described non-target genes differentially expressed in begomovirus-infected plants, were characterized in our agro-infected tomato models under controlled conditions using quantitative RT-PCR.
Among the identified targets, several transcription factors appear to be modulated by begomovirus infection in agro-infected tomato plants. These findings suggest that the proposed begomovirus-infected models are suitable for further validation.
Overall, our work highlights the value of integrating transcriptomic analyses with experimental virus infection models to elucidate the molecular mechanisms underlying plant–begomovirus interactions.

Oral Presentations

MicroRNA Analysis of Transgenic Tomato Plants Under Different Environments

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Previously, RNA interference (RNAi)-based transgenic tomato plants that target C1 gene of Tomato yellow leaf curl virus were analyzed by Illumina small (s)RNA sequencing to characterize antiviral RNAi under greenhouse and field environments (Fuentes et al. 2016; doi: 10.1094/MPMI-08-15-0181-R). Here, we further characterized the sRNA-seq data to identify microRNAs (miRNA) and to establish their expression profiles.
To this purpose, *Solanum lycopersicum* miRNAs from the database miRBase and recently described miRNAs (Arazi et al. 2022; doi: https://doi.org/10.3390/ijms231911979) were retrieved, totaling 491 mature miRNA species, and analyzed by mapping and counting the Illumina sRNA reads. The preprocessing and normalization of the count matrix were performed. A differential expression analysis (DEA) was completed, comparing transgenic-greenhouse (tgn-gr) vs transgenic-field (tgn-f) samples, and control-greenhouse (c-gr) vs transgenic-greenhouse (tgn-gr). miRNAs with a fold change greater than 2 (|log2FC| >=1) and adjusted P-value<0.05 were considered as differentially expressed (DE). Target genes of the DE-miRNAs were subjected to the functional enrichment analysis.
The comparison between tgn-gr vs tgn-f samples displayed 44 DE-miRNAs. Among the top differentially expressed miRNAs was mir399, which is involved in stress response and in phosphorus signal transduction pathways, miR156 and miR157, which are involved in regulation of fruit development and ripening, and miR396 and miR398, which are involved in plant development and stress response. The miR166 was by far the most abundant in all samples and up-regulated in greenhouse, compared to field conditions. This miRNA was previously proposed as a stress biomarker and to be involved in development processes.
Additionally, we identified potential new small regulatory RNAs by analyzing abundant unassigned reads, some of them derived from the chloroplast genome, which are potentially regulating photosynthesis pathways.
In conclusion, the above presented results point to the complex behavior of tomato transgenic plants in greenhouse and field environments, where the regulation of several miRNAs could balance the growth under different cultivation pressures.

Oral Presentations

A century of diagnostic improvements in a sugarcane pathology changing context: from symptom observation to molecular assays

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Sugarcane germplasm has been moved between geographical regions of the world for numerous centuries, but the first record of a sugarcane disease in the literature dates only to 1869 when symptoms and signs of gumming were described in Brazil. At the end of the 19th century and the first half of the 20th century, diagnosis of sugarcane diseases was solely based on symptom observation (including cytology and microscopy investigations), isolation and characterization of the pathogens (fungi and bacteria) isolated on culture media, and inoculation assays (including for viruses). Application of these methods resulted in the identification of pathogens that caused important disease outbreaks such as leaf scald, mosaic, orange rust, red rot, smut, etc. A first major breakthrough in disease diagnosis occurred during the second half of the 20th century when serological assays were developed. This allowed better and faster detection of pathogens, especially viruses such as sugarcane mosaic viruses and bacteria such as *Xanthomonas albilineans* and *Leifsonia xyli* subsp. *xyli*. At the end of the 20th century, the advent of nucleic acid sequencing resulted in the development of molecular diagnostic methods based on DNA/RNA probing and amplification assays. All these molecular methods targeted specific genome sequences of a pathogen and were developed for most diseases threatening sugarcane production. A new and transformative breakthrough occurred at the beginning of the 21st century with the development of high-throughput sequencing (HTS) technologies that permitted the detection of known but also unknown pathogens. HTS was used to investigate the virome of sugarcane and to discover new viruses but also to solve a ninety years mystery regarding the causal agent of sugarcane chlorotic streak. Numerous and powerful diagnostic methods are currently available to detect and identify sugarcane pathogens, as well as to ensure safe movement of germplasm among sugarcane producing locations.

Oral Presentations

Metagenomic revolution: the contribution of the VANA approach and high-throughput sequencing technologies (Illumina and Nanopore) for the detection of emerging plant viral diseases

Denis Filloux

With the increasing prevalence of viral diseases affecting cultivated plants and the continuous emergence of new pathogens, traditional detection methods are reaching their limits. Viral metagenomics, combined with high-throughput sequencing technologies, now offers a powerful, untargeted, and comprehensive approach to detect, identify, and characterize the full range of viruses present in a sample.
In this context, our laboratory developed the VANA (Virion-Associated Nucleic Acids) approach about fifteen years ago, with the aim of specifically enriching encapsidated viral nucleic acids from plant tissues or insect vectors. By selectively targeting genomes protected within viral particles, this method significantly reduces contamination from host and environmental DNA or RNA, thereby improving both the sensitivity and precision of metagenomic analyses.
Combined with high-throughput sequencing platforms—initially Roche 454, then Illumina, and more recently Nanopore, whose portability allows for easy deployment even in basic laboratory settings—the VANA approach has enabled the rapid and detailed detection and characterization of numerous known and novel viruses across a wide range of studies (Moubset et al., 2022). A key strength of our method also lies in its scalability, as we routinely multiplex several hundred samples per sequencing run, allowing for high-throughput analysis while minimizing costs and technical variability.
Illumina technology, thanks to its high depth and sequencing quality, is particularly well-suited for detecting viral variants. Nanopore sequencing, with its long-read capabilities, facilitates the assembly of complete genomes (Filloux et al., 2018), the differentiation of co-infecting strains, and the identification of segments in multipartite viruses. It also allows for the exploration of intra-host diversity in segmented RNA viruses (Yvon et al., 2023) and circular DNA viruses (Otron et al., 2025), and even allows for the direct sequencing of native viral nucleic acids.
We will illustrate this approach through several concrete examples from our recent work, highlighting the efficiency and complementarity of these tools in addressing current challenges in plant virology.

Oral Presentations

The development of next generation sequencing and specific e-probes for pathogen diagnosis in sugarcane

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Accurate pathogen detection in imported sugarcane material is critical for the security of our industry. Current practices can be expensive and lengthy, and pathogens are often tested individually. Next Generation Sequencing (NGS) has the potential to detect all organisms in a given sample but the bioinformatics can be difficult. E-probe libraries based on pathogen specific sequences should allow for the detection of multiple pathogens in unassembled sequence data. We tested the MinION sequencing device (Oxford Nanopore) and specifically designed e-probes libraries developed by Oklahoma State University (OSU) to detect multiple pathogens in a single sample. The Flongle, a lower capacity but economical flow cell, was evaluated for the production of needed amount of sequence data. Since many plant RNA viruses lack a poly-A tail, random hexamers were investigated as a method to prepare double stranded cDNA libraries. Libraries prepared from sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (SCYLV) infected plants were tested separately and in mixed samples. E-probe libraries developed for the OSU MiDetect system were used to query the sequences generated. SCMV and SCYLV in single and mixed infections were found using sequencing data as low as 24MB (116.21K reads). To determine the sensitivity of the method, infected plant tissue was diluted from 1:10 to 1:10,000 in healthy tissue. Both viruses were detected up to the 1:10,000 dilution. E-probe libraries constructed with probes made for other related potyviruses and luteoviruses all failed to detect SCMV or SCYLV. A set of six RNA samples containing SCYLV, SCMV, Fiji disease virus, or sugarcane streak mosaic virus alone or in combination were also sequenced and, in each case, the pathogen was detected. Consequently, NGS using the MinION device coupled with the user-friendly OSU MiDetect system appears to be suitable for detection of multiple pathogens in a single sugarcane sample.

Oral Presentations

Non-invasive imaging of salicylic and jasmonic acid activities in planta

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Modern plant disease diagnostics demand accurate, non-invasive, and cost-effective methods for early stress detection. This study introduces a non-invasive imaging system that employs genetically encoded autoluminescent reporters based on a fungal bioluminescence pathway, enabling in planta synthesis of luciferin. By using promoters responsive to salicylic and jasmonic acids to control luciferase expression, the system monitors both local and systemic hormonal changes. Experiments with Arabidopsis thaliana and Nicotiana benthamiana showed strong luminescent responses to infections by pathogens such as Pseudomonas syringae and Pectobacterium carotovorum, as well as to mechanical wounding and whitefly infestation. This non-invasive, field-deployable approach—with its compatibility with consumer-grade cameras—allows early detection of metabolic changes before visible symptoms occur, offering promising applications in precision agriculture and enhanced plant defense management.

Oral Presentations

Loop-mediated isothermal amplification (LAMP) as a viable PCR substitute for diagnostic applications in agriculture.

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Sugarcane is one of the most important crops for the production of sugar, alcohol and derivatives. Leaf scald, caused by the bacterium *Xanthomonas albilineans*, is a major disease that affects the production of this plant. Control measures for this disease depend on timely diagnosis to prevent the pathogen's spread. Isolation on selective media, serological and DNA-based molecular assays are among the methods that have been used for early detection of *X. albilineans*. Molecular methods are efficient, sensitive and specific but the most widely used have disadvantages in terms of speed and feasibility for timely use in the field. Loop-mediated isothermal amplification (LAMP) has emerged in recent years as a promising tool for its direct implementation in the field as it requires minimal equipment, is isothermal, rapid, and less susceptible to inhibitors.
In the present work, the use of the LAMP technique was assessed for the detection of *X. albilineans*. Direct amplification of DNA from bacterial isolates was performed using four primers. The presence of the expected amplification products was confirmed by agarose gel electrophoresis. The genetic construct pGEM-Xa, which contains a fragment of a specific *X. albilineans* gene, was obtained and successfully used as a positive control in the diagnostic assay. The efficacy of a rapid method for extracting DNA from sugarcane stalks for LAMP diagnosis was also evaluated.
Our data provide a preliminary demonstration of the feasibility of using LAMP as a diagnostic assay for field testing of leaf scald in hybridization centers, biofactories or production areas. The future validation of this methodology will allow us to have a highly sensitive, specific and fast diagnostic method for the management of an important disease in our country during sugarcane cultivation.
Keywords: LAMP, leaf scald, molecular diagnostics, sugacane, *Xanthomonas albilineans*.

Oral Presentations

Estrategia y modelo de negocios de la industria semillera del maíz en el sur - sureste de México.

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En el sursureste mexicano anualmente se siembran 2.6 millones de hectáreas con maíz para grano o forraje. El Servicio Nacional de Inspección y Certificación de Semillas – SNICS, registró un volumen de 2,100 t de semilla calificada para el ciclo PV/2023 para sembrar el escaso 4.3% de la superficie a nivel nacional. Solo están registradas en el SNICS, 36 empresas semilleras en los siete estados que integran la región, por ello la oferta de semillas de maíces mejorados proviene del occidente y norte del país. En el presente trabajo se analiza la red de valor de una empresa semillera regional, Reycoll Seeds, que integra dos Sociedades de Producción Rural de tipo Familiar, así como su modelo de negocio. Con base al diagnóstico, se integró un cuadro estratégico con las principales empresas semilleras del sursureste, y se propone una Agenda Estratégica, que incluye acciones clave: 1) Fortalecimiento de la Red de Aliados Comerciales Regionales Estratégicos (ACRE) para mejorar la distribución y comercialización las semillas; 2) Creación e inversión en Centros Regionales de Innovación Agronómica del Maíz (CRIAM) en alianza con instituciones de investigación, de enseñanza y gubernamentales, alianzas comerciales con proveedores de maquinaria, de insumos, de financiamiento, de asistencia técnica, centros de acopio y comercializadoras, de manera que aporten al fortaleciendo la propuesta de valor de Reycoll Seeds, transfiriendo tecnologías a sus clientes y fortaleciendo las redes técnicas y redes comerciales. Una tercera estrategia es la gobernanza corporativa de la empresa, fomentando la participación y apropiación de los socios y construir acuerdos familiares basados en sus valores y en la misión de la empresa que permitan un relevo generacional eficaz. Sin embargo, este proceso ha requerido alinear múltiples voluntades y aspectos legales, por lo que sus avances aún son incipientes, pero fundamentales para la certeza operativa y permanencia de la empresa semillera en el mercado del sursureste mexicano.
Palabras clave: Maíz, industria semillera, semilla mejorada, modelo de negocios, sursureste, Reycoll Seeds, Centros de Innovación, Aliados Estratégicos

Oral Presentations

Application and Development of Innovation in Grain Seed Production. A Business model propose

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Food production in Cuba is a strategic issue in a post-pandemic global context and amidst serious armed conflicts that are generating a severe food crisis, exacerbated in Cuba by the persistent economic war waged by the United States government against our country. Until 2019, Cuba imported an average of 800,000 tons of corn and 400,000 tons of soybeans to meet the demand for animal feed, spending around USD 500 million annually. Domestic production of these grains lags far behind demand, a phenomenon motivated by various reasons and resulting in a low quantity and quality of available seeds. In recent years, the CIGB has been working on developing Cuban corn varieties that allow for the development of simple hybrid corn production technology in the country, as well as the generation and registration of national soybean varieties. In this way, for the first time in Cuba, we are incorporating the use of a technological alternative for production, which currently accounts for 30% of the world's corn and 80% of its soybeans. Due to the specific conditions of our country, we have had to develop this alternative using our own resources.
The business proposal, based on the creation of a closed-loop company with a high-standard technology-based plant dedicated to the drying, processing, processing, and marketing of these grain seeds, and linked to a local agricultural production system that can guarantee high-quality raw materials (seeds), can represent a significant contribution to the country's agricultural development, replacing imports, and creating the conditions for export operations.

Oral Presentations

Shandong Lukang Heber Biotech Cuban-Chinese Joint Venture, a collaborative experience in agrobiotechnology.

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Join Venture, HeberNem, Production facilities, Lukang Heber Biotech Co. Ltd, agrobiotechnology.

In March 2016, Shandong Lukang Biopesticide and Heber Biotec established the joint venture in China: Shandong Lukang Heber Biotechnology Co. Ltd., with a corporate purpose aimed at agricultural products. China has entered a high-quality development phase, strengthened by encouraging it to redirect its productions to high-end products, the production of high quality standards and the use of safe and environmentally friendly products, all accompanied by increased regulations and compliance tracking.
The leading product is HeberNem, a safe, efficient and environmentally friendly bionematicide. This development fits very well into the concept of ecological environment. Lukang Heber has obtained the Sanitary Registration as a Biofertilizer and is registering as a Bionematicide. The company has completed the technology transfer and industrialization of HeberNem in China. We have production capabilities to meet demands. It was introduced in China and there are results in controlling nematode infestations and increasing yields.
Lukang Heber has obtained 14 registration certificates for microbial agents, compound microbial fertilizers, macro elements and microelements, products that are already marketed in China under the endorsement of the Ministry of Agriculture.
Lukang Heber develops and introduces products to the market, integrates and promotes ecological prevention technologies, prioritizes organic production and technological innovation towards bio-based products.
It seeks to make an impact with products that provide solutions to the great challenges of increasing production with an environmentally friendly approach. It reduces the use of toxic chemicals and the negative impact on health, seen with the integrated One Health approach. The partners seek to take advantage of their strengths, to achieve an impact on health, as well as an economic impact, of this joint cooperation.

Oral Presentations

Comportamiento de las variedades transgénicas de soya en el Occidente de Cuba.

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La soya, Glycine max (L.) Merrill, es una planta originaria de China. Este cultivo ha tenido una gran extensión durante los últimos 50 años, alcanzando un volumen de producción que supera a cultivos que tradicionalmente han sido las principales fuentes de riquezas en países como Argentina, Brasil, entre otros. En Cuba el cultivo de la soya ha tenido un desarrollo limitado, y solamente en los últimos años se ha ido desarrollando paulatinamente su cultivo en pequeños, medianos y grandes productores; como es el caso de Cubasoy en Ciego de Avila. El programa de mejoramiento de la soya, proveniente de diferentes instituciones del País (INCA, INIFAT, IIHLD, IIG y el CIGB), ha estado encaminado a la obtención de variedades apropiadas para su extensión en Cuba. En el presente trabajo se hace énfasis en el comportamiento de las variedades transgénicas resistentes al herbicida glifosato, a partir de sus posibilidades de manejo en grandes extensiones del cultivo. Se evalúan los indicadores de rendimiento y calidad de semillas de cinco variedades registradas y se comparan con las variedades convencionales a pequeña y mediana escala en un área del INCA. La selección de variedades se realiza en ferias de agrobiodiversidad, directamente en las áreas de extensión, con la presencia de especialistas, productores y directivos; resultados que permiten compartir experiencias entre los diferentes grupos vinculados con este cultivo. Se discute sobre las experiencias que pueden contribuir en el manejo integral del cultivo, con el objetivo de incrementar la eficiencia tanto en el rendimiento agrícola como en la calidad de la semilla.

Oral Presentations

Contribution of CENATOX to the estimation of environmental risk of genetically modified crops

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Introduction: Prior to the release of genetically modified crops (GMC), it is necessary to assess the risk associated with their introduction into the agroecosystem. This must be assessed under semi-field and field laboratory conditions, including the pre- and post-commercialization stages. Each trial focuses on detecting the environmental effects associated with the technology, including: the emergence of new weeds, loss of biodiversity, contamination of centers of origin, changes in gene frequencies, the emergence of resistant insects, as well as other alterations that affect the integrity of the agroecosystem. The National Center for Toxicology (CENATOX) is the entity that develops a group of trials that provide information on the environmental risk of GMCs. **Objective:** to achieve an approach to the contribution of CENATOX in the estimation of environmental risk of CGM. Materials: The works related to ecotoxicological studies at the laboratory level carried out on the different GMCs developed in the country were reviewed and presented, as well as the actions related to the monitoring and surveillance of adverse effects post-commercialization. **Results:** CENATOX results are essential for the approval of CGMs and for the authorization of large-scale releases. **Conclusion:** CENATOX's work is essential for assessing the environmental risk of CGMs.

Oral Presentations

Regulación de las Nuevas Técnicas de Mejoramiento (NBTs)

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Resumen: La regulación de la llamada “biotecnología moderna” aplicada al mejoramiento de plantas y animales, principalmente con fines agrícolas, comenzó a establecerse alrededor del mundo a partir de finales de la década de los noventa y durante la primera década del siglo XXI. Durante dicha etapa fundacional, en la práctica esta regulación sólo se aplicaba a una tecnología, la de la transgénesis para la generación de “Organismos Genéticamente Modificados” u OGM, también llamados Organismos Vivos Modificados en el contexto del Protocolo de Cartagena sobre Seguridad de la Biotecnología del Convenio sobre la Diversidad Biológica. A posteriori, durante la segunda década del nuevo siglo surgieron nuevas técnicas que hacían uso de la ingeniería genética para el mejoramiento de plantas y animales, pero sin dar lugar a un organismo transgénico, y cuyo encuadre en el concepto de OGM y OVM resulta motivo de análisis y debate. La más famosa de estas técnicas es la edición génica, particularmente a través de la herramienta CRISPR-CAS; sin embargo, existen otras técnicas de edición génica y de mejoramiento con ayuda de la ingeniería genética, por lo cual se ha acuñado el término “Nuevas Técnicas de Mejoramiento” (NBTs, por sus siglas en inglés) y otros similares como “Nuevas Técnicas Genómicas” (NGTs) para englobarlas. Hasta el momento actual, estas nuevas técnicas son objeto de extensos análisis y debates respecto de cómo deben ser reguladas. En esta conferencia se abordarán los principales puntos de vista y el estado actual de dichos debates.

Oral Presentations

Pesticide Effects of Highly Stable Green Synthesized Silver Nanocomposites to be Used in Organic Tomato Crops

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Greenhouse whitefly, Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae) together with the negative incidence of fungi such as Oidium neolycopersici and phytopathogenic bacteria, are responsible for causing serious economic losses in organic tomato crops. Silver nanoparticles (AgNPs) are a promising solution to problems caused by these pests due to their insecticidal and bactericidal properties. However, these compounds are unstable and tend to form agglomerates. This fact causes them to lose their properties so, preventing its use as an alternative to chemical pesticides in organic cultures. In this research, a novel one-step green synthesis method to obtain silver stable nanocomposites using rosemary extract (Rosmarinus officinalis L.) as green reducing agent was stablished. The polymer polyvinylpyrrolidone (PVP) was used additionlly in the same synthesis reaction as AgNPs stabilizing agent. With this scalable one step synthesis, the obtained PVP-AgNPs nanocomposite showed particle sizes of 10.8 nm being highly stable during 326 days. At different assayed doses, this highly stable PVP-AgNPs nanocomposite, was able to control whitefly specimens efficently with an average mortality rate of 98% after 10 days of the nanocomposite application to naturally infested tomato leaves grown under greenhouse conditions. Additionally, in a diffusion inhibition assay on agar plates, inhibition of Bacillus amyloliquefaciens, Pseudomonas syringae, and Xanthomonas sp growth was found. PVP-AgNPs nanocomposite was also effective to control Oidium neolycopersici in greenhouse grown tomato plants. To our knowledge, this is the first well-founded report related to a PVP-AgNPs nanocomposite obtained by green synthesis using rosemary extracts as reducing agent able to control whitefly and tomato powdery mildew, being a potential alternative to chemical pesticides in organic tomato crops.
Keywords AgNPs, PVP, nanocomposite, thermogravimetry, thermal stability, whitefly, fungi

Oral Presentations

A project for the validation of the efficiency of a nanobiopesticide for commercial use in agriculture.

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In the last years, silver nanoparticles are the most widely synthesized and used to prevent plant pests, and constitute a non-toxic, safe pest control agent with greater pesticide power compared to other forms of silver. In the other hand, biopesticides are products derived from living organisms used to naturally control agricultural pests. Recently, the integration of these biological control methods with nanotechnology has led to the development of nanobiopesticides. We are developing and scaling a nanobiopesticide (as well as the protocol for its application) based on stable silver nanoparticles an essential oils which resulted highly efficient against insects and microorganisms affecting plants. We are carrying out a project to scale our succeeded preliminary results in tomato (*Solanum lycopersicum* L.) to greenhouse and field in tomato and common bean (*Phaseolus vulgaris* L.), both vegetables with high economic value for Latin American countries due to their high production and also consumption of their fruit, but frequently affected by numerous pests and diseases. In such research, we first aim to validate, on the basis of more robust experimental and analytical designs, several effects of the nanobiopesticide, such as its high antiparasitary character, the induction of a better development of the fruit and changes in transcript profiles. So far, transcriptomics seems to indicate an overexpression of biotic and abiotic stress-related genes and subexpression of chloroplastic and mitochondrial genes. Also, we wish to make rigourous comparisons with chemical pesticides from bromatological, safety and antiparasitary efficiency points of view. In a second phase of the project, transference of the technology to field producers will be carried out.

Oral Presentations

Bioproducts production and commercialization in Colombia, AGROSAVIA's experience

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AGROSAVIA has considerable expertise in developing products based on microorganisms. Over the past 25 years, AGROSAVIA has licensed biopesticide formulation technologies to foreign companies such as Lallemand Biocontrol Ltda (Brazil), Lallemand Biologicals GmbH (Germany), and CERTIS Biologicals (United States). AGROSAVIA has two registered biofertilizers based on nitrogen-fixing bacteria: MONIBAC® based on the bacterium *Azotobacter chroococcum* for biofertilization of cotton and grass, and RHIZOBIOL® based on *Bradyrhizobium japonicum* for use in soybean. Likewise, we have registered several biopesticides: BACULOVIRUS Corpoica® based on *Phthorimaea operculella granulovirus* for control of *Tecia solanivora* in potatoes in storage; TRICOTEC® based on the antagonistic fungus *Trichoderma koningiopsis* for control of root pathogens in tomato, lettuce, rice, and ornamental fruits; LECABIOL® based on the entomopathogenic fungus *Lecanicillium lecanii* for whitefly control; SPOBIOL® based on *Spodoptera frugiperda nucleopolyhedrovirus* for control of *Spodoptera frugiperda*; and ERYTEC® based on the *Erinnyis ello granulovirus* for control of *Erinnyis ello*. We also produce the probiotic Rumitec® for improved animal health.
Development of bioproducts in AGROSAVIA follows a five-step process. In the first stage, we analyze farmers demands, generate solutions, and formulate action plans. Then, microorganisms, small molecules, or other active ingredients are obtained through bioprospecting or GBFA, and standardized methods and bioassays. If the upcoming bioproduct is economically feasible, it advances to a development phase. Here, the research groups perform further characterizations to determine the compatibility of the active ingredient with diverse excipients, its storage stability, and its biological activity under contrasting conditions; simultaneously, we develop and optimize culture media, formulation prototypes, and perform dosage studies. When the technology is robust at laboratory scale, we adjust the conditions for its mass production using our pilot bioproducts facility. At this point, we perform a newer economic analysis for business model optimization. Once the bioproduct successfully passes these first four stages, it is registered with ICA; ICA validates the bioproduct's quality and promised features using semi-commercial evaluations. Upon notification of the commercial registration, the bioproduct is ready for its distribution, production, and commercialization.

Oral Presentations

HeberNem: characteristics and uses.

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HeberNem-S has been used commercially for more than 20 years in Cuba and in other tests abroad. It is a bioproduct (Wettable powder) which active ingredient is the soil isolated bacterium reevaluated (by 16S method) as *Brevibacterium celere* (fomerly *Tsukamurella paurometabola*), strain C-924.
HeberNem-S has a remarkable nematode control activity if it is applied before planting, due to a combined production of hydrogen sulfide, chytinases, proteases and forming bacterial "biofilm" on roots, by the bacterial strain C-924. It has also demonstrated Plant Growth Promoting (PGP) effects and shown biological control activity against certain kinds of fungal agents affecting plants, and finally as floral inducer in some crops.This bioproduct has been registered in Cuba, Spain and China and considered as ecological bioproduct supported by 25 toxicological and ecotoxiological tests.
The objective of this work is to present the HeberNem uses as: bionematicide, biofertilizer, plant disease antagonist and floral inducer. It has been used in different crops as: tomato, cucumber, pepper, melon, lettuce, banana, guava, Sacha inchi and others. Potential uses of HeberNem for mosquito control and antiparasitic for veterinary use are also presented in this work.

Oral Presentations

Biostimulation of *Pseudoxanthomonas indica* H32 on chard, Chinese cabbage and cucumber crops in organic farming system.

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The use of biostimulants based on microorganisms in crops for human consumption is one of the most promising technologies for sustainable agriculture worldwide. The formulation based on *Pseudoxanthomonas indica* H32 has shown potential as a nematicide and biostimulant in protected crop houses. In this work, some experiments were carried out in “Amalia Simoni and “Nitrogen” organoponics to demonstrate the potential of the bioproduct in cucumber, chard and Chinese cabbage crops at no protected areas of urban agriculture system. In all trials, water was used as a control or negative control. The bioproduct was applied before sowing and 21 days after transplanting. Leaf length, plant length and plant weight were evaluated in Chinese cabbage and chard. In cucumber, the number of branches and female flowers was determined. The fresh mass of chard treated with biostimulants increased by 33.3% compared to plants treated with water. H32 caused the elongation of the lateral buds and therefore a greater number of branches and increased the number of female flowers by more than 75% with respect to the control treatment. Under semi-protected growing conditions, the increase in fresh mass in both chard and Chinese cabbage was 25%. The application of this composition implies the introduction of a new biostimulant for crops, which improves agricultural yields without adverse effects on the environment.

Oral Presentations

Bioestimulantes de oligosacarinas en la activación del crecimiento, la nutrición, el rendimiento y la protección antiestrés en soya

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Los bioestimulantes son compuestos naturales y microorganismos que mejoran la eficiencia de la nutrición vegetal, la calidad de los cultivos y la tolerancia antiestrés en las plantas. Los bioestimulantes de Oligosacarinas del INCA (Azofert®, Quitomax® y Pectimorf®) aplicados en forma simple o en combinaciones promueven importantes beneficios en los cultivos. **Problemática a resolver**. La soya (Glycine maxcarece de alternativas nacionales para mejorar su desarrollo y rendimientos en Cuba. El Grupo de Productos Bioactivos del INCA está enfrascado en desarrollar un nuevo bioproducto o tecnología de aplicación para soya mediante la adecuada combinación de bioestimulantes microbianos y no microbianos. **El objetivo** de este trabajo fue demostrar las potencialidades bioestimulantes del quitosano y del producto comercial Quitomax® en soya inoculada con Azofert®-S, a través de resultados experimentales y validaciones en variedades de soya. **Métodos y resultados**. Previamente se estudió en experimentos *in vitro*, la compatibilidad quitosano-Bradyrhizobium para su posible aplicación conjunta y se demostró en experimentos en condiciones controladas y de campo, la estimulación por quitosano de indicadores de la nutrición, el crecimiento, del metabolismo primario y secundario y del rendimiento en soya inoculada. Se definieron las mejores concentraciones de quitosano en la bioestimulación y se confirmaron posteriormente con el bioestimulante Quitomax®. La aplicación combinada de quitosano y Azofert®-S (aplicados a la semilla o por aspersión foliar) mejora la toma de nutrientes en soya inoculada, el crecimiento y el rendimiento, resultado que se validó con Azofert®-S y Quitomax® en diferentes variedades y localidades en condiciones de producción. **Conclusiones**. De acuerdo a los resultados es posible diseñar una tecnología de aplicación de bioestimulantes nacionales en soya que permita su protección y mejore hasta en 50% los rendimientos del cultivo.

Oral Presentations

Valorization of agro-industrial waste: Use of citrus peels for agricultural pest control

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Agricultural, agro-industrial, and market waste could constitute 25-35% of the volume of products in the food sector. In a world where the population is constantly increasing, these volumes of waste not only represent a significant burden for economies due to the need for treatment but could also be a valuable source of bioactive substances of great interest. On the other hand, the extensive use, and at times, overuse of chemical pesticides has contributed significantly to soil erosion and ecosystem contamination. In the present work, a candidate botanical pesticide was obtained from the peels of mandarin (Citrus reticulata L.), which demonstrated insecticidal and fungicidal activity against pests of interest in crops such as potato (Solanum tuberosum L.), achieving yields close to 73% of those obtained with conventional chemical treatment, which employed six chemical insecticides and three chemical fungicides. It was also demonstrated that formulations based on mandarin peel extracts did not affect beneficial entomofauna such as bees (Aphis mellifera), in contrast to conventional chemical treatments. Therefore, formulations based on Citrus reticulata peel could be a good candidate as an ecological botanical pesticide and be very useful for small and medium agricultural producers of Andean 'chakras' committed to organic agroecological production.

Oral Presentations

KestoZyme: basis of an integral solution to the main constraints of the current fungal-based technology for short-chain FOS production from sucrose

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Short-chain FructoOligoSaccharides (scFOS) are natural soluble fibbers of relative sweet taste and proven prebiotic effects in humans and animals. Current fungal-based systems for scFOS production from sucrose yield a mixture of transfructosylation products (GF_2<GF_3>GF_4), display substrate hydrolysis and have operational limitations. The presentation will be focused on the development of a novel free-enzyme biocatalyst that efficiently converts sucrose into scFOS syrups with a primary composition of most valuable 1-kestose (GF_2). The enzyme of choice was a plant sucrose:sucrose 1-fructosyltransferase (1-SST) produced to high levels in the yeast *Komagataella phaffii* via constitutive expression of multi-copy gene insertions. After fed-batch fermentation, the culture supernatant containing the recombinant enzyme (1-SSTrec, commercially named as KestoZyme) to initial purity above 85% was dialyzed and concentrated by a single ultrafiltration step and then submitted to a lyophilisation process. The freeze-dried enzyme (soluble powder) can be stably stored at room temperature for at least one year. Discontinuous batches with KestoZyme operating in a stirred tank reactor at high sucrose concentrations (600-800 g/L), pH 5.5-6.0 and 40-50ºC yielded high FOS levels (55-60% w/w) with negligible fructose release. The achieved 1-kestose content was three-fold higher than the values reported in the reaction products of fungal enzyme systems. The use of KestoZyme offers enzymatic and technical advantages over the current fungal-based technology for high-scale production of scFOS from commercial sucrose sources and other commercial sucrose sources

Oral Presentations

Kestozyme, an opportunity for the production of fructooligosaccharides in a circular economy in the environment of a sugarcane biorefinery.

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This study evaluates the technical and economic feasibility of synthesizing fructooligosaccharides (FOS) from sucrose, catalyzed by the recombinant enzyme sucrose: sucrose 1-fructosyltransferase (1-SSTrec), marketed as Kestozyme. Developed by CIGB, Kestozyme is a unique commercial enzyme preparation designed for industrial FOS production, with no comparable alternatives currently available. Experiments conducted in stirred tank reactors at laboratory and pilot scales demonstrated conversion yields of 55–60%. The synthesized carbohydrate composition consisted of 1-kestose (50–54%), nystose (4–8%), sucrose (15–17%), glucose (23–25%), and fructose (≥0.5%). Comparable results were achieved using refined sugar, unrefined sugar, or decolorized liquor (≥99% purity). However, lower yields (34.5–45.8%) and reduced FOS content (28.12–38.78%) were observed when using sugar syrup or final molasses. The evaluation of technological alternatives for FOS production within a sugarcane biorefinery confirmed process sustainability. Production costs were highly competitive relative to commercial FOS prices, enabling added value incorporation into molasses. High-purity FOS syrup (95%) achieved a 65% profit margin in independent plants, based on a reference price of $7,200/t. Implementing a circular economy model within a refinery environment led to a 25% cost reduction and an increased profit margin of 74%. These findings highlight the viability of integrating FOS production into existing sugar industry infrastructures.
Key words: fructosyltransferase, fructooligosaccharides, 1-kestose, circular economy and production cost.

Oral Presentations

Purification of fructooligosaccharides obtained from sucrose by a recombinant yeast expressing a mutated levansucrase.

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During the process of FOS synthesis from sucrose, D-glucose is released as a by-product of the transfructosylation process, while residual sucrose remains in the mixture at the end of the reaction. Due to its caloric contribution and its effect on caries and diabetes it is desirable to remove both sugars. Cells of a recombinant Komagataella phaffii expressing a double mutated levansucrase were immobilized in calcium alginate. The beads were then incubated with a FOS solution at different total carbohydrate concentrations (300-600 g/L) at 35°C. It was observed that the rate of sucrose hydrolysis decreased with an increase in the total carbohydrate concentration. However, the glucose consumption rate was higher at 400 g/L than at 300 g/L and decreased in the rest of the concentrations. The best results were obtained at a total carbohydrate concentration of 400 g/L, where a significant enhancement in the purity of the FOS was observed, rising from 48% to 92% (w/w) as monosaccharides were metabolized and sucrose was hydrolyzed in the single step of operation. Consequently, 90% of the initial FOS were recovered at the end of the process with a 1-kestose/nystose ratio of 8:2. This pioneering one-step approach holds significant market potential, offering a novel and efficient method for enhancing the purity of 1-kestose as a functional food ingredient.

Oral Presentations

Removal capacity and enzyme stability, two variables influencing recombinant dextranase enzyme performance

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Conditions under which dextranase works in the sugar mill (pH, temperature, Brix, enzyme dosage), as well as those inherent to the substrate itself (branching degree and molecular weight), are of notorious relevance to guarantee effective dextran removal during sugar production process. In this study, a quadratic mathematical model relating the enzymatic activity of recombinant dextranase to the aforementioned variables was determined. Using a multifactorial ANOVA the following equation was obtained as AE = -118,04 + 10,04*pH + 3,43*T +0,128*[E] - 0,979*pH^2 + 0,014*pH*T 0,008*pH*[E] - 0,032*T2 -0,0009*T*[E] + 0,0006*[E]^2. Likewise, the study evidenced the inversely proportional influence of the polymer molecular weight and enzymatic activity. Enzymatic treatment of both simulated juice (containing a mixture of T 110, T 200 and T1500 dextran) and real sugar mill juice showed that polymer removal levels always averaged 64 %. Thus, in the simulated juice, the removal level ranged between 38 and 90 % (reaching the best removal condition in the clarified juice, at pH = 7.2, temp = 55 °C and 13.6° Brix). For mill 'real juice the removal averaged 75 % (maximum being 96,2%) obtained in a concentration dependent manner (higher dose 0.550 U/ml) when enzyme was applied to remove dextran in the range of 580 to 1000 ppm. Arrhenius equation allowed estimate enzyme stability, thus results evidenced that enzyme can preserve 85% of its initial activity for 9 months and 1.5 years when stored at 25 and 30°C, respectively.
Key words: Dextranase, mill dextran remotion, *Pichia pastoris*, dextran remotion, stability using Arrhenius.

Poster Presentations

Avances en la obtención de cultivares mejorados de frijol común (*Phaseolus vulgaris* L.) para cosecha mecanizada

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En Cuba, varios factores determinan los bajos rendimientos agrícolas del frijol común, entre otros, dificultades para el control de arvenses y que la cosecha principalmente se realice manual o semi-mecanizada. En virtud de mejorar esta situación, se desarrolla un proyecto conjunto entre el Centro de Investigaciones Agropecuarias (CIAP), el Instituto de Biotecnología de las Plantas (IBP) y el Centro de Ingeniería Genética y Biotecnología (CIGB), cuyo objetivo es obtener cultivares con altos potenciales de rendimiento agrícola, aptos para cosecha mecanizada y transformados genéticamente con resistencia al herbicida Basta®. El Material Base fue una accesión conservada en el CIAP, con semillas de testa de color negro, y presencia de individuos de hábito de crecimiento Tipo I con buen despeje. Para obtener líneas estables y homogéneas se realizaron dos ciclos de selección masal continua, seguidos por selecciones individuales de plantas élite, adoptando el método de selección de progenies. Las mejores progenies "C" se han seleccionado según el hábito de crecimiento Tipo I, altura de la planta de 35-45 cm, altura del ápice de las legumbres bajas (despeje) de 9-15 cm, diámetro del tallo de 5-8 mm, número de legumbres totales por planta de 16-22, número de semillas por planta entre 55 y 85, y masa de semillas totales por planta entre 10,5 y 13,0 g. Se prevé obtener cultivares con potenciales de rendimiento agrícola entre 3,4 y 3,6 t ha^{-1}, al utilizar altas densidades (317 000 a 320 000 plantas ha^{-1}), lo cual es posible al utilizar cultivares de hábito de crecimiento determinado arbustivo (Tipo I).

Poster Presentations

Development of a platform for the evaluation in vitro of the activity of biological compounds on the growth of *Sporisorium scitamineum.*

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Plant fungal diseases represent a significant menace to agriculture. Several reports describes loses between 20 and 40 percent of the global annual harvest. Unfortunately, once they become visible it is a symptom that they have progressed too far and sometimes it is difficult to save them. Smut is caused by *Sporisorium scitamineum*, the characteristic symptom is a structure called “whip”, induced by the fungus, which develops from stalk apical meristem. In Cuba, this disease has led to the disuse in the sugar industry of highly productive varieties, such as Ja60-5, due to their susceptibility. The genetic modification of plants would allow to obtain crops tolerant to this disease. Having a platform for the evaluation of the in vitro biological activity of different compounds on the growth of S. scitamineum would be a useful tool for the selection of the most promising transformation events or molecules for the control of the disease. This work describes the methodology for the in vitro evaluation of different biological compounds on the growth of *S.scitamineum*. Culture media, inoculum preparation and suitable incubation conditions were established for the growth of the fungus under in vitro conditions. In addition, different commercial fungicides were evaluated in terms of inhibition of in vitro growth of the fungus. Of the fungicides evaluated, three showed a certain level of growth inhibition: Zampro, Previcur and Nagata, the latter being the one with the highest activity. This last result has not been described before. In addition, extracts of plants transformed with a gene described for resistance to fungi such as *Phakopsora pachy*rhizi and *Colletotrichum truncatum* were evaluated. Different inhibitory capacity of plant extracts on the growth of *S. scitamineum* was observed.

Poster Presentations

Efficient *Agrobacterium*-mediated transformation of soybean meristematic explants by phosphomannose isomerase/mannose selection.

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Selection markers are necessary for the development of plant transformation technology due to their ability to protect transformed cells in the presence of a toxic substrate. Few cells are transformed in most experiments and the probability of distinguishing transgenic cells without selection is usually very low. Within these genes, one of the most used in soybeans is the *cp4epsps* gene, which confers resistance to the herbicide glyphosate. Nevertheless, the search for new selection markers for the production of transgenic plants is necessary. In this research we have adapted the PMI/mannose system for the genetic transformation of soybean (*Glycine max* [L.] Merrill). First, mannose sensitivity was evaluated in meristematic explants of soybean genotype DT84. Increasing mannose concentrations from 5g L^{-1} to 20g L^{-1} were tested, as well as different mannose-sucrose combinations. Subsequently, the *Escherichia coli manA* gene (E.C. 5.1.3.8) that encodes a phosphomannose isomerase that converts mannose-6-phosphate (glycolysis inhibitor) to fructose-6-phosphate (glycolysis intermediate) was isolated from the MIR162 event. This gene was cloned in the commercial plasmid pCAMBIA3300 under the CaMoV 35S constitutive promoter. The gene of interest was introduced into the plants by transformation with *Agrobacterium tumefaciens* strain LBA4404, generating a transformation efficiency greater than 20%. Transgenic plants rooted in a selection medium containing 20g L^{-1} mannose were molecularly confirmed by PCR. The relative expression of the *manA* gene was confirmed by real-time RT-PCR and the enzymatic activity of phosphomannose isomerase was evidenced by the chlorophenol red method. This is the first time that soybean plants have been transformed with *manA* gene, which will allow the use of this selection system to obtain transgenic plants that carry genes of interest.

Poster Presentations

Establishment of an efficient sugarcane transformation protocol using *Agrobacterium tumefaciens.*

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Sugarcane is the most important source of sugar around the world representing the 80% of sugar's production. This culture has economic importance in other industries due to the use of his derivates as a biofuel, food and feed animals, in cosmetics, plastic among others. In Cuba sugarcane industry was successful until the last 20 years where it was observed a diminish in sugar production levels. One of the causes of this phenomenon was the increase emergence of diseases a caused by fungus or bacteria's. There are many strategies for improve sugarcane productions; traditional crossing could be hard working nevertheless biotechnology most be a promising tool, in particular genome modification. In the trans-genesis process, sugarcane is well known as a recalcitrant culture so improve the efficiency of this process will result in obtaining a high number of plants with the desired traits. In this work it is proposed the optimization of the transformation protocol of Jaronu 60-5 variety. A binary vector was developed carrying a gene for fungal resistance and the bar gen for herbicide resistance. For the transformation we used Agrobacterium tumefaciens strain LBA 4404.Some modifications involve changes in culture media composition. In addition, it was changed the incubation condition, the types of sugarcane callus and the osmotic media previous and during of infection. The use of calluses R0 increased the number of plants obtained by grams initial of callus, similar result was obtained using controlled incubation conditions in climacell. The phosphinotricin as the selection agent after the selection in darkness diminishes the possibility of false positives during the next steps of the plant's development. 51 plants were obtained during the transformation process, 47 of them were analysed by PCR showing the expected amplicon of 407 pb corresponding with a fragment of the gen bar.

Poster Presentations

Evaluation of the drought resistance potential of glyphosate-resistant transgenic soybeans.

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Soybean is one of the most economically important crops worldwide, primarily due to its contribution of protein meal and vegetable oil. It is considered among the five main crops that sustain the global economy; however, drought is an environmental factor that limits its growth and development. This abiotic factor implies changes in the morphology, physiology, and metabolism of the plant, which leads to a decrease in its yield. The objective of this research was to evaluate the potential for drought resistance in five transgenic soybean varieties selected from 20 varieties, after evaluating which ones presented the greatest resistance using leaf area as a morphological marker. An experimental design was used that consisted of in vitro germination of transgenic seeds, using 3% polyethylene glycol-6000. The dry matter index and chlorophyll levels were determined. The results obtained showed that the CA20 variety was the one with the smallest difference in dry matter and chlorophyll index compared to its controls, with a dry matter index of 16.6% and a chlorophyll concentration of 0.28 mg/g, being the one with the best results. Among the five study varieties, CB11 with an 18.7% dry matter index and a chlorophyll concentration of 0.38 mg/g was the one that obtained the greatest difference compared to its control. It is concluded that this in vitro experiment is useful for studying plant resistance, although a more extensive study under field conditions will be necessary to corroborate these results.
Keywords: Glycine max, Drought, Chlorophyll, Polyethylene glycol, Abiotic Stress

Poster Presentations

Expression of the artificial microRNA156 in soybean leaves using a simple and efficient agroinfiltration protocol.

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MicroRNAs (miRNAs) are post-transcriptional regulators in plant immune systems. Nevertheless, little is known about their roles in plant immune response to fungal pathogens. The use of artificial microRNAs (amiRNA) has been an alternative to improve plant resilience, particularly resistance against pathogens. The analysis of transient expression of amiRNAs would allow a rapid evaluation of their functions as a repressor or activator of the molecular mechanisms that underlie the response to biotic stress. However, the application of this *in vitro* assay in soybean leaves constitutes a challenge, due to the recalcitrant characteristics of the crop. Previous studies revealed the differential expression of miR156 in soybean plants infested with *Fusarium oxysporum*, which served as the basis for synthesizing amiRNA156. In the present work, amiRNA156 was cloned into the pC3300 vector to transform soybean using *Agrobacterium tumefaciens* and express it in leaves. For this, an Agroinfiltration protocol of unifoliate soybean leaves was optimized using vacuum. The relative expression of amiR156 was quantified by qRT-PCR with specific primers and the results showed the highest expression at 5 days after leaf infiltration. Here, the expression of artificial microRNA156 was achieved for the first time in vacuum-agroinfiltrated soybean leaves. These results can serve as a basis to quickly discern soybean microRNAs involved in resistance to fungi of the Fusarium genus.

Poster Presentations

Histidine-tagged defensin fusion construct for integration in tobacco plants as model

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Soybean (*Glycine max* (L.) Merryll) is a strategic crop for both human and animal nutrition. The productivity of this crop is threatened by fungal pathogens such as *Phakopsora pachyrhizi* (Asian soybean rust) and *Colletotrichum truncatum* (anthracnose). Defensins are small, cysteine-rich antimicrobial peptides with potent antifungal activity, acting primarily through membrane disruption and pore formation, leading to fungal cell death. The developed transgenic soybean plants expressing plant defensin genes, such as *NmDef02*, have demonstrated enhanced resistance to those pathogens under field conditions, as was previously reported (Soto et al., 2020). For the registration and widespread adoption of the resistant soybean plants it is crucial to establish robust analytical tools for the detection and quantification of defensin. To develop methods for detection and quantification of defensin in transgenic plants, in the present work we aimed to produce defensin in alternative host as *Nicotiana tabacum*, as a feasible model to this purpose. To this goal, we have developed a histidine-tagged (His-tag) defensin fusion construct. Therefore, a set of primers were designed to introduce a C-terminal His-tag into the defensin gene by polymerase chain reaction (PCR). The obtained amplicon was cloned into an intermediate vector under transcriptional regulation of the 35S promoter. Finally, the expression cassette was ligated into a binary vector. Sanger sequencing confirmed the correct sequence of the defensin-His fusion. The construct was subsequently introduced into *Nicotiana tabacum* via *Agrobacterium*-mediated transformation. Transgenic tobacco plants were regenerated and screened by PCR using the specific primers, confirming the presence of the expected amplicon corresponding to the defensin-His fusion gene, demonstrating it successful integration. As an outcome of this work, the transgenic tobacco plants will provide a valuable resource of defensin for future studies on its accumulation in soybean plants, enabling it precise quantification and functional analysis.

Soto, N., Hernández, Y., Delgado, C., Rosabal, Y., Ortiz, R., Valencia, L., Borrás-Hidalgo, O., Pujol, M., Enríquez, G.A., 2020. Field resistance to *Phakopsora pachyrhizi* and *Colletotrichum truncatum* of transgenic soybean expressing the NmDef02 plant defensin gene. Frontiers in Plant Science 11, 562.

Poster Presentations

In vitro propagation of Citrus spp. a tool for plant production and genetic improvement

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Citrus is an important crop for human nutrition and health, its fruits are a source of vitamins and minerals such as, vitamin C, carotenoids, folates, potassium. World production of citrus fruits is 103.7 million tonnes. Globally, citrus is affected by biotic and abiotic stresses that reduce fruit production and area under cultivation. Grafting techniques using resistant rootstocks are a common practice in citrus cultivation. Biotechnology can contribute to the recovery of citrus plantations by increasing the availability of rootstocks for grafting, rapid introduction of new cultivars and providing tools for genetic improvement of the crop. Given the importance of citrus and the need for rootstock availability, the aim of the study was to develop protocols for in vitro propagation and ex vitro acclimatization of citrus plants. Segments of in vitro germinated seedlings were propagated on Murashige and Skoog (MS) medium supplemented with 6-benzyladenine and rooted on MS medium supplemented with naftalene acetic acid. Immature seeds were also used for callus formation and direct embryogenesis on MS medium supplemented with 6-benzyladenine and vitamins. MS and Woody Plant (WP) media were tested for embryo conversion. Plants, from both regeneration systems, were successfully acclimatized *ex vitro*. The protocols used allowed to obtain plants from in vitro culture with excellent development of the aerial part and their root system.

Citrange Carrizo, Citrange C35, Citrus macrophylla, micropropagation

Poster Presentations

Increasing genetic diversity in a seed bank of transgenic corn by traditional techniques

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Crop genetic diversity in the field is an important element to assessment the production of it. One of the characteristics of the modern corn productions is the uses of single hybrids with high yield. These cultivars are homogeneous populations that under specific unforeseen conditions could be susceptible to biotic or abiotic stresses. To avoid this problem, the number of the varieties in the field most be increase. In the present work we stablished several approaches for increase the diversity of our corn germplasm bank. We developed new single hybrids from the cross between our transgenic line and others with several origins. It´s new combinations most be well synchronic of it's parents during the flowering and good adaptation to the tropical conditions.

Poster Presentations

Molecular characterization by RAPD markers of different plant varieties obtained by mutations.

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Current breeding programs are developing new varieties that resist diseases and adverse environmental conditions since many of them have become vulnerable. Among the species included in Cuban program are beans and tomatoes, because they their production yields are very low.
The common bean (*Phaseolus vulgaris*) belongs to the legume family and is a plant native to the Americas that is grown all over the world and is one of the most important because it is an indispensable nutritional supplement in the diet.
The tomato (*Solanum lycopersicum*), is an herbaceous plant species of the genus Solanum of the Solanaceae family. It is an edible plant native to America that today is consumed worldwide due to its nutritional properties, such as high-water content, low in carbohydrates and calories and rich in fiber, vitamins and some minerals. Medicinal properties such as digestive, disinfectant, antiscorbutic are also attributed to it, and is a valuable source of lycopene, which plays an important role in disease prevention.
In this work, the results of genetic variability among varieties are presented based on the analysis of the presence or absence of bands obtained with different decamers. The RAPD markers used allowed the molecular characterization of the varieties. The use of this tool in breeding programs is recommended.

Poster Presentations

Obtaining bean plants resistant to the herbicide LifeLine.

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In Cuba the climate favors the proliferation of weeds. The high prevalence of these plants causes a productive and agronomic decrease of beans. Obtaining herbicide resistant plants through genetic transformation via *Agrobacterium tumefaciens* could offer an effective solution to enhance the cultivation of this legume. Genetic transformation of beans goes through a complex regeneration process, described by several authors as inefficient. The purpose of this work was to obtain transformed bean plants, regardless of the low efficiency of the regeneration process bean exhibits. To achieve this goal, the embryogenic axis with the partial node was used as an explant to be infected with *Agrobacterium tumefaciens*, the bacteria carrier of the expression cassette that confers resistance to ammonium glufosinate, which is the active component of the LifeLine herbicide. Given the low regeneration efficiency of the explant, the transformation methodology was focused on the enhancing the area of the explant infected with the bacteria. The transgenic clones obtained were evaluated by their resistance to LifeLine at a dose of 10 mg/L of the active component of the herbicide, which is twice of that used during selection of the transformants *in vitro*. Although 39.02% of explants developed shoots resistant to the LifeLine, only 1.1% of plants were adapted successfully to soil. The resistant clones were analyzed by PCR using specific primers to target the bar gene. A 15% of these clones produced a 591 bp amplicon, which demonstrated the presence of the bar gene in the transformed bean plants. Several lines of transgenic plants were selected by evaluating their resistance to LifeLine. From these plants, presumably transgenic seeds were obtained for two generations T2 and T3. In summary, the methodology used ensured the production of transformed plants resistant to LifeLine in spite of the low regeneration/transformation efficiency.
Keywords: Agrobacterium tumefaciens; embryonic axis; bean; bar; herbicide, transformation

Poster Presentations

RNA interference-based resistance in transgenic tomato plants against geminiviruses

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The tomato plant is one of the most consumed vegetables in the world. However, its cultivation is complex due to being affected by numerous pathogens and pests. Among them, viruses have caused significant losses to tomato farming, especially those transmitted by insect vectors, particularly the whitefly (*Bemisia tabaci*). Thus, there is great interest in developing genetically modified tomato plants resistant to viral diseases. In this context, the objective of this study was to produce genetically modified tomatoes via *Agrobacterium tumefaciens* using the RNA interference technique, with sequences of the *rep* gene from four main species of begomoviruses. PCR analysis revealed the presence of both transgenes (4GEMpdk and *nptII*) in all 27 transgenic lines obtained. Through bioassays, some of the transgenic lines were exposed to viruliferous whiteflies in order to test resistance to the tomato severe rugose virus (ToSRV). Among the tested lines, two stood out for their resistance to begomovirus compared to the control lines: line 5 (61.54% of individuals uninfected) and line 7 (74% of individuals uninfected). Other transgenic lines generated are being evaluated, and the individuals showing resistance will be selected to generate homozygous populations for the transgenes, aiming to obtain 100% ToSRV-resistant GM tomato plants.

Poster Presentations

B3 transcription factors from *Nicotiana* spp. activate the β-phaseolin promoter in *N. benthamiana* vegetative tissue

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Type B3 transcription factors are a family of plant-specific proteins that play a critical role in gene regulation of essential processes in plant growth, particularly seed maturation. Their key feature is a highly conserved B3 DNA-binding domain, responsible for binding to specific DNA sequences in target gene promoters. The β-phaseolin promoter is widely used in plant biotechnology to express transgenes; nevertheless, it is mostly limited to seed expression. This study focused on obtaining *Nicotiana benthamiana* seeds genetically engineered to constitutively express transcription factors originally from *N. tabacum* and *N. rustica*, which are capable of activating the β-phaseolin promoter in vegetative tissue. To achieve this, RT-PCR isolation of the transcription factor genes of *N. tabacum* and *N. rustica* was performed, using specifically designed primers. Resulting fragments were individually cloned into the commercial vector pGEM-T Easy, and amino acid sequence analysis was conducted to compare these B3 factors with those from other plant species. The fragments were subsequently cloned into the pBPF-Ω7 intermediate vector, with a cMYC tag, and then into the binary vector pCAMBIA3300. The final construct was used for *Agrobacterium*-mediated transformation of *N. benthamiana* cotyledon leaves and expression of both transcription factors was confirmed through Western blot using an anti-cMYC antibody. *N. benthamiana* plants were agroinfiltrated to express the light and heavy chains of the CB-Hep1 monoclonal antibody, which recognizes a linear epitope in the AgsHB (Hepatitis B antigen surface), under control of the β-phaseolin promoter. Expression of both chains and assembly of the functional antibody in leaf tissue were confirmed by Western blot and ELISA. Type B3 transcription factors from *N. tabacum* and *N. rustica*, when constitutively expressed, activate the β-phaseolin promoter in vegetative tissue. This demonstrates the feasibility of using seed-specific promoters for targeted expression in vegetative tissues, providing a promising tool for plant-based production of recombinant proteins.

Keywords: B3 transcription factors, *Nicotiana*, β-phaseolin promoter, plant-based protein expression

Poster Presentations

New products with a dermo-regenative effect derived from sericin hydrolysate

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The use of *Bombyx mori* as a bioreactor has been enhanced for the production of recombinant proteins, with the sericin promoter being the most widely used. The natural polymer sericin, produced by this lepidopteran, has biological properties like good oxygen permeability, moisture-regulating capacity, resistance to UV radiation, ability to promotes cell growth and biocompatibility. All these characteristics make possible its use as a component of pharmaceutical formulations with several biomedical applications. In the present work, two new pharmaceutical products in the form of cream and jelly were designed, containing 5% sericin hydrolysate as the active ingredient. A D-Optimal experimental mixture statistical design, adjusted to a linear model, was employed using Design Expert software version 12.0.3.0. For the cream, the independent variables evaluated were the amounts of stearyl alcohol, stearic acid, and sodium hydroxide; for the jelly, the variables were Carbopol 940 and sodium hydroxide. The dependent variables were pH and spread ability. The cream formulations were prepared using the fusion method, while the jelly was obtained via the incorporation method. Physical and chemical evaluations were conducted over 30 days of shelf life, with the best formulation selected for each pharmaceutical form. Three pilot batches were prepared, and their physicochemical parameters were evaluated at baseline and after 30, 60, and 90 days of shelf life. The cream batches were subjected to thermal stress and destabilization by centrifugation to assess emulsion stability. In both designs, the selected formulations were similar in qualitative and quantitative composition to the prepared technological variants, validating the effectiveness of the developed design. The three batches of cream and jelly formulations maintained their properties during the 90-day stability study under ambient temperature (30 ± 2 °C) in the tested packaging. Furthermore, both formulations were classified as non-irritating to skin or eyes and exhibited a dermo-regenerative effect.
sericin, dermo-regenerative, cream, jelly, pharmaceutics products.

Poster Presentations

Production of recombinant hemagglutinin from avian influenza in plants.

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The emergence of a new outbreak of avian influenza is causing millions in losses to the global poultry industry and generating high import costs. The main countries with export capacity are implementing vaccination campaigns with inactivated recombinant viruses, the production of which requires high levels of biosecurity. Plants as a protein expression system constitute a viable alternative in pandemic scenarios for the production of recombinant veterinary vaccine candidates in relatively short periods of time. Previously, aqueous soluble total protein extracts from seeds of a transgenic *Nicotiana tabacum* line, expressing hemagglutinin gene from the highly pathogenic avian influenza Viet Nam/1203/2004/H5N1 showed low to mild hemagglutination inhibition titters in immunized chickens. In this study, the hemagglutinin was purified by cation exchange chromatography from aqueous seeds extracts increasing the concentration of hemagglutinin from 13% in TSP extract to 70% in purified fraction. However, size excluded chromatography analysis of purified HA/H5 revealed low proportion of trimeric structures, which would be related with low capacity to generate an adequate immune response. To increase the immunogenicity of hemagglutinin a new HA/H5 antigen fused to chicken CD154 was designed to be expressed in seeds. Transient expression of this protein fused antigen was confirmed in *Nicotiana benthamiana* agroinfiltrated leaves mediated by the co-expression of seed transcription factor like ABI3. Stable transformation of *Nicotiana tabacum* was performed and the stable integration of HA-CD154 gen in transgenic plants was confirmed.

Poster Presentations

Rapid production of bovine FSH protein in *Nicotiana benthamiana* leaves

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Follicle-stimulating hormone (FSH) is a hormone that plays a significant role in sexual development and reproduction. The FSH is produced by the anterior pituitary gland, being released into the bloodstream to the ovaries where it plays its fundamental role in the maturation process of the oocyte, which is subsequently released during ovulation. In cattle, the exogenous administration of FSH (natural or recombinant protein) for reproduction during the follicular wave stimulates the growth of a large number of follicles. In this study, two plant expression vectors for constitute production in agroinfiltrated *Nicotiana benthamiana* plants, each carrying separately the alpha and beta chain genes that comprise bovine FSH fused to His tag were designed. The expression of the genes encoding bovine FSH was characterized by Western blot analysis and the recombinant hormone was subsequently purified using Immobilized metal affinity chromatography (IMAC). The identity of alpha and beta chains of the bovine FSH protein was confirmed using mass spectrometry. The purified and quantified recombinant protein will be evaluate in order to confirm the capacity to stimulate superovulation in female cattle, supporting the use of plants as a system for the expression of complex heterologous proteins.
Keywords: bovine FSH protein, *Nicotiana benthamiana*, transient expression

Poster Presentations

Characterization of a new isolate of *Rhynchosia golden mosaic Yucatan virus* (RhGMYuV) identified in soybean crop in Havana.

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The soybean crop, *Glycine max* (L.) Merrill holds global significance as a primary source of protein and oil for animal and human feeding. Its productivity can be compromised by various fungal, bacterial, and viral diseases. Begomovirus—the main genus of the *Geminiviridae* family—is widely distributed around the world; these viruses can cause severe damage to economic important crops; some of them have been identified in soybean plants in Cuba.
In this work, the etiology of the symptoms observed in a soybean plant with prominent dwarfism, wrinkling with blistering, greening of blisters and bright yellow mosaic in a plot in Havana was studied. Using Rolling Circle Amplification (RCA), geminivirus-sized DNA sequences were isolated. Subsequent sequencing and BLAST analysis identified the bipartite begomovirus components A and B, showing the highest sequence identity with *Rhynchosia golden mosaic Yucatan virus* (RhGMYuV). Key genomic features, including the nonanucleotide motif, iterons, common region, and open reading frames, were characterized. To evaluate infectivity, hemidimer constructs of DNA-A and DNA-B were cloned into small vectors and used in biolistic inoculation of soybean sprouts. Symptom development, along with PCR and RFLP analyses, confirmed successful infection, fulfilling Koch's postulates.
These results not only have diagnostic/epidemiological implications, but could also imply a major scope in the aim of studying the complex geminivirus-plant interactions and plant defense mechanisms.

Poster Presentations

Clustering and differential expression analysis of small RNAs; an expression profiling of microRNAs in greenhouse and in field cultivated transgenic tomato plants.

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The microRNAs (miRNAs) are small RNA molecules that regulate gene expression in plants and other eukaryotes. Understanding miRNA expression under different conditions could provide valuable insights of biological processes under study. In this work, we compared the miRNA profiles in tomato yellow leaf curl virus-resistant transgenic tomato plants cultivated under field and greenhouse conditions, using our previously-obtained Illumina small RNA sequencing data (Fuentes et al. 2016; doi: 10.1094/MPMI-08-15-0181-R). To this aim, we implemented a read counting procedure and performed preprocessing of the count matrix. We used a hierarchical clustering, implemented in Complex Heatmap package in R, and tested various distance metrics (Euclidean, Pearson, Spearman) and association methods. We conducted Principal Component Analysis (PCA) of standardized data to plot samples in terms of the two firsts principal components. Using the edgeR package, we identified differentially expressed miRNAs (|log2FC| >=1 and adjusted P-value<0.05) and visualized the results using volcano plots to highlight miRNAs differentially expressed between greenhouse and field conditions. The bidimensional hierarchical clustering analysis revealed distinct patterns of miR expression and all the experimental conditions were perfectively grouped. The Spearman's rank correlation and the average linkage method provided the most robust unsupervised clustering. PCA successfully identified the more relevant features, with the first two components explaining 58% of the variance, reflecting the important molecular changes underlying greenhouse and field conditions. Differential expression analysis identified 39 miRNAs that were up- or down-regulated in greenhouse versus field conditions: the most statistically significant miRNAs were related to fruit development, abiotic stress tolerance, pathogen resistance and phosphate starvation in plants. This study highlighted the feasibility of the applied bioinformatics methodology to uncover miRNA expression dynamics in transgenic tomato plants. Our findings could contribute to understanding the behavior of tomato plants in greenhouse and field conditions under complex environmental stresses.

Poster Presentations

Evaluación de genotipos de tabaco frente a *Peronospora tabacina* D. B Adam. Identificación del fragmento BRM1 asociado a la resistencia del moho azul.

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El objetivo del presente trabajo fue evaluar el comportamiento de cultivares comerciales de tabaco cubano y genotipos de interés frente a P. tabacina y la presencia del marcador molecular estrechamente ligado al locus de resistencia al moho azul (BRM1). Se emplearon cultivares de tabaco Negro, Burley y Virginia además, de incluirse los cultivares “Bel 61-10” como control resistente y “Bel B” como testigo susceptible para un total de 69 genotipos. La resistencia al moho azul se evaluó por el Protocolo de Colaboración de CORESTA mediante la escala 1 – 9. Las extracciones de ADN se efectuaron en 38 cultivares, considerando el comportamiento de los cultivares frente a la enfermedad. Para la identificación del fragmento de BRM1 de talla aproximada de 205 pb y proveniente de N. debneyi se utilizaron los cebadores (Mil275-3.1 y Mil275-2.2). Se efectuó una prueba de independencia basada en el estadístico χ2 (chi-cuadrado) (p=0,05) para determinar la asociación entre el marcador BRM1 y la respuesta de resistencia Todos los cultivares comerciales cubanos de tabaco Negro fueron identificados como resistentes, excepto Criollo 2010, que se clasificó tolerante. Para tabaco Virginia cubano se demostró diferentes comportamientos frente al moho azul, donde Virginia isogénica, San Luis 21 y Virginia Resistente manifestaron ciertos niveles de resistencia, sin embargo, para loa cultivares Burley no se identificaron genotipos resistentes. Existió una asociación significativa entre la presencia del fragmento BRM1 y el grado de resistencia a moho azul donde el 100 % los genotipos de tabaco resistentes mostraron la presencia del fragmento BRM1 de 205 pb, no así para los cultivares susceptible donde en el 100% de los casos el marcador no fue identificado. Para los cultivares con respuesta de tolerancia al patógeno se observó que el 80 % no mostró el fragmento BRM1 mientras que el 20 % de estos cultivares si fue identificado.

Poster Presentations

Fusarioides pathogens causing wilting of chili peppers (capsicum spp.) in Mexico.

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Wilt in chilies is the most significant disease affecting cultivation of Capsicum spp. It is caused by a complex of soil-borne fungal pathogens that leads to partial or total loss of the root system resulting in subsequent chlorosis, foliar necrosis and plant death. Fusarioid genera includes several species involved in this pathosystem. Organisms within this genera are known to genetically evolve to strains carrying higher virulence, hence, failure of many management strategies against the pathogens. Studies are required to determine fungi associated with this disease in order to characterize the diversity of fusarioid species present in chili wilt in two regions of Mexico.Two producing areas were surveyed during this study one in the State of Zacatecas, and the other in the State of Puebla. Samples of roots from chili plants exhibiting wilt symptoms were collected, superficially disinfected, and processed for the isolation of phytopathogenic fungi. Identification was performed through polyphasic taxonomy, via amplification and sequencing of the TEF-1α gene, and morphological characteristics analysis. Sequence similarities analysis were performed using Basic Local Alignment Search Tool (BLAST), the Fusarioid-ID database and phylogenetic analysis with reference sequences confirmed the presence of two species complexes of the genus Fusarium in addition to the Neocosmospora complex. Furthermore, six different fusarioid species were identified across both producing regions. Pathogenicity tests conducted on chili seedlings revealed symptoms similar to those observed in the field. This study reaffirms the phytopathological threat that fusarioid fungal species continue to pose for chili production in Mexico.
Keywords: Fusarium; Neocosmospora; Capsicum spp.; chili wilt; TEF-1α gene; fungi.

Poster Presentations

Artificial Parthenogenesis: A Strategy for Obtaining Transgenic Lines in *Bombyx mori* L. (Lepidoptera, Bombycidae)

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Only 1 % of the planet's species can develop eggs without fertilization through parthenogenesis. In *B. mori*, this phenomenon was first observed in the 18th century, and its artificial induction was achieved in 1847 using females exposed to sunlight. The processes for establishing transgenic lines of *B. mori* could be simplified through the use of parthenoclones. The advantage of using parthenogenetic lines is that a single female initiates a clonal lineage with a genome identical to the mother's, which can easily be maintained as populations of pure females without sexual reproduction. Subsequent generations exhibit the same expression of the inserted gene. The aim of this study is to evaluate the effectiveness of two thermoparthenogenesis variants for obtaining parthenogenetic eggs in *B. mori*. Three pure bivoltine races of *B. mori* were used: J7, J3, and J13. Eggs from virgin moths were subjected to thermal treatments at 46 °C for 18 minutes. Rapid cooling was performed in variant A1 at 25 °C for 10 minutes and in variant A2 at 15 °C for 30 minutes. Effectiveness was assessed based on color changes indicating embryo fertility, as well as the hatching of parthenogenetic larvae. Microphotographs from optical and scanning electron microscopes of parthenogenetic eggs are presented. Fertile eggs from parthenogenetic individuals were obtained for all three races in both variants, with the A1 variant for the J7 race achieving the highest effectiveness values for artificial parthenogenesis.
Keywords: Thermoparthenogenesis, Pure races, Bivoltine, Effectiveness.

Poster Presentations

Design of a molecular method for the diagnosis of *Phyllosticta citricarpa,* based on the amplification of *MAT* genes.

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Molecular diagnostic assays have a significant impact in agriculture by enabling rapid and accurate detection of pathogens and disease outbreaks. Accordingly, the present work aimed to design a method for the molecular diagnosis of *Phyllosticta citricarpa*, based on the amplification of the *MAT* genes. For this purpose, the primers Gcmat1-56f/Gcmat1-686r and Gcmat2-686f/Gcmat2-1185r were evaluated, as well as the conventional PCR conditions described by Wang *et al.,* (2016) for the *MAT*1-1–1 and *MAT*1-2–1 loci of *P. citricarpa*, respectively. Samples were collected in groves or private yards affected by citrus black spot (CBS), in four Cuban provinces. Genomic DNA was extracted from 20 *P. citricarpa* isolates and 100 fruit samples with typical CBS lesions using the "Wizard Genomic DNA Purification Kit" (Promega). As results, the expected amplicon of 500 bp, corresponding to the *MAT*1-2-1 gene of *P. citricarpa,* was detected in all samples assayed. Amplicon of *MAT*1-1-1 gen (630 bp) was only generated when the strain *P. citricarpa* SA-KZN 35, from South Africa, was used as a template. The specificity of the method was demonstrated by using the Primer-Blast tool, which confirmed that these primers do not amplify with the genomes of *Citrus* spp. or other microorganisms. This was also corroborated *in vitro*, as no amplification occurred with the gDNA of *P. capitalensis* or pathogenic fungal isolates from citrus. This greatly simplifies the diagnosis, since PCR can be performed using the DNA extracted directly from CBS symptomatic lesions. An RFLP method was also designed to complement the PCR method, for the discrimination between species of the *Phyllosticta* genus associated with citrus. This diagnostic method is available as part of the National Citrus Certification System and can be useful for the international trade of citrus fruits and the disease management

Poster Presentations

Expression, purification and characterization of the capsid protein of the PMWaV-2 viral variant for the development of an immunodetection method associated with pineapple wilt in Cuba

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Pineapple wilt is the main biotic factor of viral origin limiting global pineapple production. This disease is caused by a viral complex primarily composed of three variants: PMWaV-1, PMWaV-2, and PMWaV-3, belonging to the genus *Ampelovirus* in the *Closteroviridae* family. Previous research indicates that only plantations infested with *D. brevipes* or *D. neobrevipes* alongside PMWaV-2 exhibit symptoms, suggesting that this variant encodes suppressor proteins of the host's RNA silencing mechanisms. This study focused on the expression, purification, and characterization of the capsid protein of PMWaV-2 (VP2). The pQE-30 VP-2 construct was inserted into the *Escherichia coli* strain M15. Protein expression was induced with 1 mM IPTG, resulting in the protein being expressed in the insoluble fraction. This fraction was purified by IMAC, and the eluted fractions were analyzed by SDS-PAGE and Western Blot, achieving high yield and adequate purity. With the obtained protein, a specific monoclonal antibody was generated, which was subsequently used in an indirect ELISA for recognition in samples from infected plants. These results provide a solid foundation for obtaining VP2 protein, essential for developing specific antibodies against PMWaV-2. These antibodies will be used in immunodiagnostic methods such as Dot-blot, Tissue-blot, and ELISA. Implementing these serological techniques will enable timely detection and management of the disease in pineapple crops, improving phytosanitary control strategies.

Keywords: virus, pineapple, viral protein, PMWaV-2, IMAC, Western Blot and ELISA.

Poster Presentations

Noninvasive diapause disruption by corona discharge: applications in *Bombyx mori* L., transgenesis

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The use of diapausing strains of *Bombyx mori* L., in transgenesis faces complex technical challenges due to limitations in the diapausing egg manipulation. The most widely used method currently combines hydrochloric acid with relatively high temperatures (> 48 °C), which accelerates the slow egg hatching process, but contributes to decrease the embryo survival and the success of microinjection of exogenous genetic material. To addressing this technical challenge, a corona-generating instrument was adapted to break diapause in eggs laid by *B. mori* butterflies at different times, in this study. For such purpose, *B. mori* diapausal Japanese strains were used, whose eggs were treate d with high-voltage electrical pulses at room temperature. As result, treated diapausal eggs were activated within 2 h of being oviposited facilitating the microinjection of exogenous genetic material. The eggs had > 95 % hatching, (1.23/1.42 g) cocoon weight, (17.82/19.99%) cocoon quality and (1/3 pupae per treatment) deformity, being in all parameters similar to observed in eggs traditionally treated with hydrochloric acid at 48 °C. In conclusion, the corona effect is a promising innovation to further expand the already demonstrated broad biotechnological applications of *B. mori*, in modern sericulture and in the production of recombinant proteins to be used in food, cosmetic and pharmaceutical industries.
Keywords: *Bombyx mori*, diapausing strains, pharmaceutical

Poster Presentations

Obtaining biological reagents for the development of a diagnostic method for leaf scald in sugar cane.

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Sugarcane is an economically important crop, essential for the production of sugar and biofuels. However, it faces serious threats due to diseases such as leaf scald, caused by the bacterium *Xanthomonas albilineans* (Ashby) Dowson. This pathology manifests itself through defined streaks, wilting and necrosis of the leaves, which can ultimately lead to plant death and considerable yield losses. Recently, several key factors in the pathogenicity of *X. albilineans* have been discovered, including the outer membrane protein XaOmpA1. This study focused on obtaining the XaOmpA1 protein for use as an antigen in developing an effective serological method for diagnosing leaf scald. To achieve this, PCR amplification of the XaOmpA1 gene was conducted using specifically designed primers, on symptomatic sugarcane samples exhibiting leaf scald.The resulting fragment was cloned into the commercial vector pGEM-T Easy and then into the expression vector pQE30, with correct gene insertion confirmed by sequencing. For recombinant protein production, the M15 strain of *Escherichia coli* was used and expression was induced with 1 mM IPTG. The recombinant protein obtained with a histidine marker, accumulated in the insoluble fraction, was solubilized under denaturing conditions. It was then purified using immobilized metal affinity chromatography and characterized by SDS-PAGE and Western Blot with a peroxidase-conjugated antihistidine antibody. Results showed a polypeptide of approximately 39 kDa, corresponding to the expected molecular weight for XaOmpA1, with high purity. These findings suggest that the protein is expressed at high levels and well-purified. The characterization of XaOmpA1 confirms its production and purity and sets a precedent for standardizing a diagnostic method through immunodetection techniques, offering a promising approach to control leaf scald in sugarcane.
Sugarcane, Leaf scald, *Xanthomonas albilineans*, XaOmpA1, diagnosis.

Poster Presentations

Obtaining positive controls for the detection of two polymorphic regions in Cuban strains of '*Candidatus* Liberibacter asiaticus'

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"Huanglongbing" (HLB) is currently the most destructive disease of citrus worldwide. The detection, identification and genetic diversity studies of strains of its main associated agent '*Candidatus* Liberibacter asiaticus' were limited to the use of housekeeping genes. Because of the increasing availability of complete genome sequences of different '*Ca*. L. asiaticus' strains from different countries, regions inside the genome of these bacteria having greater variability have been successfully implemented for its characterization, including microsatellites, prophage genes and miniature inverted repeat transposable elements (MITEs). In the present work, six Cuban strains of '*Ca*. L. asiaticus' with different geographical origins (Western, Central and Eastern regions) were included. Two polymorphic markers (prophage types and MITEs) were used to verify the presence of genetic diversity among the strains. The combination of the information obtained from detecting markers allowed verifying the strain differentiation according to the length of the amplified bands. The detected strains are important controls necessary to guarantee the performance and interpretation of tests using these molecular markers. The PCR systems used will allow a fast and improved characterization of the bacterial populations present in Cuba. This is the first report of the detection of polymorphic regions in the genome of Cuban strains of '*Ca*. L. asiaticus'.

Poster Presentations

Biological tools to enhance Cuban coffee production.

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Coffee is a very important crop and one of the most popular non-alcoholic beverages. Two coffee species are cultivated: Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*), both belonging to the Rubiaceae family. In the post-harvest process, the fermentation stage is crucial for coffee quality, as various biochemical reactions occur. Enzymes from microorganisms present in the bean oxidize sugars and produce substances such as alcohols, acids, aldehydes, ketones, and esters, among others. This produces changes in the color, aroma, density, acidity, pH, and chemical composition of the bean, all of which are essential for the final quality of the coffee. Recently, yeasts have been used as commercial products in coffee fermentation processes, increasing the variety of flavors and aromas and improving the overall characteristics of the coffee.
In Cuba, coffee production is one of the most important agricultural sectors, not only for its contribution to the country's economic development but also for its sociocultural impact. With this in mind, the Cuban industry is exploring new strategies to improve coffee quality. This work describes the isolation and characterization of yeasts obtained through biotechnology processes in the production of fermented coffee.

Poster Presentations

Changes in the Sensory Profile of Robusta Coffee Through Controlled Fermentations with Yeasts and the Use of Natural Additives

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Robusta coffee, although more resistant than Arabica to the imminent climate change that affect these cultures, is less appreciated for its flavor. This study explored the fermentation of Robusta to enhance its sensory characteristics. Natural and controlled fermentations were conducted using commercial Lallemand yeasts (Denmark) and their re-isolates as starter cultures, along with additives such as tropical fruits and sugarcane juice. The processes included natural cherry coffee and depulped, washed coffee, with fermentation times ranging from 24 to 96 hours. Controlled fermentation, through precise time management and the use of yeasts and pressed fruits, resulted in more complex flavor profiles. These exhibited mild fruity notes, light acidity, and reduced bitterness and astringency. Aroma quality depended on the method and ingredients used. Two main profiles were identified: one sweet, fruity, and vinous, and another featuring very sweet and pure coffees with classic spice and chocolate notes. Natural cherry coffee fermentations highlighted fruity and vinous nuances, whereas washed coffee fermentations produced cleaner profiles. Fermentations incorporating yeast and sugarcane juice proved particularly interesting, demonstrating a well-balanced interplay of sweetness, acidity, and bitterness, emphasizing their potential to enhance Robusta's sensory perception.
Keywords: Robusta coffee, fermentation, sensory profiles

Poster Presentations

Chemical macroelements in soils of protected cultivation houses treated with *Pseudoxanthomonas indica* H32.

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Microorganisms play an important role in soil composition. They are capable of varying the bioavailability of important macronutrients for plant development, such as potassium, phosphorus, and nitrogen. The bacterium *Pseudoxanthomonas indica* H32 has demonstrated biostimulant properties in horticulture. Based on the above, this study determined the composition of the main macronutrients required for plant development in soils from the CIGB (Central Biosystems Institute) protected cultivation facility in Camagüey after the application of a liquid formulation based on *Pseudoxanthomonas indica* H32 during tomato cultivation. A mixture of soils from different parts of the flowerbeds was taken to the soil institute for determination of pH, conductivity, percentage of organic matter, and ions such as phosphorus, potassium, and magnesium. Water was used as a control treatment. The flowerbeds where the *P. indica* H32-based formulation was applied had 3.8 times higher potassium oxide content than the control treatment. Calcium oxide content and conductivity also increased. However, the treatment reduced organic matter content by approximately 3%. The *P. indica* H32-based formulation can be used to improve potassium bioavailability in crops that require it.

Poster Presentations

Effect of HeberNem-S Application on Papaya Cultivation Under Open-Field Conditions

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In recent decades, there has been a growing trend towards the use of beneficial microorganisms as biostimulators and biological controllers in agricultural activities. The bionematicide and biofertilizer HeberNem-S has been successfully applied in protected and semi-protected crops, demonstrating its efficacy in stimulating germination, growth, and flowering. However, its application in unprotected crops remains insufficiently studied.

This study represents the first evaluation of the effects of HeberNem-S on vegetative development and agricultural productivity in the cultivation of papaya (Carica papaya L., Maradol Roja variety) under open-field conditions. The trial was conducted at El Jibao farm, Camagüey Province, using a schedule of four applications of HeberNem-S: at transplanting, and subsequently at 40, 100, and 160 days post-transplantation.

Compared to the untreated control group, treated plants exhibited significant improvements in morphophysiological indicators such as plant height and stem diameter. Additionally, flowering and fruit set occurred earlier in treated plants, compared to both the control group and previously reported observations for this variety. Agro-productive parameters, including the number of flowers and fruits per plant, fruit length and diameter at technical maturity, and average fruit weight, were also significantly enhanced. An estimated yield exceeding 85 tons per hectare was achieved—substantially higher than the national average.

Poster Presentations

Effect of HeberNem-S application on papaya seedlings

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HeberNem-S is a biofertilizer, bionematicide, and plant biostimulant developed at the Center for Genetic Engineering and Biotechnology in Camagüey. It has been used in protected and semi-protected crops in the country, particularly in short-cycle vegetables. However, its application in long-cycle crops has not been widely studied. In this context, application trials of HeberNem-S were conducted on in vitro papaya plants, representing the first attempt to use this product for this crop. The trial was carried out on a private plot in the Puerto Príncipe neighborhood of Camagüey, using seeds of the Maradol Roja variety. The seeds were pre-hydrated and incubated for eight hours in a HeberNem-S solution. The application schedule began at seed sowing (day 0). The seeds treated with HeberNem-S exhibited 100% germination, with germination times ranging from 7 to 10 days, compared to 11 to 15 days for untreated seeds. Regarding height, plants treated with HeberNem-S grew more rapidly than untreated plants. Other parameters evaluated, such as the number of leaves, the length-to-width ratio of the last true leaf, and stem diameter, showed significant improvements in treated plants compared to the control group. These results demonstrate that HeberNem-S effectively stimulates germination, growth, and leaf development in papaya seedlings.

Poster Presentations

Effect of *Pseudoxanthomonas indica* H32 on the growth of *Beta vulgaris* var cicla in organoponic

Dulemy Carrazana Granado[1], Ileana Sánchez Ortiz[1], Danalay Somontes Sánchez[1], Idania Wong Padilla[1], Ramón Franco Rodríguez[1], Raúl Gonzalez Rios[1], Aylin Nordelo Valdivia[1], Ruthdaly Segura Silva[1].

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Microorganisms are one of the most promising tools to improve crop growth and productivity naturally in urban agriculture. On this basis, the objective of this work was to determine the effect of the bacterium *Pseudoxanthomonas indica* H32 as a biostimulant on the growth of chard, by measuring the morphoagronomic indicators of the organoponic crop and determining the economic feasibility. The treatments *Pseudoxanthomonas indica* H32 and water as a control were applied, two days before sowing and 21 days later, in the semi-protected beds of the Nitrogen organoponic. After 36 days, the morphoagronomic variables were evaluated: length, width, number of leaves and fresh mass of the plants of 30 random samples for each group and the yield per bed was determined. The application of *Pseudoxanthomonas indica* H32 increased, with statistically significant differences, the length, width and weight of the leaves of the chard plants compared to the control treatment. It also favored an increase in the number of leaves compared to the water treatment. This led to an increase in yield of 4.8 kg per m2 of bed compared to the control and an increase in profitability by 235.33%. Therefore, the formulation based on the *Pseudoxanthomonas indica* H32 bacteria stimulates the growth of chard by increasing the morphoagronomic indicators of the crop and the application in semi-protected organic systems is economically feasible.

Poster Presentations

Effectiveness of Cuban bioproducts on the acclimatization of plantain and sweet potato cultivars propagated by *in vitro* culture

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Biotechnology and the use of bioproducts make an important contribution to agricultural sustainability. The study aimed to determine the dosage, application mode, and effect of several Cuban bioproducts on the overall development of *in vitro*-produced plantain and sweet potato plants during their acclimatization. Using completely randomized designs and several cultivars of interest, trials were conducted to determine the optimal dosages of each bioproduct and the most appropriate number of applications for the optimal vegetative development of plants. In plantain, a trial combining two doses of VIUSID Agro® with two of Icibiop Glu® was studied. An absolute control (no applications) was always used, and all applications were sprayed onto plants and substrate every seven days. At 50 days after planting (dap) were evaluated: plant height, pseudostem thickness, width and length of the developed leaf and its leaf area. The optimal dose was determined for each product; four applications allowed for a better plant response. All bioproducts stimulated the vegetative development of plantlets, always with statistical differences ($P \leq 0.05$) with respect to the control (without applications), for the plant height, width and length of the developed leaf and leaf area. Applying 2.5 $ml.L^{-1}$ of VIUSID Agro® or combining 1.5 $ml.L^{-1}$ with 10 $ml.L^{-1}$ of Icibiop GLU® showed an special increase of 3.5 times the leaf area. For the first time, it was demonstrated that four applications of Icibiop Glu®, Lebame®, Nitrofix® and Fitomas-E® favor the optimal development of plantain and sweet potato cultivars in acclimatization. This alternative streamlines the currently recommended fertilization process and replaces mineral fertilizer, contributing to the sustainability of agricultural production.
Keywords: agriculture, biofertilizers, biostimulants, horticulturae.

Poster Presentations

Effects of HeberNem-S on tobacco germination in seedbeds.

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Cuban tobacco represents around 70% of cuban agricultural exports, it is marketed in more than 150 countries currently and demand continues growing. However, the economic situation has led to a 10% reduction in plantation hectares, due to the lack of fertilizers and other inputs necessary for cultivation, some alternatives are being sought to increase yields. The influence of HeberNem-S on germination, rooting and stimulation of plant growth is well known, so it was decided to carry out a trial in the greenhouse, using a seedbed with 400 tobacco seeds of the BHMN variety in floating trays method. HeberNem-S was added to the pond water at a concentration of 2.5 g/L. In parallel, a germination study was carried out using filter paper discs soaked in HeberNem-S solution at equal concentration. Germination counts were carried out on days 7, 10 and 15. No significant differences were obtained in the number of germinated tobacco plants treated with HeberNem-S compared to the control. However, two trials show greater development in the emerged tobacco plants that were subjected to treatment with the product. The plants showed greater vigor, root development, stem thickness and more developed leaves, which guarantee success in their transplant and reduction of time in seedbeds. Therefore, it is concluded that HeberNem-S is potentially applicable as a plant growth promoter in tobacco seedlings

Poster Presentations

Efficacy of *Pseudoxanthomonas indica* H32 for root-knot nematode (*Meloidogyne* spp.) control and growth promotion in protected horticultural crops.

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The *Pseudoxanthomonas indica* strain H32, developed within the Agro-Use Bioproducts project, exhibits both nematicidal activity and the capacity to produce indole-3-acetic acid (IAA). This study evaluated its potential to promote plant growth and control *Meloidogyne* spp. nematode infestations in protected squash, lettuce, and tomato crops. Results demonstrated a significant reduction in nematode infestation across all crops, decreasing both the gall index and the number of galls per gram of root. In lettuce, application of H32 at 1 × 10^8 CFU/mL reduced the number of galls by 74.3%. For squash and tomato, concentrations of 1 × 10^8 CFU/mL and 1 × 10^7 CFU/mL achieved notable technical efficacies of 77.6% and 50.8% in squash, and 66.9% and 64.1% in tomato, respectively. In addition to its nematicidal activity, H32 stimulated plant growth and development. Significant increases were observed in the dry weight of squash roots, leaf area and plant height in lettuce, and the dry weight of leaves and roots in tomato. Furthermore, in tomato, H32 significantly improved yield by increasing the number of inflorescences and flowers, resulting in 2.6 times more fruit per plant than the control, with a total weight 2.5 times greater. These findings suggest that H32 is a promising agricultural bioproduct for nematode control and enhanced crop productivity.

Poster Presentations

Ensayos *in vitro* del efecto antagónico de diferentes cepas bacterianas frente a dos cepas de *Phytophtora parasitica* Breda de Haan.

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El incremento alarmante del número de plagas y enfermedades en las plantas, producen crisis en los sistemas agrícolas y alimentarios. Además, inciden en la productividad y ponen en riesgo la seguridad alimentaria en las zonas afectadas, con consecuencias económicas, sociales y ambientales. El empleo sistemático de productos químicos en la agricultura provoca el resurgimiento de plagas, la contaminación del medio ambiente, problemas de salud animal y humana, así como la aparición de cepas resistentes a estos compuestos (Mendez *et al.*,2017). Por esta razón, en los últimos años, se fomentan los estudios dirigidos al empleo de microorganismos antagonistas como controladores biológicos de estas plagas (Mohamad *et al.*, 2018). Por lo que el objetivo de estos ensayos fue evaluar el efecto antagónico de diferentes cepas bacterianas frente al fitopatógeno *P. parasitica*, que permita posteriormente seleccionar un candidato promisorio para el biocontrol del mismo para el cultivo de la piña. Se evaluaron en dos ensayos diferentes, la capacidad antagónica mediante cultivo dual de 8 cepas bacterianas provenientes de la colección del laboratorio IPM (4 cepas) y del cepario del laboratorio de Fisiología del INCA (4 cepas) contra dos aislados de *Phytophthora parasitica*. Se pudo comprobar que las cepas bacterianas proporcionadas por el laboratorio IPM no mostraron capacidad para inhibir el crecimiento micelial de las dos variantes de *P. parasitica* analizadas. La cepa *Pseudomonas prosekii* mostró un efecto antagónico contra las dos cepas de *Phytophthora*, reforzando su potencial para el manejo sostenible de enfermedades causadas por este patógeno.
Palabras claves: biocontrol, oomicete, pudrición del cogollo

Poster Presentations

Good practices to stablish agricultural bioassays.

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Good Practices are a set of principles, standards and technical recommendations applicable to the research, processing and production of goods and services. Quality Management System (QMS) standards based on ISO 9001 guidelines are applied in the development of Agricultural Research at the Center for Genetic Engineering and Biotechnology (CIGB).
This work describes the implementation strategy for these standards for obtaining plants as experimental models in the Functional Genomics Laboratory of Agricultural Research Department. The use of plant models is essential for understanding the mechanisms of plant-microbe interactions, as model plants can advance our knowledge of the plant immune system.
We investigate and establish temperature, light, humidity, and substrate conditions for different crops, such as, *Arabidopsis, Nicotiana spp., Solanum lycopersicum spp, Citrus* L., *Ananas comosus*, among others. This strategy allows for the successful development of scientific activity with higher levels of reproducibility and reliability.

Poster Presentations

Evidence of the safety of the HeberNem-S bioproduct for its national and international commercialization

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HeberNem-S™ was characterized and formulated from *Brevibacterium celere* strain C-924 as an alternative to the use of chemicals in agriculture that contaminate the environment. HeberNem has been shown to be an effective controller of plant parasitic nematodes such as *Meloidogyne* sp., *Radopholus similis* and *Platilenchus coffeae*; In addition, it exhibits results as an antiparasitic for veterinary use against *Haemonchus* sp and *Trichostrongylus* sp. Knowledge of its metabolism, behavior and effects on different crops allowed applications as a biofertilizer and flowering inducer, properties that have allowed its protection as a biopesticide, antiparasitic and floral inducer, with patents and use in other countries. It has been placed under the requirements of the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA) and the Department of Agriculture (USDA), who develop the plan of regulations, supervision mechanisms and specific standards, for the protection of human health and the environment, as well as Decree No. 76/2022.- Regulation of Decree-Law 64 “On the production, development and use of biofertilizers, biostimulants and biopesticides for agricultural use” in our country. This has been complemented with a total of 19 toxicological studies and 11 ecotoxicological studies, representative of different environments of the ecosystem, with positive results in favor of the product, which show that HeberNem-S is not toxic, not infestive, is not pathogenic and does not cross the natural barriers of non-white organisms. In addition, it was shown that it does not accumulate in the soil, the populations of the bacteria are maintained between 15 and 21 days after application, after which it is reduced to concentrations similar to the dynamics of untreated soil, where its nematicidal activity is not evident. So we can conclude that HeberNem-S is an environmentally friendly biological product.

Poster Presentations

Hebernem /Aikexian Product Technology Transfer. Introduction to China's Agricultural Production

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The Technology Transfer for the Production of the Bionematicide product based on the bacterium *Brevibacterium celere* strain C924 was satisfactorily concluded. Trade name in Cuba Hebernem and in China Aikexian. During fermentation, yields of more than 450 g/L were obtained, which is a production record in high-density cultures. The exposure time of the cells to the outlet temperature in the dryer was reduced by parameter adjustment at this stage of the process, influencing the number of viable cells to be higher and 93% recovery was obtained. It has production capacity to respond to product demands. Process throughput was superior at 70%, and production costs decreased by 20%. With this cost, it has been possible to penetrate the Chinese fertilizer market, which is highly competitive, together with the design of a commercial strategy in China as a high-end product. The introduction in the field has led to a training strategy from sales managers to producers, promotional tests with technical accompaniment. The monitoring of field use found results in its action to control the damage caused by nematodes, with similar effectiveness with the chemicals most commonly used today. Taking into account the importance of encouraging the use of Safe and Friendly Bio Products for Man, Animals, Plants and the Environment, in the **One Health Concept**.

Poster Presentations

New strains with antagonistic activity against phytopathogens with potential for protection against abiotic stress.

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Microorganisms, such as certain bacteria and fungi, stand out as promising agricultural biostimulants for improving crop growth and productivity, especially under adverse conditions (drought, salinity, extreme temperatures). This study sought to identify strains with antagonistic activity against phytopathogens and potential to mitigate biotic stress in tomato seeds. Twenty-eight strains were plated on Tryptone Soy Agar medium with sodium chloride at concentrations of 0.5%, 1%, 5%, and 10%. Bacteria capable of growing at 10% were cultured for 12 hours and refrigerated for 24 hours. A decimal dilution was made, and the seeds were immersed for 1 hour. Seeds treated with each bacterium were placed in two sterile Petri dishes with filter paper and cotton covering the bottom, and seeds were placed at a rate of 25 seeds per dish. The dishes for each treatment were moistened with sterile distilled water. Seeds treated with Tryptone Soy Broth diluted 1/10 were used as a positive control for fungal infection. Seeds treated with distilled water were used as a non-diseased control. Infested seedlings and total germinated seeds were counted. It was determined that the PK2 strain increases seed germination by more than 25%, while the C1 strain controls fungal infection by more than 50% compared to the treatment without control microorganisms. As a result, 9 strains were able to grow on Tryptone Soy Agar with 10% Sodium Chloride. Treatment of seeds with the C1 strain allowed the infection of the seedlings that emerged to be only 9.45%, while in the infested control it was 60.55%. The selected strains have a high potential to mitigate abiotic stress and improve productivity in tomato crops.

Poster Presentations

Phylogenetic comparison of serine proteases from *Pseudoxanthomonas indica* H32 with those from other nematicidal microorganisms.

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Serine protease enzymes are of great importance in the biological activity of many microorganisms that biocontrol phytopathogenic nematodes. The bacterium *Pseudoxanthomonas indica* H32 releases serine proteases with gelatinase and caseinase activity, which have nematicidal activity. Since phylogenetic analysis of proteins allows us to identify sites conserved throughout evolution, the objective of this work was to use this tool to predict serine protease enzymes in the H32 genome sequence with nematicidal potential. The serine protease sequences of the *Pseudoxanthomonas indica* H32 strain, obtained from the complete genome (Microsynth), were aligned by serine protease groups (S9, S41, S46, serine hydrolases and serine of the rhomboid family) with the nematicidal serine protease sequences (obtained from NCBI) of microorganisms that biocontrol nematodes. Nematicids with the aim of determining whether any of the H32 serine proteases in the genome sequence are phylogenetically related to any of the previously described nematicids. Trees were constructed using the Maximum Likelihood method in the Mega 6 program. It was determined that only the serine hydrolase homologous to the WP 139381367 protein of *Pseudoxanthomonas indica* P15 has a common evolutionary origin with the rest of the nematicidal enzymes. The rest of the H32 enzymes do not form a coherent group with any of the previously described nematicidal serine proteases. This result demonstrates the potential of evolutionary genomics and the use of phylogeny to predict the function of genes and proteins with unknown activity.

Poster Presentations

Predictive models to optimize coffee sensory profiles through metabolic interactions in bean fermentation with microbial consortia.

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Coffee fermentation plays a crucial role in determining important sensory characteristics, which are influenced by specific metabolites generated during this process. This work introduces a novel method that combines microbiology, metabolomics, and artificial intelligence to predict coffee sensory profiles based on the interaction of three known microorganisms: Saccharomyces cerevisiae, Pichia kluyveri, and Lactobacillus plantarum.

Predictive models allowed for the identification of sensory profiles linked to important metabolites: Saccharomyces cerevisiae provides fruity and floral notes thanks to the production of esters and higher alcohols; Pichia kluyveri produces volatile compounds with a spicy flavor; and Lactobacillus plantarum increases acidity by producing organic acids such as lactic and acetic acids. Interactions within the microbial consortia showed significant synergies, resulting in complex profiles that balance acidity, sweetness, and spicy notes in combinations of the three species.

Optimized fermentation protocols were suggested to test the predictions, adjusting factors such as temperature, pH, and culture ratios. Furthermore, metabolic maps were developed that depict the fundamental pathways and connections between metabolites, providing a comprehensive view of microbial dynamics.

This study not only expands scientific knowledge about fermentation, shortening research timelines, but also presents practical applications for producers seeking to innovate and manage complex profiles in the high-quality coffee market. The combination of advanced science with practical application underscores the importance of the proposed predictive approach.

Poster Presentations

Recent progress in the synthesis of silk fibroin nanoparticles: a novel delivery system for bioactive molecules

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Silk fibroin-based nanoparticles are emerging as promising carriers for delivery purposes of bioactive molecules due to their biocompatibility and biodegradability. The present investigation aims to set up a desolvation or nanoprecipitation method to synthesize silk fibroin nanoparticles. Silk skein was boiled for 30 min in an aqueous solution of 0.02 M sodium carbonate, and then rinsed thoroughly with distilled water. After air-drying, the extracted silk fibroin was dissolved in 9.3 M LiBr solution at 60°C for 4 hours. The solution was dialyzed against distilled water using Snake Skin Dialysis Tubing (3.5K MWCO, 35 mm dry I.D., 35 feet) for 72 hours to remove the salt and centrifuged to remove silk aggregates as well as debris from skein. The final concentration of silk fibroin aqueous solution was approximately 4.2 % (w/v). A 3% silk fibroin solution was added drop by drop in an excess of acetone at room temperature while stirring continuously, and the ratio of silk fibroin solution to acetone was 1 : 5 (v/v). Added silk solution get precipitated which was then centrifuged at 12500 rpm for 10 minutes at 4°C. The pellet was suspended in 10 ml of ultrapure water by ultrasonication at 80 watts of power for 10 min. After that, the silk fibroin nanoparticles formed were freeze dried and stored at 4 °C. Finally, a silk fibroin nanoparticle solution (1 mg/ml) was characterized by Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS). The results showed a solution of spherical and porous silk fibroin nanoparticles with an average size of 129.8 nm and a Zeta Potential of -24.8.
Keywords: silk, fibroin, nanoprecipitation, carriers, nanoparticles.

Poster Presentations

Secondary metabolites and extracellular proteases contribute to the antagonistic action of indigenous *Trichoderma* strains against *Botrytis cinerea*

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Trichoderma spp. have numerous applications in agriculture, as they have the ability to employ multiple mechanisms of action including mycoparasitism, antibiosis, competition for space and nutrients, and induction of defense responses in plants. The aim of this work is to evaluate different molecular strategies deployed by indigenous isolates of *Trichoderma* in their interaction with the phytopathogen *Botrytis cinerea*. In vitro antagonism assays, determination of volatile and diffusible compounds, and the relative expression of the prb1 gene, which codes for an extracellular protease, before and during the stage of direct contact between the two fungi, were carried out; the characterization of this protease was also performed. All 17 Trichoderma strains tested showed high levels of inhibition against *B. cinerea* growth in dual culture, with overgrowth of antagonist colonies on top of pathogen colonies being observed in most cases. Pathogen growth inhibition by antagonist-released volatile compounds ranged from 17 to 100 %, while the inhibition linked to the production of diffusible compounds ranged from 13 to 100 %. The prb1 gene was shown to be three-fold upregulates compared to growth alone before direct contact between the two fungi was established and then its transcript levels declined again at the direct contact stage. In the Trichoderma culture supernatant, the presence of elastase-type serine proteases (SP) associated with the initiation of the mycoparasitism process could be observed

Poster Presentations

Alternativas para el control del almidón en la industria azucarera

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En la investigación se estudia el proceso de hidrólisis enzimática con la α-amilasa libre e inmovilizada para el control del contenido de almidón en la industria azucarera, por su relevancia en la comercialización del azúcar crudo. Para ello, se determina el comportamiento del contenido de almidón en el proceso de producción de azúcar crudo; se inmoviliza la enzima por el método de adsorción no específica, y se determinan sus propiedades estructurales, funcionales y operacionales; se estudia la efectividad de la α-amilasa libre e inmovilizada en el desdoblamiento de almidón en jugos simulados e industriales; se realiza un análisis técnico- económico de su aplicación en la industria. El almidón entra al proceso con la caña de azúcar, se elimina en la clarificación entre un 40 y 68 % del mismo, mientras que se retiene entre un 17 y 40 % en el azúcar crudo. El conjugado Quitina-Qitosana-α-amilasa incrementa la estabilidad al pH, disminuye 2,5 veces la afinidad al sustrato e incrementa 1,2 veces la velocidad máxima de reacción con respecto a la enzima nativa; su reusabilidad queda demostrada al retener más del 60 % de su actividad inicial después de 19 ciclos de operación. La selección de la corriente de aplicación de la α-amilasa libre en el proceso de producción de azúcar crudo va a depender de las necesidades de calidad y tecnológicas; las dosis más factibles para su aplicación son de 0,49, 0,08 y 0,01 kg de enzima por t de caña molida en jugo alcalizado, clarificado y meladura respectivamente. El conjugado demostró ser efectivo en la remoción de almidón en el jugo alcalizado.
Palabras claves: almidón; α-amilasa; inmovilización enzimática; hidrólisis enzimática.

Poster Presentations

Bi-enzymatic transformation of sucrose for the diversified production of fructooligosaccharides

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Fructooligosaccharides (FOSs) are soluble prebiotic fibers with proven health-promoting effects in humans and animals. Among the currently commercialized linear inulin-type FOSs, the sweet-tasting 1–kestotriose (1K; DP3) stimulates the growth of probiotic bacteria faster than 1,1–kestotetraose (1,1K; DP4) and 1,1,1–kestopentaose (1,1,1K; DP5). Both the degree of polymerization (DP) and the type of bond appear to influence the efficiency of fructooligosaccharide fermentation by the intestinal microbiota. We have developed a bi-enzymatic cascade system that allows complete depletion of the substrate sucrose and yields short-chain FOSs of varied structures with their sum representing 60–65 % (w/w) of the total carbohydrates in the reaction mixture. Two sequential reactions are performed in the same reactor using fructosyltransferases of different substrate/product specificities. In the first step, sucrose is partially converted into 1K and 1,1K at a ratio 9:1 by the enzyme sucrose:sucrose 1–fructosyltransferase from *Schedonorus arundinaceus* (*Sa*1–SST) produced in the yeast *Komagataella phaffii*. The initiation of the fructose peak in the monitored reaction reflects 1K hydrolysis and indicates the optimal moment for thermal inactivation of the reaction. In the second-step reaction, the remaining sucrose is transformed via hydrolysis and fructosylation by a mutated variants of *Gluconacetobacter diazotrophicus* levansucrase (*Gd*_LsdA). The resulting mixture of linear and branched FOSs of short chains (DP 3–5) and varied linkages may stimulate a wider spectrum of intestinal probiotics comparing to traditional inulin-type FOSs.
Key words: Fructooligosaccharides, prebiotics, fructosyltransferase, levansucrase

Poster Presentations

Determinación de las propiedades estructurales y operacionales del biocatalizador Quitina-Quitosana-Invertasa

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En la presente investigación se sintetizó un conjugado de Quitina-Quitosana-Invertasa inmovilizada (Qui-Qsa-Inv) mediante la combinación de dos métodos absorción no específica y entrecruzamiento. Se realizó un diseño experimental para obtener las mejores condiciones de síntesis, donde las mismas son: tiempo contacto con glutaraldehído 5 min y concentración de la enzima de 100 U/mL. Se determinó el pH óptimo de la enzima nativa y la modificada en 5,5, apreciándose un incremento de actividad en la enzima modificada hacia la zona de pH ácido. La invertasa inmovilizada incrementó su temperatura óptima en 10 ºC. El conjugado de Quit-Qsa-Inv retiene un 60% de la actividad inicial después de transcurridos 10 ciclos de reuso frente a una disolución de sacarosa de 20 ºBrix. Para una disolución a 40 ºBrix la actividad relativa disminuyó a 79% al finalizar el cuarto ciclo. Se caracterizaron los productos hidrolizados de dichos ciclos en cuanto a pH, contenido de sólidos solubles y contenido de azúcares reductores, obteniéndose una correspondencia en la disminución del contenido de azúcares con la pérdida de actividad enzimática.

Palabras clave: enzima, inmovilización, invertasa, siropes invertidos.

Poster Presentations

Estabilidad de β-mananasas en el extracto enzimático producido por *Bacillus subtilis* E44

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Las β-mananasas son enzimas con aplicación en la industria de alimentos y piensos, blanqueamiento del papel, producción de biocombustibles, entre otras. El objetivo de la presente investigación fue determinar la estabilidad de las β-mananasas en el extracto enzimático producido por *Bacillus subtilis* E44. Las enzimas se obtuvieron por la fermentación en estado sólido del bagazo de caña de azúcar. La estabilidad térmica se determinó al incubar el extracto a 40, 50, 60, 70 y 80 °C durante 48 h. Se calculó la energía de activación de proceso de inactivación térmica en el rango de temperatura establecido y el tiempo de vida media. La estabilidad a pH se evaluó en el rango de 3,0 a 7,5. En ambos casos se calculó la actividad enzimática residual. Los resultados de la temperatura evidenciaron que a 40 °C las β-mananasas conservaron el 80% de su actividad a 24 h y a 48 h el 70 %. Mientras que a 50 °C la actividad disminuyó drásticamente, aunque después de 1 h conservó más del 45 % de actividad. A temperaturas superiores (60, 70 y 80 °C) la actividad de la enzima a las 0,5 h disminuyó por debajo del 40%. Los tiempos de vida media calculados fueron 15,4 h; 1,2 h; 36 min; 12 min y 6 min para 40, 50, 60 70 y 80 °C, respectivamente. La energía de activación del proceso de inactivación térmica fue 106,2 kJ mol^{-1} (R^2=0,9172). El perfil de estabilidad de pH mostró que la enzima retuvo entre el 80 y 100% de su actividad inicial en el rango entre 4,0 y 6,0. Mientras que entre 3,0 y 4,0 y 6,0 a 7,5 se conserva entre el 60 y el 80% de su actividad respectivamente. Las β-mananasas obtenidas por *Bacillus subtilis* E44 mostraron propiedades de interés para la aplicación industrial.

Palabras claves: Enzimas, Temperatura, pH

Poster Presentations

Evaluation of competitive inhibitors on chimeric dextransucrase enzyme DSR-F-ΔSP-ΔGBD-CBM2a produced in *E. coli* JM109

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The use of 3 molecules as inhibitors of dextransucrase DSR-F-ΔSP-ΔGBD-CBM2a (recombinantly expressed in *E coli* JM109) is described. Before inhibition enzyme, purification evidenced an increase in specific activity and enzyme activity being those values 78.62 IU.mg $^{-1}$ and 50.4 IU. mL^{-1} respectively. The 3 inhibitors behaved as competitive inhibitors of the enzyme (no effect in kinetic constant Vmax and increase in apparent KM values were shown).
The enzyme inhibition capacity was shown by obtaining respective inhibition percentages on the enzyme activity of 64.43, 52.77 and 40.09 % for inhibitors 1, 2 and 3. Similarly, inhibitory effect was evident by inhibition constants (Ki) values estimated for each inhibitor. Values of 0.483; 0.729 and 1.021 mM for inhibitors 1, 2 and 3 respectively, were obtained, hence inhibitory power was associated with the order inhibitor 1 > inhibitor 2 > inhibitor 3. Notorious was the fact associated with inhibitor 1 acting on transferase activity of enzyme (decreasing in this variable being evident).
Furthermore, the size decreasing of polymer produced in presence of inhibitor 1 was shown. The effect of inhibitor 1 in diminution of enzyme produced dextran was also evaluated, reaching values of 94.49 % of inhibition in polymer formation at 7 mM inhibitor concentration. Finally, a mathematical model was obtained that optimally adjusts (R^2=0.981) the enzymatic activity as a function of different variables, including the inhibitor concentration.
Key words: Competitive inhibitor, dextransucrases, dextran, kinetic constants, Ki, recombinant enzyme, *Leuconostoc citreum*

Poster Presentations

Switching regioselectivity in the fructooligosaccharides synthesis by *Gluconacetobacter diazotrophicus* levansucrase

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Fructooligosaccharides (FOSs) are soluble prebiotic fibbers with proven health-promoting effects in humans and animals. Bacterial levansucrases (EC 2.4.1.10) catalyze fructosyl transfer reactions from the donor substrate sucrose to the natural acceptors water (sucrose hydrolysis), sucrose (FOSs synthesis), and precursor FOSs (polysaccharide formation). *Gluconacetobacter diazotrophicus* levansucrase (*Gd*_LsdA) is distinguishable for the synthesis and accumulation of the β-(2→1)-linked FOSs 1-kestotriose and 1,1-kestotetraose, with a consequent low yield of the β-(2→6)-linked polysaccharide levan. In this work, the substrate-interacting amino acids His172 and Asn306 were selected as independent targets for saturation mutagenesis aiming to decipher the structural factors involved in regioselectivity. His172 binds the fructosyl moiety of the donor sucrose (subsite -1), while Asn306 interacts with the glucosyl moiety of the acceptor sucrose or the second fructosyl moiety of the acceptor FOS (subsite +2). HPAEC-PAD analysis of mutated *Gd*_LsdA variants revealed remarkable differences in the output of the fructosyl transfer reaction. As a general behavior, the replacement of His172 decreased the fructosylation/hydrolysis ratio without changing the FOSs spectrum. More interestingly, the substitution of Asn306 by a positively charged amino acid (Arg or Lys but not His) directed the acceptor sucrose in an orientation that favored the synthesis of 6-kestotriose over 1-kestotriose. The Asn306Arg and Asn306Lys variants mostly yielded β-(2→6)-linked FOSs with degree of polymerization (DP) between 3 and 6 which were no further elongated in prolonged reactions. Our results provide insight into a key structural determinant of the regioselectivity and processivity of the fructosyl transfer reaction in levansucrases.

Poster Presentations

Analysis of the inorganic components that influence irrigation water quality in the experimental plot.

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Abstract

Water analysis should be the first step when considering irrigation water use, to ensure maximum crop yield and avoid adverse plant toxicity issues. This paper presents the characterization of the water used for irrigation in the CIGB experimental plot for the production of original seeds for transgenic soybean and corn crops, for extension in hybridization areas and introduction into national production. Sampling was carried out on the main water inlet line to the plot, and the components pH, electrical conductivity (EC), total dissolved solids, calcium and magnesium concentration, alkalinity, carbonates and bicarbonates, iron, manganese, copper, zinc, and potassium were evaluated. Results of elevated pH, alkalinity, calcium, and magnesium are presented. It is concluded that water test results should be considered in conjunction with soil study results for agronomic decision-making.

Keywords: irrigation water quality

Poster Presentations

Determination of heavy metals in original seed of transgenic corn and soybeans

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The environmental impact of heavy metals in agriculture represents a challenge for sustainable food production, due to the damage they cause to ecosystems. Heavy metals (HM) such as cadmium, lead and mercury can accumulate in the soil through wastewater sources, pesticides and fertilizers, affecting local fauna, biodiversity and soil microorganisms, which decreases agricultural fertility and productivity. These toxic elements have the potential to enter the food chain, where their bioaccumulation leads to harmful health effects. In the CIGB experimental plot, original category seeds of genetically modified crops are produced, glyphosate-resistant soybeans and corn expressing resistance to lepidopteran insects and tolerance to the herbicide Ammonium Glufosinate, which has generated controversy. Regulatory authorities in charge of risk assessment take into consideration the absorption and accumulation capacity of HM for decision making. In the present study, HM contamination was determined in transgenic corn and soybeans and conventional CT9 corn. According to the results, pewter, calcium, magnesium, potassium, copper, zinc, iron, cobalt and manganese were not found. No cadmium, lead, mercury, nitrate, aflatoxins, or organophosphate residues were detected; which placed them at lower rates than those published in the CUBAN STANDARD NC 493:2015, which establishes the principles and procedures applied and recommended in relation to metallic contaminants in foods and beverages intended for human consumption, based on those established by the Codex Alimentarius, Mercosur and the European Union. It was demonstrated that the management of genetically transformed varieties does not alter the quality of the seed for reproduction in agricultural extension areas.

Poster Presentations

Production and increase of liquid inoculant based on *Bradyrhizobium japonicum* to obtain 300 hectares of certified soybean seed.

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Soybeans (*Glycine max*) are a highly important crop in the country, and their progressive development is a response to the high levels of consumption and importation of this legume.
This crop uses high concentrations of nitrogen due to the total protein content accumulated in the seeds. Symbiotic nitrogen fixation is a key process in achieving high yields in soybean cultivation, justifying the use of a bioinoculant as a substitute for chemical nitrogen fertilization, which reduces soybean production costs and contributes to soil protection. The main target of this work was to establish a methodology for the production and growth of a liquid inoculant based on the bacterium *Bradyrhizobium japonicum*. The highly symbiotic strain SEMIA 5080 was used for this process, using a multi-platform orbital shaker. Optimizing the growth medium and establishing a system of serial and scaled inoculations allowed the culture to remain in continuous logarithmic phase and increase its volume 100-fold in 7 days. The final culture with an average viability of 5 x 10^9 c.f.u. mL^{-1} and high exopolysaccharide content did not require additives and remained stable under storage at 4ºC. The inoculant was applied to the seeds at a dose equivalent to 250 to 400 $mL.ha^{-1}$, during the 2024-2025 campaign for the production of certified seed in a total area of 300 hectares. The inoculated field plants showed high nodulation in the root collar region and maintained adequate nutritional status throughout the growing season. The resulting liquid bioinoculant favored the bacteria's survival in the soil, reduced soybean production costs, and contributed to environmental protection.
Keywords: Glycine max, inoculant, *Bradyrhizobium japonicum*, nitrogen fixation.

Poster Presentations

Protection of soil resources on the CIGB experimental plot

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Environmental protection has been a concern in recent decades. However, efforts have not prevented its deterioration, reaching alarming levels. Agriculture is identified as the most invasive activity in terrestrial and aquatic ecosystems, through the introduction of technologies to increase production in order to meet the demand for food. The CGEB experimental plot is dedicated to the production of original transgenic soybean and corn lines seeds for extension. In order to achieve adequate yields for commercialization, a strategy aimed to soil conservation was designed. The cultivable area is initially subjected to agrochemical study in order to apply fertilization that is truly needed (Liebig's Law of the Minimum). Consequently, crop residues, organic matter, and simple fertilization were incorporated to address the high levels of unavailable phosphorus accumulated in the rhizosphere; the relationship between nutrients was improved, and the deficits required by the crop were supplemented, preventing bioaccumulation. Furthermore, the land was leveled, and the furrows running across the slope were redirected to prevent the leaching of organic matter, maintaining water drainage in a less pronounced direction that would be detrimental to soil conservation. Because the soil was disturbed, a subsoiler was also used during soil preparation. These tasks had a positive impact on the soil's physical, chemical, and microbiological properties.

Poster Presentations

Strategy for increasing soybean crop productivity

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Soy culture plays an important role in the national economy, as a crop that combines nutritional value, productive efficiency, and sustainability, positioning itself as a strategic resource for food security. Soy production in Cuba has been characterized by low yields (0.8–1.2 tons/ha) due to insufficient knowledge and an undefined technological package for its cultivation. It has demand that the country importing large amounts of soybeans, including meal, grain, and oil, with imports of these products valued at more than US$1.4 million between 2014 and 2018. Under current conditions, eradicating import dependence is essential, which is why the Center for Genetic Engineering and Biotechnology developed an ongoing strategy to contribute to the growth of national production by utilizing the soybean varieties it has developed. This strategy is based on actions such as training, technical monitoring, and adjustments in crop management, ranging from the production of the original seed to the certified soybean category. Currently, the results are evident in the production of high-quality seeds with yields above 1.5 tons per hectar and germination rates of over 85%. In 2024, registered seed production was achieved for the first time, and certified seed production will be achieved in early 2025. The area dedicated to seed production increased from 2 hectares of basic seed in 2022 to 285 hectares of certified seed in the 2024-2025 season, in addition to the remaining seed categories. Continuing with this strategy will be possible to cultivate 5,000 hectares across the country and approach the expected national production levels.

Poster Presentations

Biophysical-chemical monitoring of the soil from post-sowing to post-harvest of areas planted with hybrid corn H-Ame 15.

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Case-Specific Monitoring is one of the tools for determining the adverse effects of genetically modified crops (GMC) after registration and commercialization. Due to its importance in the sustainability and functionality of agroecosystems, soil is a safeguard target. Objective: To monitor soils cultivated with hybrid corn H-Ame15 to evaluate its release into this compartment. Methods: The Jesús Sablón Moreno Sugar Company in Calimete, Matanzas, was selected. It had an area planted with conventional corn (refuge) and another with hybrid corn H-Ame15. In both areas, the soil was physically, chemically, and biologically characterized post-planting to establish a baseline by determining the associated fauna, water retention percentage, pH, temperature, and the activity of the microbial community through respiration. These determinations were repeated post-harvest, along with the quantification of ammonium levels. Results: In the physical-chemical parameters, there was similarity between the baseline and post-harvest monitoring. In the latter, the existence of a higher quantity and diversity of functional groups as well as microflora was confirmed, with a predominance of these variables in hybrid corn compared to conventional corn. Conclusion: Hybrid corn H-Ame15 favored the processes associated with soil biotic communities.

Poster Presentations

Considerations for non-regulation of transgenic soybeans carrying the GTS 40-3-2 transformation event in Cuba

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Increase in national soybean production in Cuba is required to cover animal feed needs. The average of annual costs for importing grain between 2014 and 2018 reached 283 MM USD. On the other hand, Center for Genetic Engineering and Biotechnology had developed 5 soybean varieties carrying the genetic transformation event GTS40-3-2 that confers tolerance to glyphosate and with potential yield between 3 to 4 tons/ha. Besides, bolivian varieties with similar characteristics have been introduced in Cuba recently. The introduction of these genetically modified crops in agriculture has been more complex due to the national regulations over transgenic crops. Taking into account the prolonged use of GTS40-3-2 transformation event in Cuba and the decisions adopted by other countries in relation with this event, the non-regulation proposal was presented to the National Commission for the Use of Genetically Modified Organisms in Agriculture. A technical dossier concerning to glyphosate resistance soybean was prepared, containing the pertinent data according to the current legislation. The results of ecotoxicological studies, nutritional content compared to conventional products, metal accumulation and toxin level analyses were included, demonstrating the product's safety, as well as the results of risk assessments and field monitoring. In addition, certificates from authorities at the Ministries of Agriculture and Public Health were indexed. Process concluded with the approval of this event. Final decision was published in the Official Gazette GOC-2023-388-EX33. In this way, the use of glyphosate-tolerant soybeans in the country is made more flexible, aimed at increasing the production of food as well as derivatives and byproducts.

Poster Presentations

Detección e identificación de Organismos Genéticamente Modificados: Un pilar para la seguridad alimentaria.

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Palabras claves: PCR-TR, OGM, Validación, acreditación, ISO 17025.

Cuba es beneficiaria de un proyecto GEF–PNUMA, que ha permitido crear capacidades en dos laboratorios del país, para la detección e identificación de Organismos Genéticamente Modificados (OGM), mediante ensayos moleculares de PCR tiempo real en granos de soya y maíz, que permitan estudiar OGMs obtenidos a partir de la Biotecnología en Cuba o importados como alimento para el consumo humano y animal, así como dar respuesta a la autoridad regulatoria (ORSA), para esto los ensayos deben ser validados y acreditados por la NC-ISO-17025: 2017, que permitan demostrar competencia y datos analíticos confiables. El presente trabajo tiene como objetivo: Validar los ensayos de reacción en cadena de la polimerasa en tiempo real (PCR-TR) para la detección cualitativa de los eventos específicos GTS 40-3-2 de soya y TC 1507 de maíz. Para validar ambos eventos se emplearon materiales de referencia certificados (MRC) con diferentes concentraciones, el ADN se obtuvo empleando el DNeasy plant Mini Kit (Qiagen), se siguieron las instrucciones del fabricante. Los ADN obtenidos se estandarizaron a 50 ng/µl. Todos los ensayos se realizaron mediante PCR-TR en las plataformas LightCycler 480 y LightCycler 96. Se determinó la presencia de inhibidores analizando los genes endógenos lectina de soya y alcohol deshidrogenasa de maíz. Entre los parámetros analizados durante la validación se tuvieron en cuenta: aplicabilidad, practicabilidad, especificidad, sensibilidad (límite de detección), repetibilidad (precisión intraensayo), precisión intermedia (precisión interensayo), reproducibilidad (precisión interlaboratorio), así como la Robustez. Se obtuvieron rendimientos altos y puros de ADN. No se demostró la presencia de inhibidores en las diferentes concentraciones de los MRC, contando con muestras de calidad para ser utilizadas en la validación de los ensayos. El estudio de especificidad analítica, demostró ser que ambos ensayos son específicos para el blanco propuesto (GTS 40-3-2 y TC 1507) y negativo para el resto de las matrices transgénicas ensayadas. El límite de detección del método, fue a la concentración de 0.2 % para GTS 40-3-2 y de 0.1 % para el TC 1507 (IC-95 %), los métodos analíticos mostraron buena repetibilidad y precisión intraensayo. La precisión intermedia y la reproducibilidad mostraron también, resultados satisfactorios, no mostraron diferencias significativas, en la precisión interensayo e interlaboratorio. El ensayo de robustez realizado demostró la capacidad de las técnicas de producir resultados consistentes y reproducibles ante pequeños cambios experimentales ensayados. Conclusiones: Los ensayos de PCR en tiempo real cuantitativa para los genes específicos GTS 40-3-2 de soya y TC 1507 de maíz, cumplieron con los parámetros establecidos en la validación, declarándose los métodos validados y útiles para el fin propuesto.

Poster Presentations

Installation of soil-based techniques for environmental monitoring of genetically modified crops

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Introduction: Ecotoxicology has advanced in the study of the impact of xenobiotics on the terrestrial ecosystem, where soil microorganisms are key elements. Genetically modified crops (GMCs) have a close relationship with these, hence the need to evaluate their impact on these microbial communities in the pre- and post-commercialization stages. To achieve this, it is essential to have methodologies that allow determining their diversity and functionality. Objective: To develop techniques for quantifying levels of ammonium, bacteria, fungi, and actinomycetes in different substrates as part of research for environmental risk assessment. Methods: A standard curve for ammonium (NH4+) quantification was determined using a spectrophotometric method. Microbial quantification was performed using a plate-plating technique on a selective medium (nutrient agar, potato dextrose, and Csapeck dox). Results: Regression and correlation analyses were performed, which showed a highly significant association with an R2 value of 0.9978. Ammonium levels showed similar behavior between groups. The presence of microbial activity in the substrates was confirmed by measuring ammonium, and microflora growth was determined according to the treatment group. Conclusion: The feasibility of using these techniques to assess the impact of CGM on soil microfauna was demonstrated.

Poster Presentations

International Standards and Regulatory Environment of CIGB Agricultural Products.

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Regulations for sanitary registrations, constitute an international legal instrument aimed to promoting the safe development of products and managing or reducing potential risks to living beings and the environment. Agricultural registration is the procedure for verifying the quality, efficacy, and safety of a product by evaluating and recognizing its history. The Regulatory Environment for bioproducts of agricultural use of the CIGB is very diverse and different, in relation to those for human use. Knowing this environment will allow actions to be taken into account that facilitate sanitary registration. In registration of agricultural products, various regulatory entities were identified based on their use, such as medicines and animal feed; bioinputs; varieties of seeds; and human food. This duplicates efforts in processing the same product; requires unnecessary expenditure of resources; and lacks digitalization of procedures, which impacts the delay in registering a given product. A study has been proposed for the unification of the regulatory entities responsible for agricultural products of CIGB concern, based on "One Health", as a comprehensive and unifying approach whose objective is to balance and optimize the health of people, animals and ecosystems.

Poster Presentations

Managing biosafety related to genetically modified crops through agricultural extension

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Genetically modified crops have revolutionized agriculture by offering solutions to improve yield and resistance to pests and diseases. However, these advances also pose challenges in terms of biosafety, given the potential risks to the environment and the need to ensure the responsible use of biotechnology. On the other hand, agricultural extension plays a fundamental role in educating farmers about safe practices. Therefore, we proposed integrating biosafety management into the agricultural extension program developed by the Center for Genetic Engineering and Biotechnology, through training strategies, technical assistance including advice on regulatory implementation and crop monitoring. As a result, more than thirty training activities were developed, reaching more than 200 managers and farmers and three national workshops were held, with the participation of the national authority responsible for biosafety and farmers who have adopted these crops. Biosafety has been incorporated into working procedures, and authorization applications including the risk assessments have prepared. All production areas, processing plants, and seed movement have biological safety authorizations. During technical assistance, compliance with regulatory requirements are verified, such as areas and varieties identification, establishment of refuges for target insects and emergency plans. In addition, phytosanitary monitoring and sampling are carried out for crop management, considering the new characteristics derived from genetic modification, to prevent insects and weeds resistance. Direct interaction between extension specialists and farmers has been a tool for the transfer of these technologies and maintain a balance with environmental protection and biodiversity particularly.

Poster Presentations

Monitoring and Surveillance System for Adverse Effects of Genetically Modified Organisms in Cuba

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As a State Party to the Cartagena Protocol, Cuba established Decree-Law No. 4/2020 of the National Commission for the Use of Genetically Modified Organisms (GMOs) in Cuban Agriculture as a legal framework to strengthen the GMO surveillance and monitoring system. This Decree-Law serves as a shield against the commercialization and release of GMOs, the impact of which could pose risks to two protected assets: the environment and human health. To this end, the Office of Environmental Regulation and Safety designed a Monitoring and Surveillance System for adverse effects to detect releases, imports, and other unauthorized activities involving GMOs, identify the occurrence of adverse effects, evaluate the effectiveness of specific risk management strategies, and contribute to early response to the occurrence of adverse effects.

Poster Presentations

Management and Accreditation System for the Detection and Identification of Genetically Modified Organisms in Soybeans for Human and Animal Food by Real-Time PCR.

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Abstract
The National Center for Animal and Plant Health and the Civil Defense Scientific Research Center are participating in a GEF–PNUMA international cooperation project whose objectives include building laboratory capacity for the detection and identification of genetically modified organisms (GMOs). The objective of the project was to implement a management system in the laboratories in accordance with the NC ISO/IEC 17025:2017 reference standard; this is an essential process for the accreditation of the real-time PCR assay, used for the detection and identification of GMOs in GTS 40-3-2 transgenic soybeans. A diagnostic audit was conducted to assess the level of implementation of the management system, and nonconformities and necessary corrective actions were identified. The implementation of the management system was planned, as well as the accreditation process for the selected test. Risk management was innovative, modifying the concept of preventive actions in good laboratory practices (GLP). The process approach applied to analytical activity was an effective way to ensure results traceability. Documentation in form of procedures and records supporting the system allowed for demonstration of how to perform the activities and evidence of their results. Participation in an international proficiency test demonstrated technical competence in a context of interlaboratory comparison, obtaining satisfactory results for transgenic soybeans and corn arrays. Staff training through available courses and individual preparation facilitated the implementation of the system and created the conditions for beginning the accreditation process with the National Accreditation Body of the Republic of Cuba (ONARC). The management system provided the platform for safely transitioning to the rehearsal accreditation process.

Poster Presentations

Surveys for monitoring the adverse effects of genetically modified crops

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Introduction: The Environmental Monitoring Plan for post-commercialization of genetically modified crops (GMC) aims to identify early adverse effects associated with the use of this technology. The use of a Surveillance System that allows the identification of deviations that occur from usual agricultural practices constitutes a powerful tool in the early detection of the effects of these crops. Objectives: To develop surveys for monitoring the adverse environmental effects of GMCs. Methods: We reviewed international surveys for such purposes. Three types of surveys were designed for different stakeholders. The surveys were conducted at the Jesús Sablón Moreno Sugar Company in Calimete, Matanzas, which had an area planted with the hybrid H-Ame15 corn. Results: The stakeholders for whom the surveys were designed were farmers, agronomists, and phytosanitary specialists, each with the purpose of providing information about the deviations from their perspectives. An analysis of the surveys completed in the area selected for the study, which corresponded to agronomists and farmers in equal proportions, found no significant deviations in agronomic practices, crop characteristics, or associated biodiversity between hybrid H-Ame15 maize and conventional maize. Deficiencies were detected in the interpretation of some of the questions. Conclusions: Surveys were developed to monitor the adverse effects of CGMs, which in their implementation stage require specialized support for better understanding.

Poster Presentations

Toxicological profile of hybrid corn with the transgenic events MIR162 and TC1507.

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It was necessary to carry out several toxicological and ecotoxicological studies for the registration and safe extension in the Cuban ecosystems of hybrid corn with the transgenic events MIR162 and TC1507, which confer characteristics of resistance to insects and the herbicide Ammonium Glufosinate. A bromatological quality was determined based on proteins and contaminants of the dry seed of hybrid corn, the detection of heavy metals in the original seed used for agricultural extension and investigation of the toxic effects on bioindicator organisms of the agrosystem, such as *Apis mellifera* bees exposed to the pollen of hybrid corn and the impact on the soil microfauna and microbial decomposition processes with the intervention of *Eisenia andrei* earthworms on plant residues, from the transgenic corn crop. The results did not show significant differences between the nutritional parameters contained in the hybrid corn grain, compared to the conventional one. No metallic contaminants of toxicological significance were detected in the samples of corn seeds from the production area, in charge of distribution for its extension. The exposure of the bee to the pollen of hybrid corn MIR162 and TC1507 and its administration in the diet, as well as the treatments with the active proteins Cry1Fa and Vib3Aa20, did not cause toxic effects. The quantification of CO2 emissions in the interaction with soil microfauna did not show significant differences between the control and the groups treated with the proteins under study. Exposure to plant residues did not reveal lethal effects on the earthworm *Eisenia andrei*. It is concluded that the distribution of the original seed for the extension of hybrid corn with the stacked event MIR162 and TC1507 was not toxic and its release to the agrosystem is safe.

Poster Presentations

The impact and use of artificial intelligence: scientific and biotecnology challenges for achieving sustainabke agricultura.

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El desarrollo de la Inteligencia Artificial en la actualidad, es algo que suscita inacabables debates, nos enfrentamos a desafíos científicos-biotecnológicos y civilizatorios sin precedentes. Este debate nos devuelve al escenario de los problemas ético-filosóficos de la ciencia, la tecnología y la innovación, ya que presuponen un reto en el área de la seguridad internacional para el logro de una agricultura sostenible, donde el manejo genético de variedades de plantas y animales, la gestión de los suelos y el agua, no escapan a ello en la era del cambio climático. Como sociedad, debemos examinar el impacto y uso de la IA, así como el papel de la ciencia y la tecnología en la configuración de nuestro futuro, especialmente en sectores cruciales como la agricultura y los procesos agrobiotecnológicos que a ella se les aplican. Lograr una adecuada regulación del uso de la IA, sus aplicaciones y las tecnologías, es uno de los desafíos más importantes de nuestro tiempo, ya que exige un aprendizaje mutuo basado en buenas prácticas que propicien oportunidades para transformar el sector agrícola en una agricultura sostenible.
Palabras Claves: Inteligencia Artificial, tecnología y agricultura sostenible

Poster Presentations

Integrated management system with it platform for the agricultural research area at the center for genetic engineering and biotechnology

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The Agricultural Research Department (IAP) at CIGB develops innovative biotechnological products for sustainable agriculture, focusing on food security, resource efficiency, and climate change mitigation. Important biotech products include the transgenic seeds of maize and soybean, as well as the bionematicide HeberNem-S. This study is aimed at implementing an integrated strategy which combines different processes such as: the Quality systems (NC 9001:2015 standards), the Environmental management (regulatory compliance and best practices), the Internal control (traceability and audits) and the Informatics management (document digitization, process automation, and real-timeabel monitoring). The result of the study explains how the centralized informatics system enabled streamlined documentation in accordance with the principles of Good Laboratory Practices (GLP). The findings suggest that this approach enhances product certification, ensures traceability, and improves organizational performance.
Keywords: Quality management, agricultural biotechnology, integrated systems, digitalization, sustainability.

Poster Presentations

"Tendencias clave en el sector agroindustrial y biotecnológico global: Implicaciones para la gestión de negocios y asuntos regulatorios"

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El avance científico y tecnológico está impulsando una creciente colaboración e innovación en el sector agroindustrial y biotecnológico, fortaleciendo la confianza entre los actores del mercado y fomentando la adopción de prácticas sostenibles. Esta evolución no solo involucra a diferentes regiones, sino también a competidores que buscan desarrollar tecnologías, productos y procesos biotecnológicos con impacto a largo plazo. Un aspecto clave en la gestión de negocios y asuntos regulatorios dentro de este sector es la identificación y evaluación de tendencias globales. En particular, el mundo se prepara para una transformación en el registro de productos de biocontrol, lo que promete agilizar el acceso al mercado. Este y otros cambios deben ser analizados estratégicamente, ya que representan una oportunidad para acelerar la comercialización de soluciones biológicas innovadoras. La creciente demanda global de alternativas sostenibles basadas en tecnología subraya el potencial del sector de la agricultura biotecnológica como una industria emergente y altamente prometedora. Ignorar su desarrollo significaría perder oportunidades clave en un mercado internacional en constante evolución, donde la optimización de tiempos, costos y regulaciones es esencial para el éxito comercial.
Palabras Claves: agroindustria, procesos biotecnológicos, tecnologías y negocios.

Poster Presentations

Citrus disease management using plant elicitors.

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Plants grow facing different biotic and abiotic stresses daily. They have developed diverse defense mechanisms to achieve adequate development. These include Systemic Acquired Resistance (SAR), related to salicylic acid (SA) and Pathogenesis-Related (PR) proteins; Systemic Induced Resistance (SIR), activated by bacterial strains of saprophytic rhizobacteria; and Locally Acquired Resistance, triggered by the plant's Hypersensitive Response (HR) and the production of phytoalexins. These processes include ion fluxes, production of reactive oxygen species, accumulation of phytohormones, and transcriptional activation of defense-related genes, among others. Considering these interactions, several investigations are focus to development new elicitors capable of stimulating these mechanisms and, therefore, increasing resistance to numerous diseases.
This work described the role of four new synthetic compounds (SC) as plant elicitors. They are able to activate plants signaling pathways related to immune response. Finally, the results suggest the use of synthetic compounds as an alternative to enhance plant immunity to control citrus greening or other citrus diseases.