Improvement of Recommended Sugarcane Varieties Using Nuclear Technology and Biotechnology

Progress Report for RAS/5/070 - **Developing Bioenergy Crops to Optimize Marginal Land Productivity through Mutation Breeding and Related Techniques**

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Presentation outline

- Project Team
- Background
- Project Rationale
- Objectives
- Methodology
- Activities and Results
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- Future Plan

Project Team

Philippine Nuclear Research Institute

(Mutation breeding and Soils Group)

- Mary Jayne Manrique- (PNRI) Molecular Breeder & Biotechnology –Project Leader
- Ana Maria Veluz- (PNRI) Tissue Culture
- Arvin Dimaano- (PNRI) Plant Pathology
- Jorge Sahagun- (PNRI) Agriculture Biotechnology
- Roland Rallos- Soil Science
- Faye Rivera- Chemistry
- Ruben Dacoco- Contractual -Tech. Assistant
- Vivian Maguide- Contractual –Research Analyst
- Consultant- Plant Physiologist
- Consultant- Molecular Biologist

Sugar Regulatory Administration research staff

- Ma. Theresa Alejandrino- (SRA) Tissue Culture (Plant propagation)
- Rimmon T. Armones- (SRA) Plant Breeder
- Nora Meneses- (SRA Plant Pathologist)

Background

- Major Concerns
  - Global warming
  - Kyoto Protocol (commit to reducing emissions of six key green house gases by at least 5%)
  - Oil price hike
  - Energy Security

Government Response

Republic Act 9367- known as the Biofuel act of 2006 that mandates the use of biofuel in the country and signed into law on January 2007

At least 5% bioethanol shall comprise the annual total volume of gasoline fuel actually sold within two years of its affectivity and increased to 10% for the next two years.

A minimum of 1% of biodiesel by volume shall be blended onto diesel engine fuels within 3 months from affectivity and will be increased to 2% 2 years from affectivity

Gasoline Demand and Targets

Year	Gasoline Demand (In Million Liters)	Bioethanol Blend (Target)	Supply Requirement (In Million Liters)	No. Of Bioethanol Plants Required
2011	3,775.66	10%	377.57	-
2012	4,008.20	10%	400.82	10
2013	4,268.03	10%	426.80	-
2014	4,555.92	10%	455.59	1
2015	4,877.32	10%	487.73	1
2016	5,203.83	10%	520.38	1
2017	5,629.43	10%	562.94	1
2018	6,073.70	10%	607.37	1
2019	6,536.82	10%	653.68	1
2020	7,017.43	20%	1,403.49	27

Source: DOE Biomass Sector Roadmap

Bioethanol Plant Capacities

Company	Plant Capacity (Mli/Year)	2014	2015	2016	2016-2020
GreenFutureInnoInc.	54	54	54	54	54
LeyteAgri Corp	9	9	9	9	9
RoxolBC	30	30	30	30	30
SanCarlosBioenergyInc	40	40	40	40	40
UniversalRobinaCorp	30	30	30	30	30
BalayanDistilleryInc.	30	30	30	30	30
KoolCompanyInc.	12	12	12	12	12
FarEastAlcoholInc.	15	-	15	15	15
CaviteBioenergyPI	38	-	-	38	38
CanlaonAAI, AbsolutAsianAlcohol, ConsolidatedDistillery	Combined 185	-	-	-	185
TOTAL	443	205	220	258	443

Project Rationale

- Sugar cane is a C4 plant and considered as one of the world's most efficient crops in converting solar energy into chemical energy.
- It is an important crop for food and energy production
- It is the single major source of manufactured white sugar the source of 80% of world's sugar
- Breeders have been focused on increasing sugar content and gave less importance to fiber content
- Cultivars typically have 13-14% sucrose and 15% or less fiber and about 70% water
- But with evolving industry of biofuel and biobased chemicals, the aim is to breed for "Energy-Cane".

- The major byproduct of sugarcane are bagasse and molasses
- Bagasse contains 48% cellulose and ca be converted to bioethanol
- Ethanol is obtained either as a primary or secondary by product
- Conventional breeding could take very long time
- The narrow genetic diversification in sugarcane is one of the underlying difficulty in breeding new improve cultivars
- Sugarcane biomass is a versatile renewable energy source that can provide environmental and economic benefits and can play an important role to reduce greenhouse gas emission.
- Development of high sugar-high fiber content sugarcane through nuclear technology and biotechnology will increase production of biofuel for power generation.

Research Aim

General objective: To improve SRA recommended sugar cane varieties in Batangas, Negros and Bukidnon using Nuclear Technology and Biotechnology targeting traits for bio-ethanol production efficiency.

The specific objectives are as follows:

- Increase sucrose content of the mutated recommended varieties by 5-10%
- Decrease lignin content in cell wall by at least 5%
- Increase average millable stalks by at least 20%
- Increase yield (tons/hectare) by 20%

Methodology

- **Identify varieties**
  - Phil 99-1793, Phil 2000-1011, Phil 97-3933,Phil 6607, Phil 7544……
- **Radiosensitivy test**
  - Exposure of plantlets to gamma radiation/ion-beam using different dose
- **Bulk irradiation**
  - Bulk irradiation of samples using optimum dose
- **Advance generation using tissue culture**
  - From M1V1 to atleast M1V4
- **Phenotypic selection**
  - Observing traits like sugar content, height, cane diameter and cellulose content….
- **Genotypic selection**
  - Incorporating marker assisted selection, RAPD including the genes for lignin and sucrose
- **Multi-location trial**
  - Negros, Batangas and Bukidnon

Activities and Results

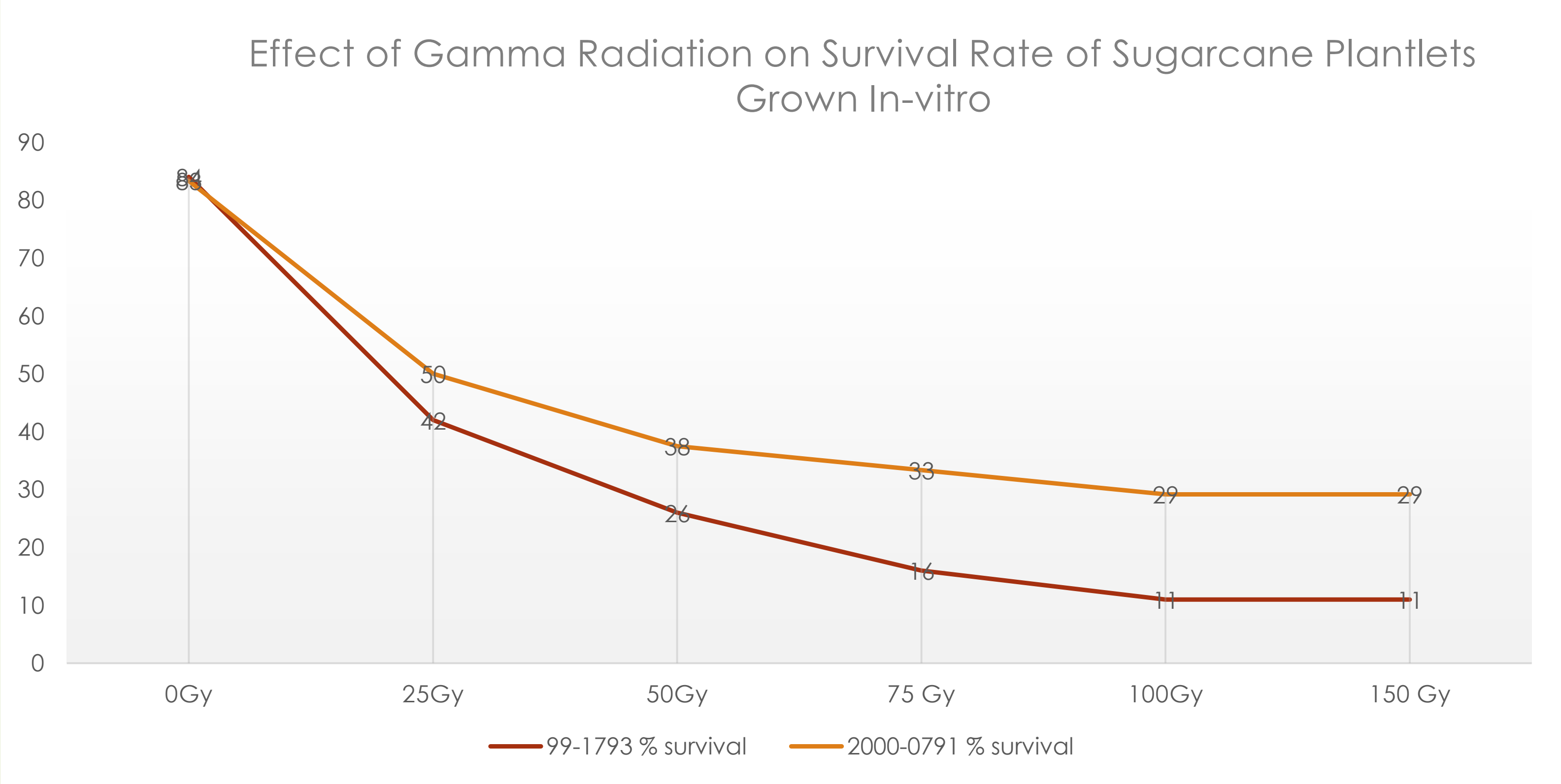
- **Varietal collection**

Three popular varieties from each sites was used in the mutation induction experiment using gamma radiation with a total of eight varieties with Phil 8013 as the check variety. All plant samples was in-vitro propagated and was acquired from La Granja research station in Negros.

Experimental Sites	Sugarcane Varieties
Bukidnon	Phil 6607 Phil 99-1793 Phil 2003-1389
Batangas	Phil 7544 Phil 99-1793 Phil 8477
Negros	Phil-2004-1011 Phil 99-1793 Phil-2000-0791
Check variety: Phil 8013	

Radiosensitivity test of varieties from Negros

- Radio-sensitivity test was conducted using Gammacell 220 irradiator with the following doses: 25, 50, 75,100 , 150 Gy and control
- For variety Phil 2000-0791 LD_{50} was achieved at 25Gy while for variety Phil 99-1793 LD_{50} is at slightly less than 25Gy.



From tissue culture to plantlet hardening

Irradiated Plantlets

Conduct short training course on In-vitro Propagation of sugarcane using shoot tips and meristem for PNRI Staff

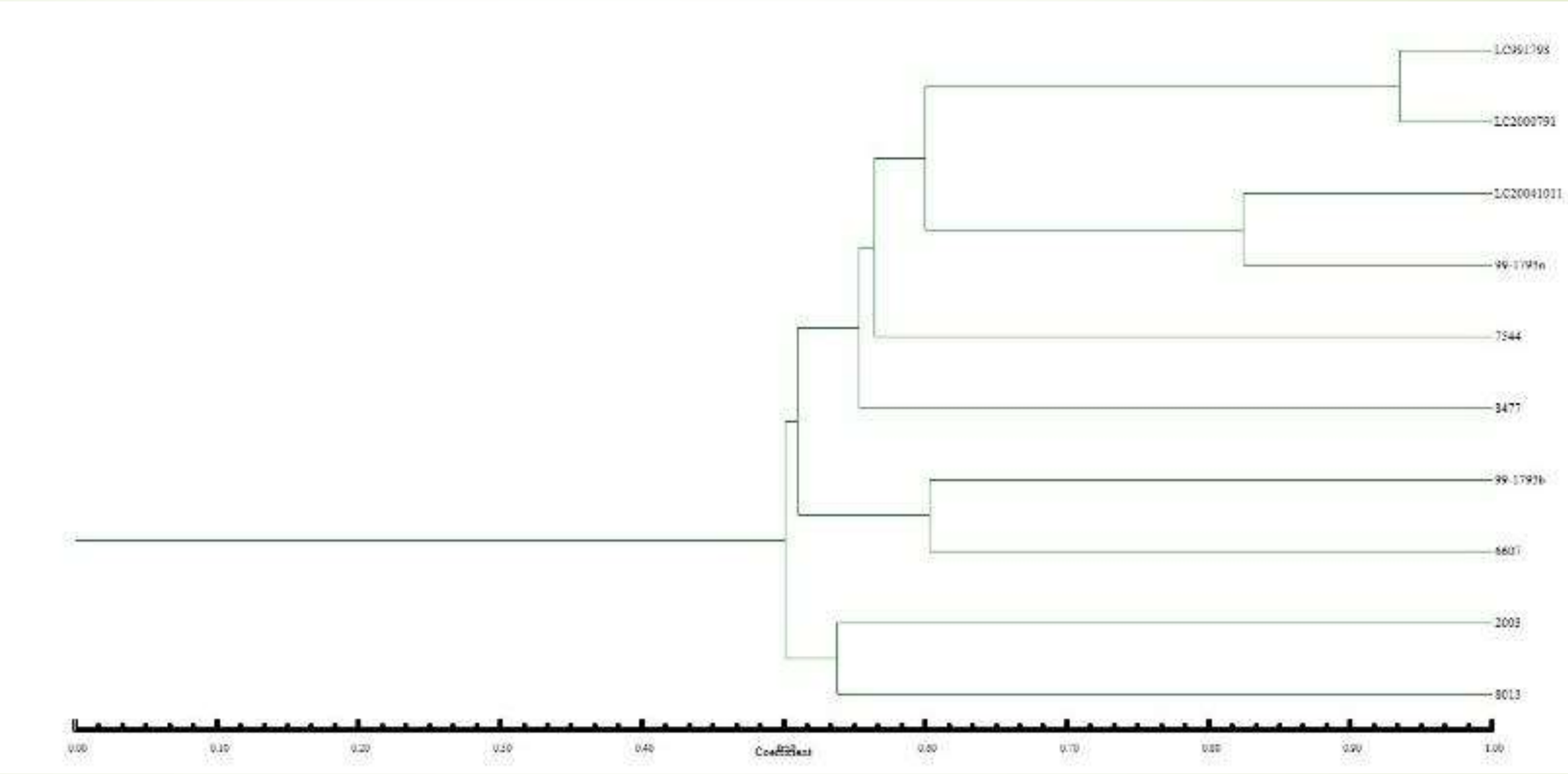
Field inspection and site selection

Nasugbu, Batangas (Luzon)

La Carlota, Negros Occidental (Visayas)

Maramag, Bukidnon(Mindanao)

RAPD Analysis to determine genetic variation of the 8 genotypes from different sites



Newly renovated molecular laboratory

Thermal cycler and Gel doc

Gel Electrophoresis

Real Time PCR

Sample preparation

Hot Room

Oven and Freezers

Lesson learnt

- ***Challenges***-
  - Sugarcane genome size being octoploid,
  - RAPD analysis sometimes are hard to replicate
  - first time to handle sugarcane crop(lack of expertise in sugarcane agronomic practices)
  - problems in micro-propagation because of chemical-sanction for nitrates,
  - delayed release of funds and long process of procurements for supply and materials.
- ***What was new?*** Our newly renovated laboratory with the acquirement of new equipment have increase our capability to incorporate the molecular marker techniques in our breeding projects.
- ***What was positive?*** With the use of the marker techniques, we can start molecular characterization even at earlier generation (M1V2 or M1V3).
- ***How to move forward?***
  - We should establish enough irradiated sample material to have successful selection,
  - design a marker to select for our target trait (reduce lignin and sucrose content)

Future work plan

- Advance generation
- Screen and select for target traits
- Stablish selected plants in field condition
- Multi-location trial
- Plant registration
- Strengthen collaborations with stakeholders
- Introduce and turn-over technology (mutant plants) to farmers and bio-ethanol producers through extension works

Thank you!!!