

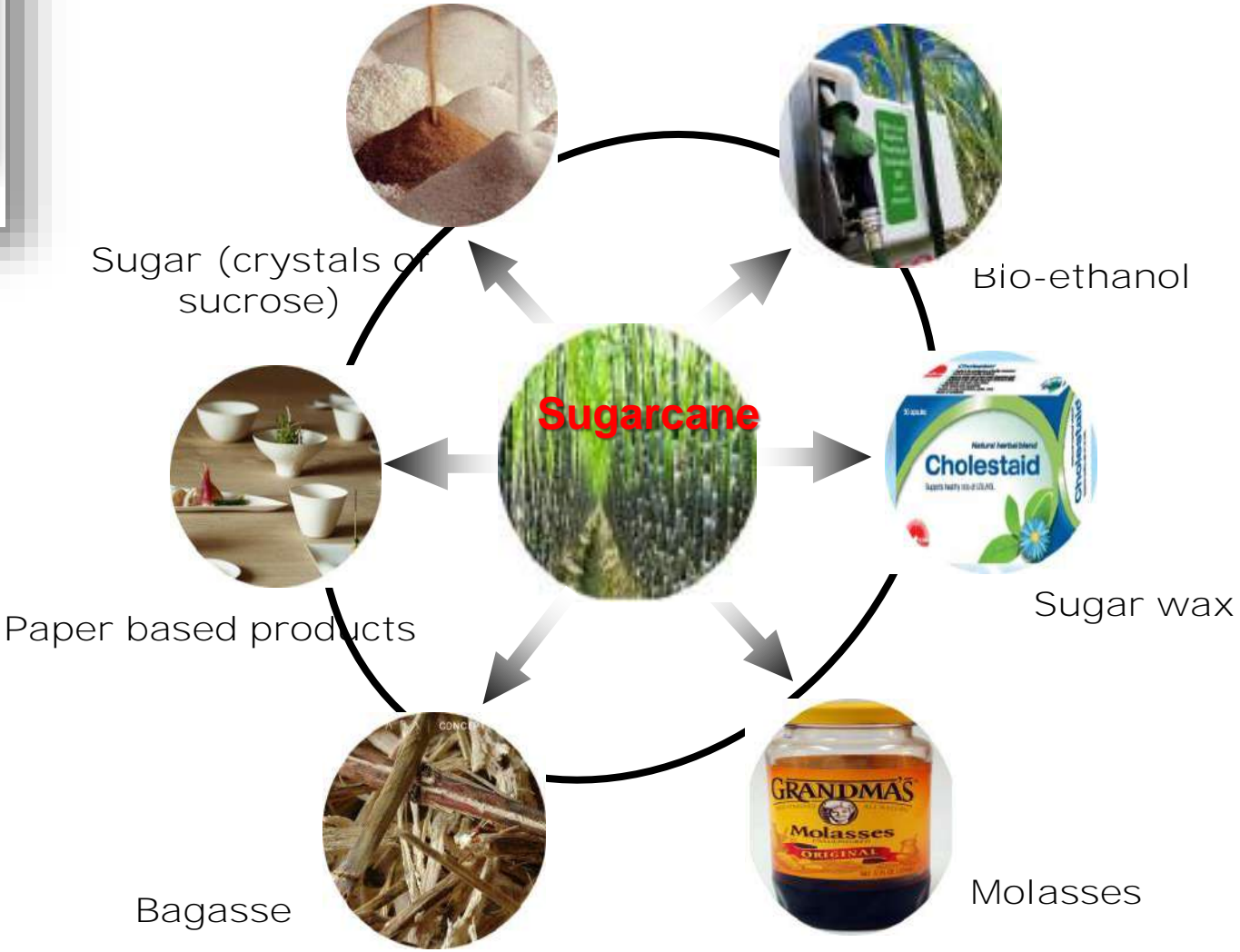
**International Seminar and Workshop on
Biotechnology and Biosafety
Jember University, 10-12 July, 2019**

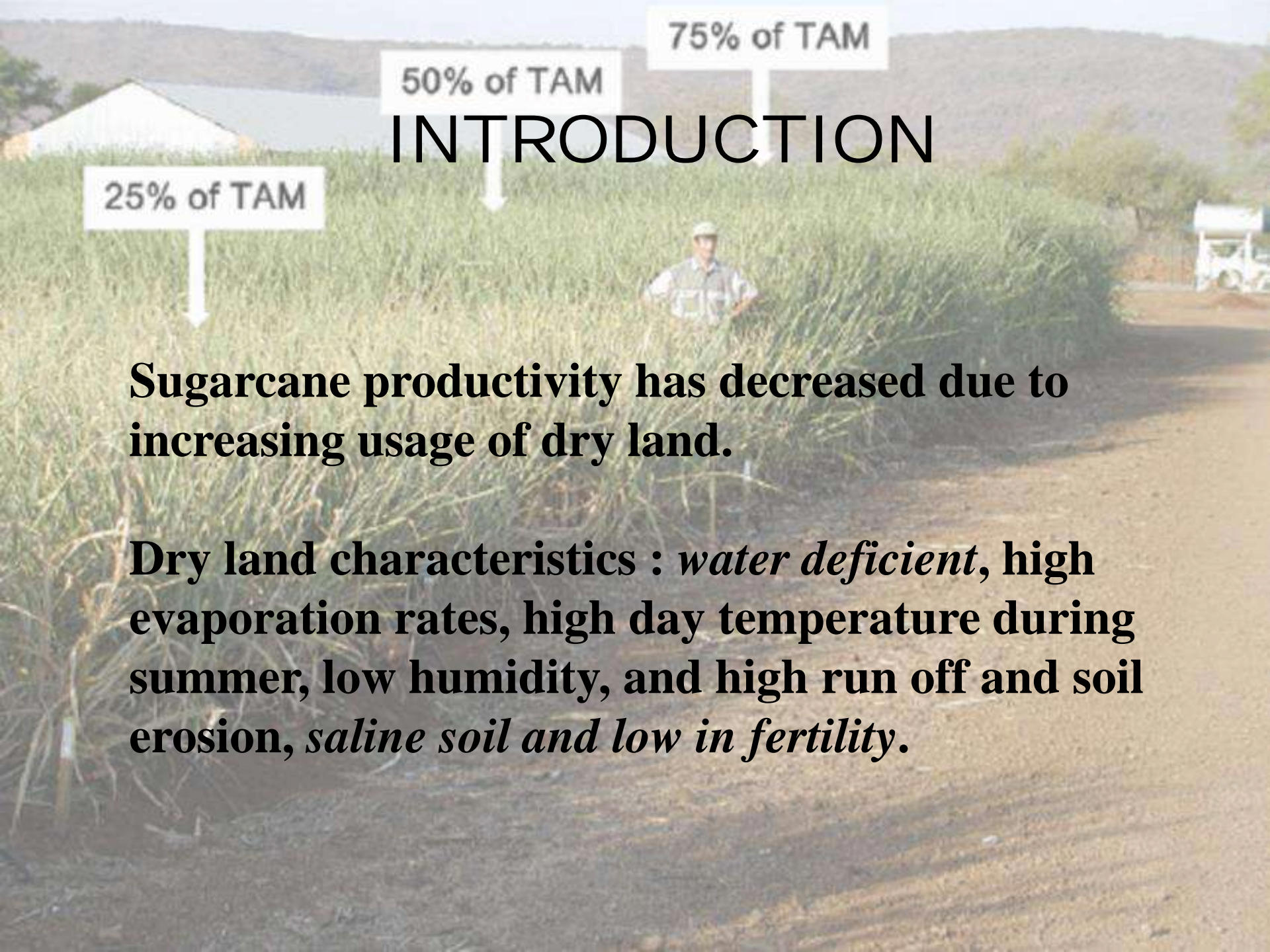
**BIOSAFETY CERTIFICATION
GENETICALLY MODIFIED PLANTS :
Drought Tolerance GM Sugarcane
A Case Study**



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THE BENEFITS OF SUGARCANE





INTRODUCTION

25% of TAM

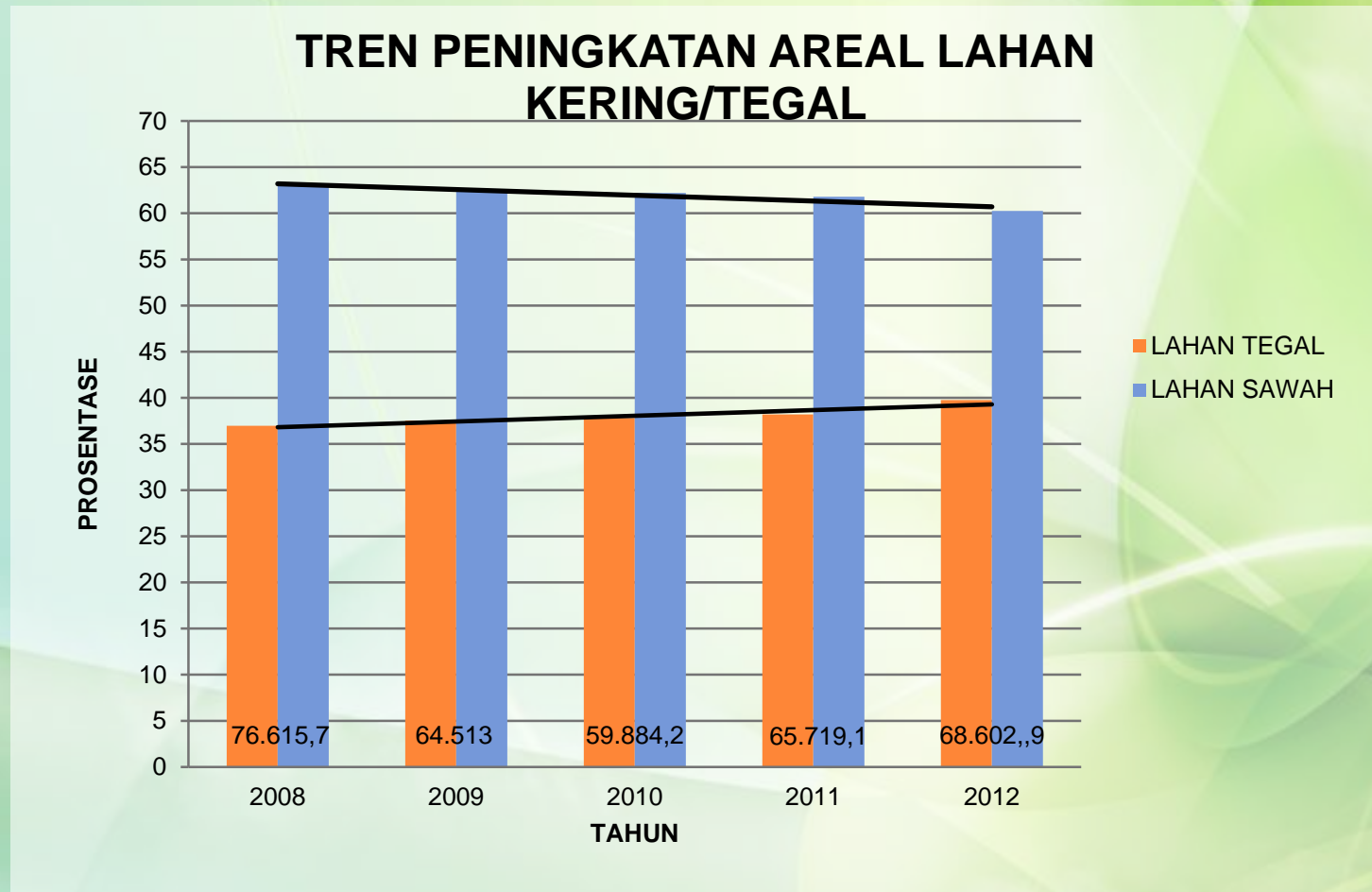
50% of TAM

75% of TAM

Sugarcane productivity has decreased due to increasing usage of dry land.

Dry land characteristics : *water deficient*, high evaporation rates, high day temperature during summer, low humidity, and high run off and soil erosion, *saline soil and low in fertility*.

INTRODUCTION



Gambar 2. Trend Peningkatan Areal Lahan Kering / Tegal di PTPN XI
(Sumber : Bidang Tanaman PTPN XI)

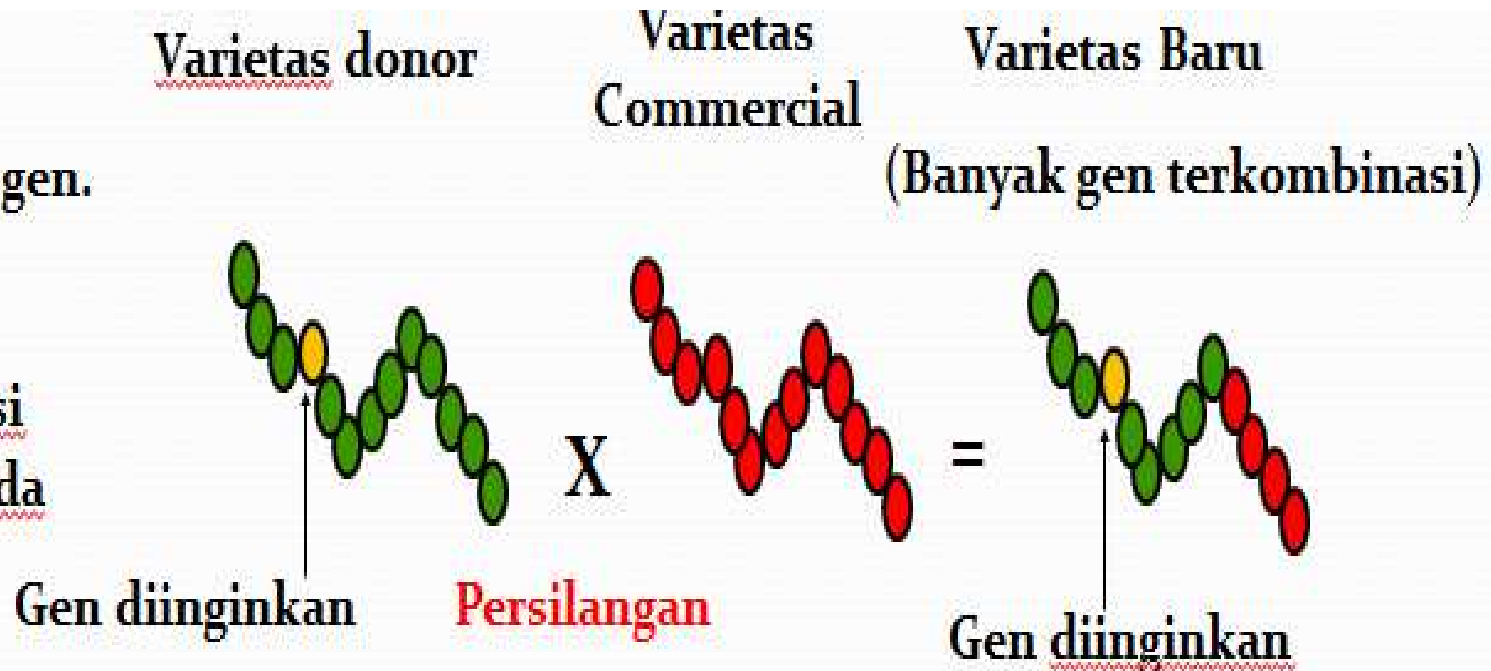
INTRODUCTION



Gambar 3. Produktivitas per Kategori Lahan
(Sumber : Bidang Tanaman PTPN XI)

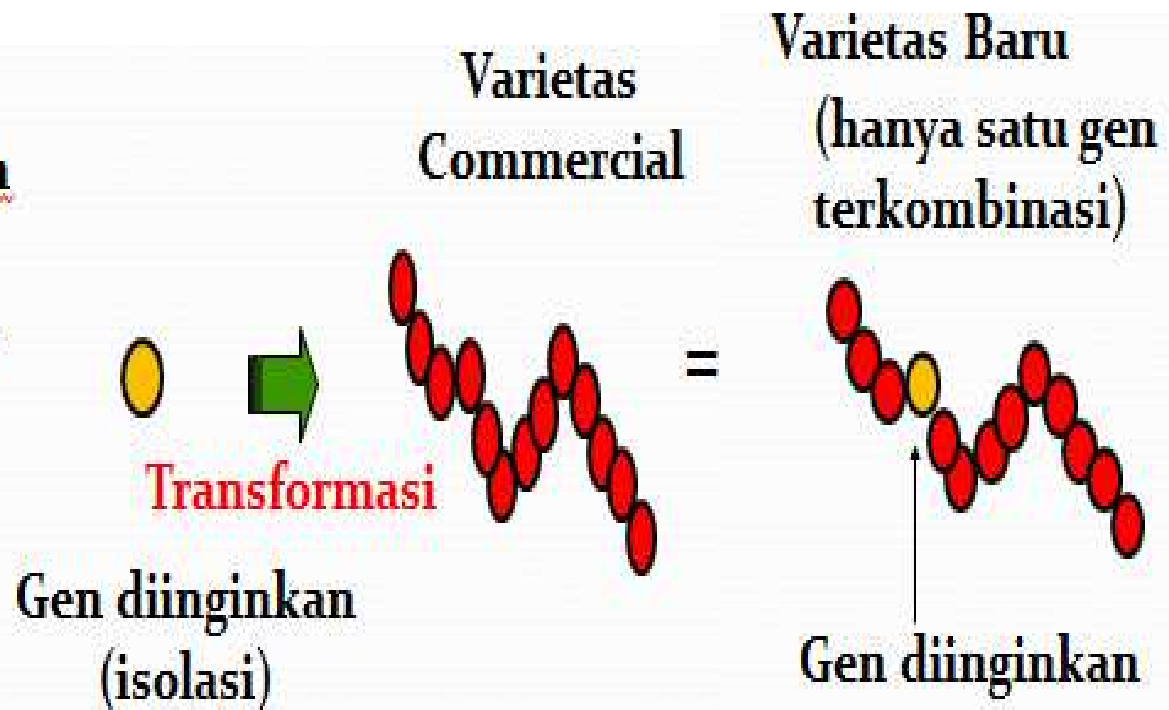
CONVENTIONAL BREEDING

DNA adalah kumpulan gen-gen.
Pemuliaan Traditional mengkombinasi seluruh gen pada anakannya.



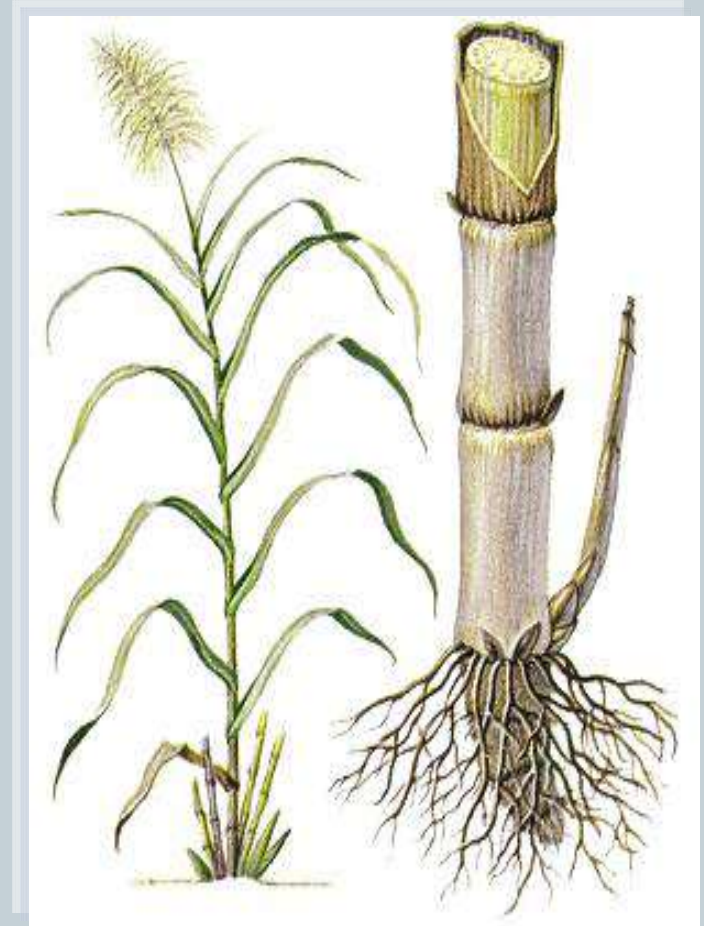
BIOTECH BREEDING

Bioteknologi
Tanaman menambah
single spesifik gen
ke genome tanaman.



Limiting Factors of Conventional Breeding

1. Sugarcane pollination in natural condition is very rare :
 - there's no animal spreading pollen,
 - different time of flower maturity
 - low viability of pollen
2. Sugarcane seeds are very small, low viability (only few minutes), have no dormancy periode, and cannot be grown on land naturally



Developing New Varieties

1. Gene Technology has been considered as a powerful method to anticipate a shift in planting sugarcane to dry land, addressing issues of water availability and climate change.
2. Specific character can be introduced into plant tissue through genetic engineering
3. Cooperations with other institutions (foreign & domestic) are needed
4. precise, rapid, efficient and cost effective



PTPN XI Strategy for Developing New Varieties through Biotechnology

THE SELECTION OF COMMERCIAL SUGAR CANE VARIETIES AS PARENTAL

SUGARCANE TISSUE CULTURE

GENETIC TRANSFORMATION

DROUGHT TOLERANCE, HIGH
SUGAR CONTENT SUGARCANE

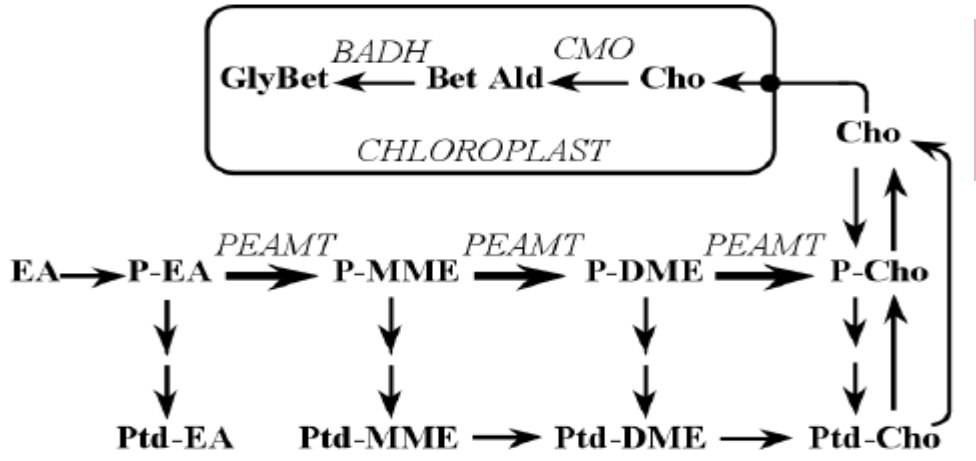
EXAMINATION OF MOLECULAR BIOLOGY AND PHISIOLOGY CHARACTER

FASILITAS UJI TERBATAS (LFT) DAN CONFINED FIELD TRIAL (CFT)

FOOD SAFETY AND ENVIRONMENT SAFETY ASESSMENT (PP 21 TH. 2005)

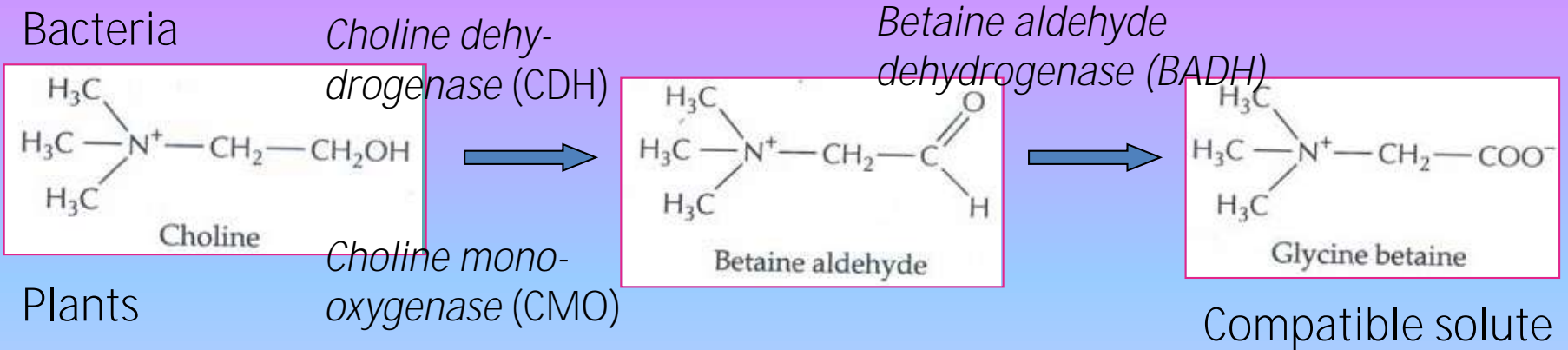
Releasing New Variety

GM Sugarcane using *betA* gene encoding choline dehydrogenase



Synthesis of choline and GB in plants. Some plants can synthesize GB.

Synthesis of glycine betaine (GB) from choline in bacteria

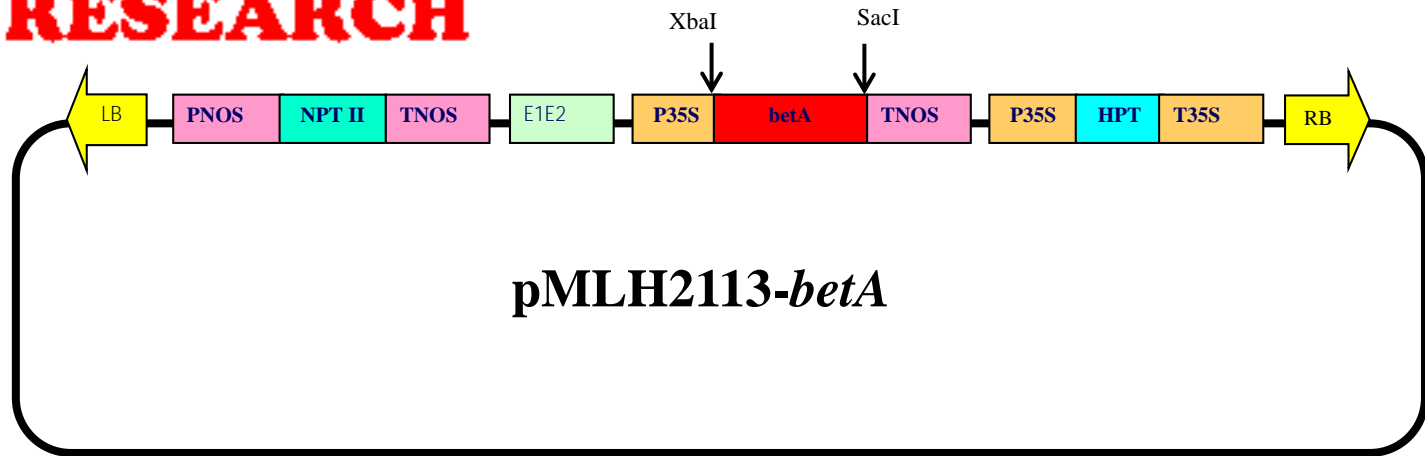


LAB RESEARCH

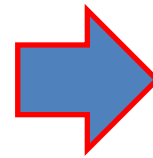
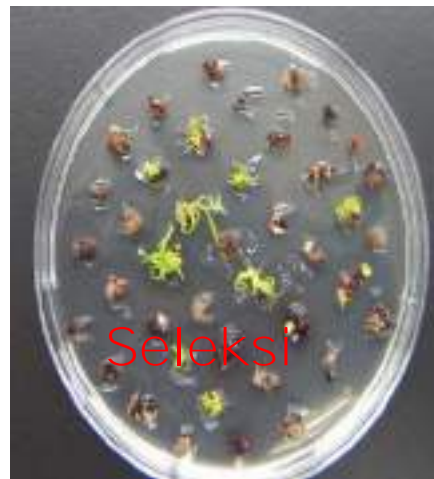
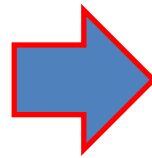
LAB RESEARCH

- a. Parental : BL (Bulu Lawang)
- b. Character : *betA* gen coding for protein choline dehydrogenase (CDH). *betA* gen was taken from *Rhizobium meliloti* (soil bacteria) by Ajico, Intl. cloned to pMLH213 with *Agrobacterium tumefaciens* strain LB 4404 as host. Genetic transformation (biological method) to sugarcane by PT. Perkebunan Nusantara XI

LAB RESEARCH



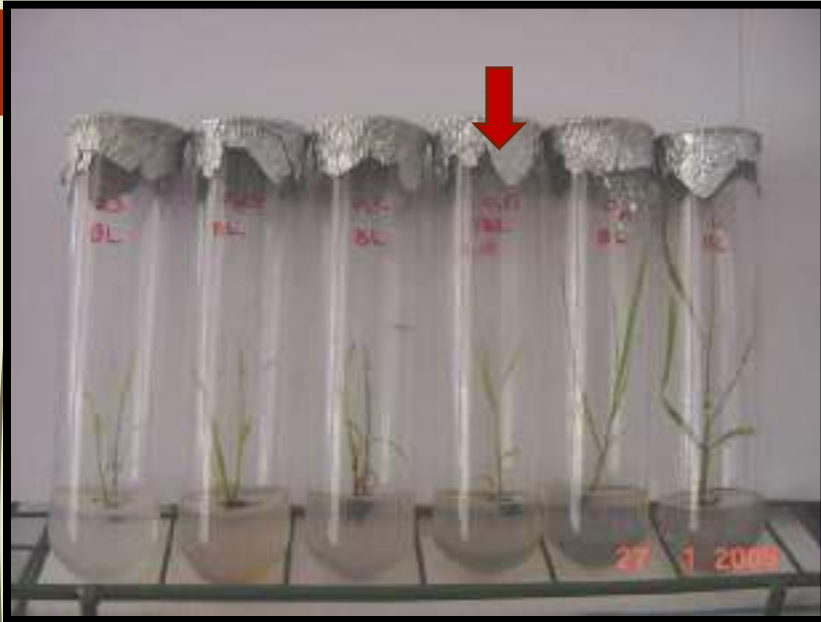
Peta konstruk plasmid rekombinan pMLH2113-*betA*



Tahapan transformasi genetik metode biologis melalui *Agrobacterium tumefaciens*

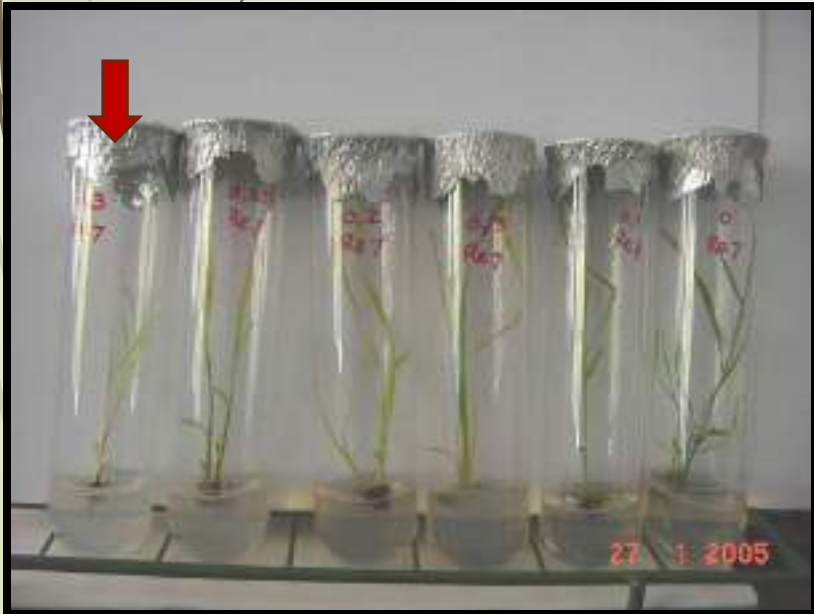
LAB RESEARCH

Tolerance to osmotic stress



Gambar 10.

Plantlet BL dalam media dengan konsentrasi NaCl (dari ki-ka : 0,3; 0,25; 0,20; 0,15; 0,1; 0) pada hari ke-21 (minggu ke-3)



Gambar 11.

Plantlet NXI-4T dalam media dengan Konsentrasi NaCl (dari ki-ka) 0,30; 0,25; 0,20; 0,15; 0,10 ; 0) pada hari ke-21 (minggu ke-3)

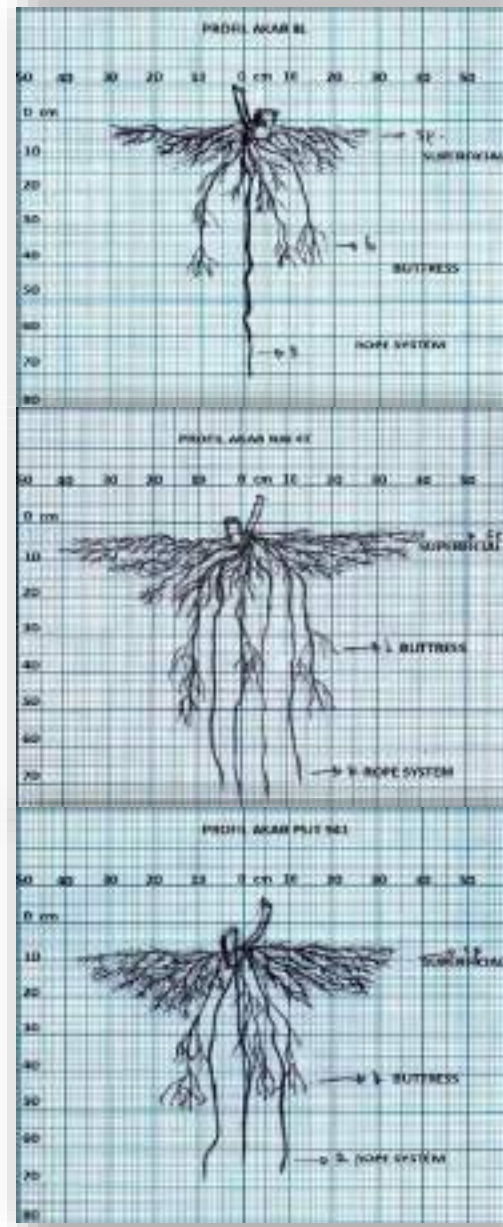
(Sumber data : *Laboratorium Bioteknologi PTPN XI*)

GH RESEARCH

Growth response in water deficiency

Sugarcane Cultivars	Length of water stress (days)		
	Wilting	Permanent Wilting	Dried
NXI-3T	12	> 30	> 30
NXI-4T	19	> 36	> 36
NXI-5T	14	> 30	> 30
BL579-NT (Control)	6	19	19
PSJT 941 (non GM drought tolerant cane)	19	> 36	> 36

Uji kering dilakukan dgn penghentian pemberian air



Profil akar –
tebu non PRG

Profil akar -
tebu PRG NXI-
4T

Profil akar –
PSJT 941

ADMINISTRATOR:

Biosafety Commission for GM Product (by President Regulation) and its organs :

1. Environment Safety Division
2. Food Safety Division
3. Feed Safety Division
4. Assessment team of social economic impact (2012)

BASIC REGULATION :

1. Laws of the RI no. 21/2004 : Ratification of Cartagena Protocol about biosafety for biodiversity convention
2. Govt regulation no. 21/2005 : Biosafety of GM Product
3. Minister of Agriculture regulation no. 61/Permentan/OT.140/10/2011 : Examination, assessment, release and discharge of plant varieties

(Website Indonesia Biosafety Clearing House : indonesiabch.or.id)

FIELD TESTING

Environment Safety Assessment



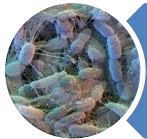
Genetic Stability



Genetic expression



Plant performance



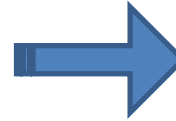
Study of gene flow, displacement



Study of invasiveness



Study of non target organism



1. Molecular testing (using Southern Blott)
2. Biochemistry testing (using HPLC)
3. Physiological Character test (NaCl test)
4. Morphology character (shoot, root, nodes length)



Pollen
and Seed

Soil
Bacteria

Soil
Parameter

Diversity of Soil
Microbe

LIMITED FIELD TRIAL

Definition :

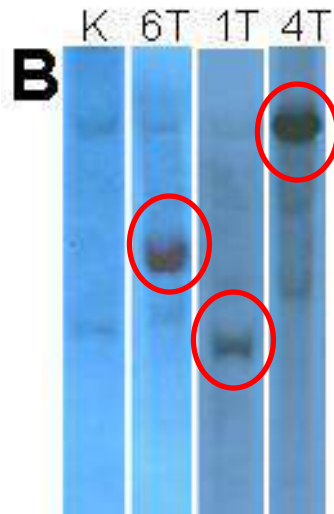
A study area of genetically modified plants that require restrictive measures against planting material and new gene in order to remain in its study site.

LFT is a mandatory if GM plants intended to be released commercially

Permentan no. 61/2011

Genetic stability and Gene Expression

Detection of inserted gene (Southern Blot method)



betaA

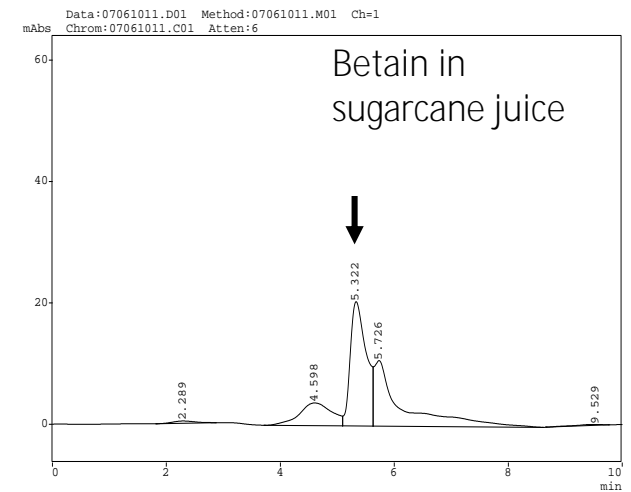
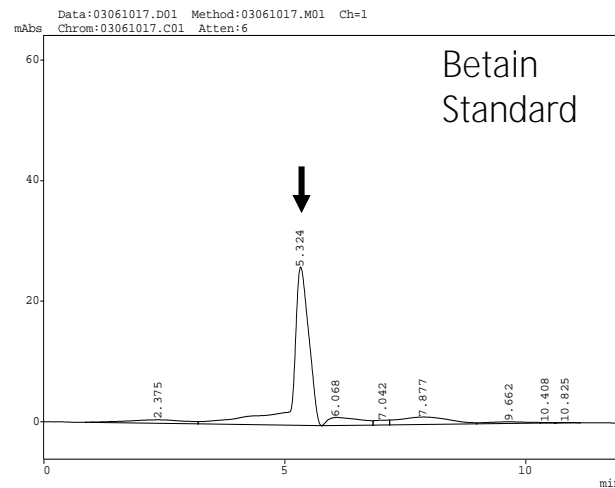


CaMV



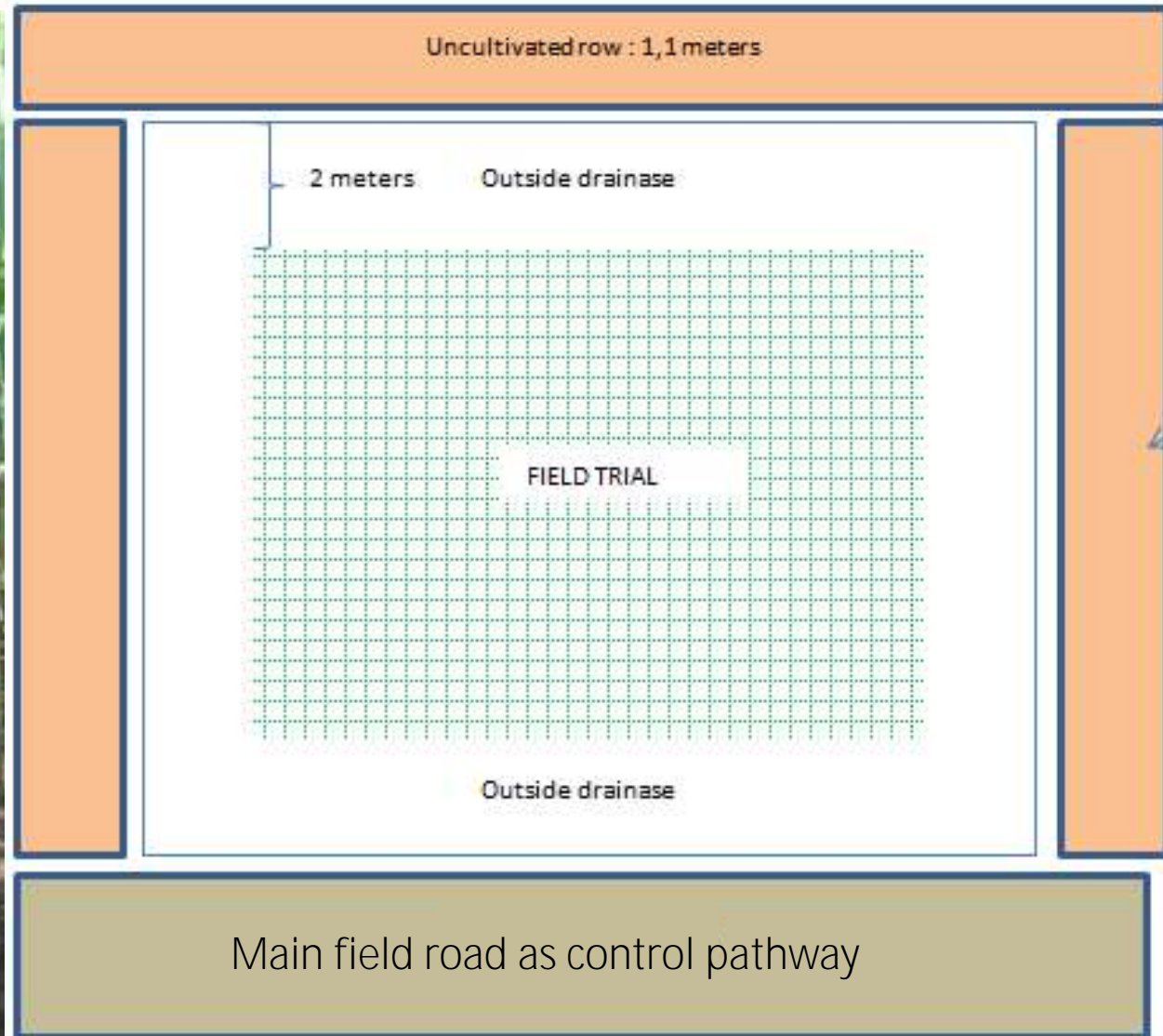
PCR analysis for inserted gene detection (3 vegetative generation)

Betaine content on sugarcane juice (HPLC method)



LIMITED FIELD TRIAL

Layout LFT



Adjacent field with
different period of planting



LFT GM sugarcane



Outside drainage



Uncultivated row

Distance isolation to avoid
contamination or gene flow