


# Exploitation of *in vitro* culture techniques for the development of new genetic combinations in sugarcane

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group




# What is *in vitro* culture techniques?

*How about some definitions*

**General Definition**

**The application of technology to improve a biological organism**

**Detailed Definition**

**The application of the technology to modify the biological function of an organism by genes modification**

# What controls this natural variation?

***Allelic*** **differences at** ***genes*** **control a specific trait**

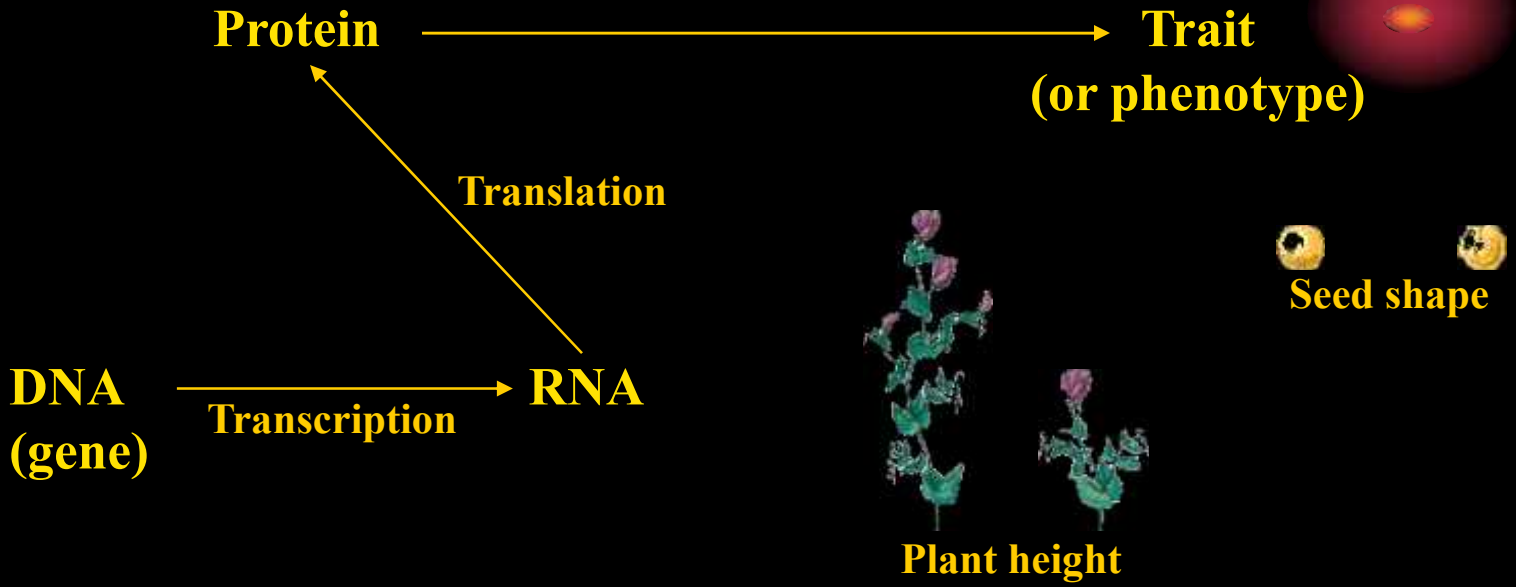
**Definitions are needed for this statement:**

**Gene - a piece of DNA that controls the expression of a trait**

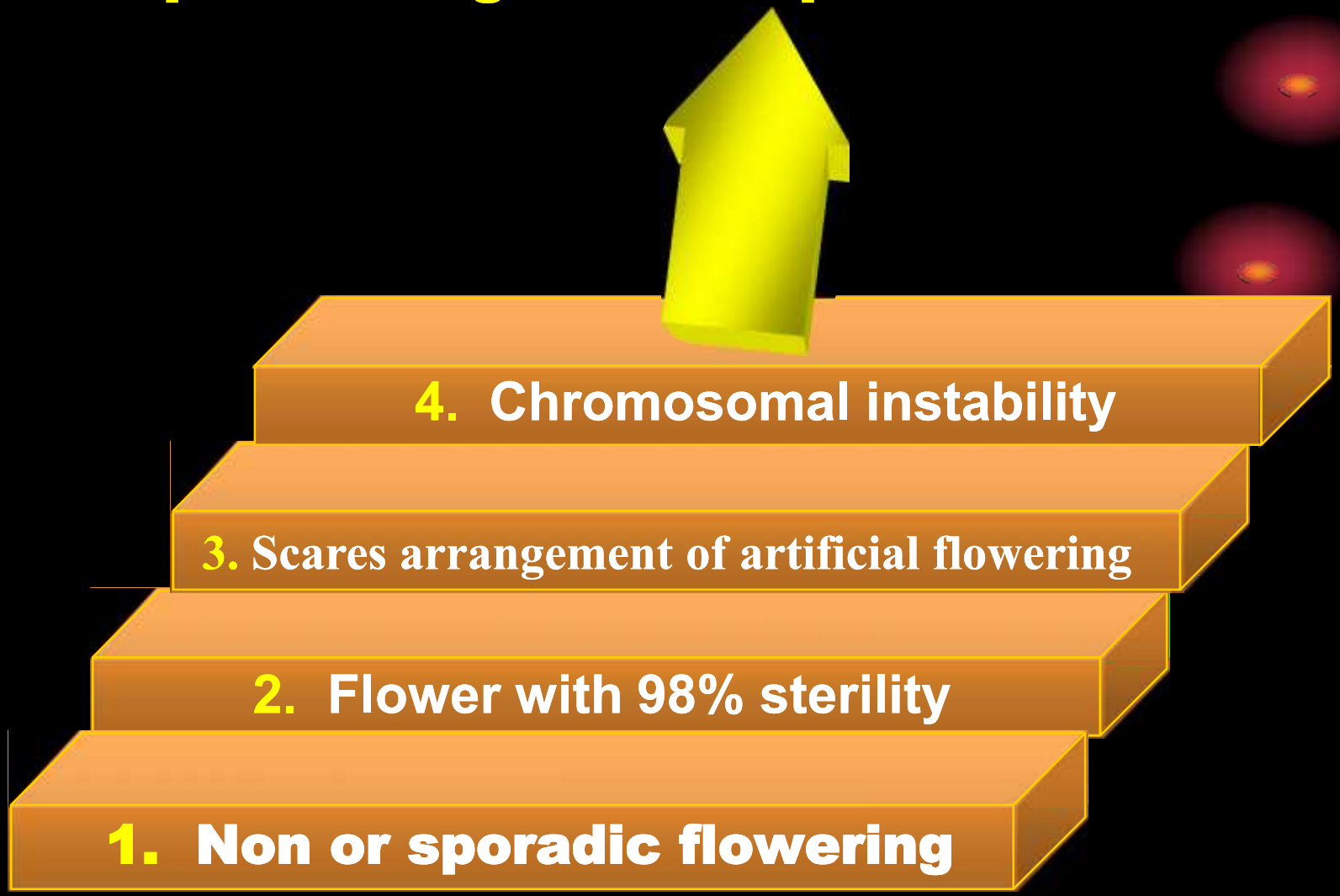
**Allele - the alternate forms of a gene**

# Central Dogma of Molecular Genetics

## (The guiding principle that controls trait expression)



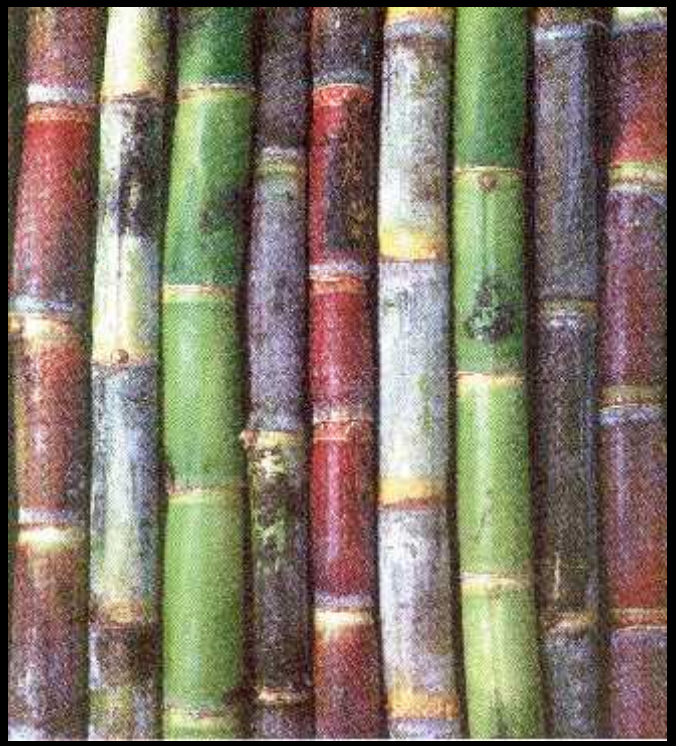
# Rational of applying biotechnological techniques for sugarcane improvement



# Sugarcane improvement through biotechnological techniques

Callus culture

In vitro mutagenesis

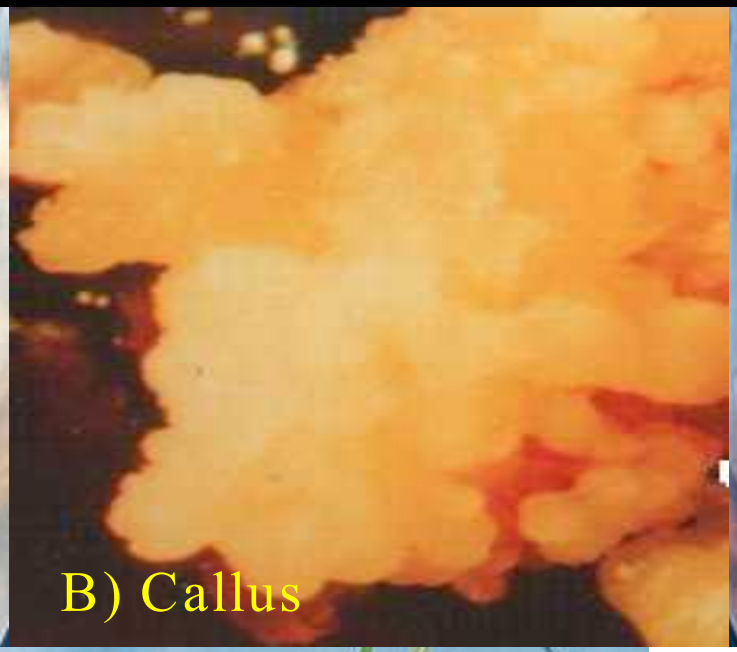


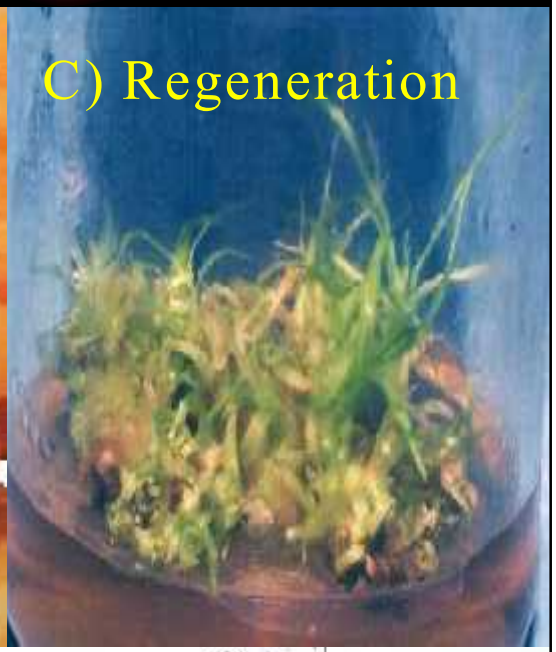
Micropropagation

Direct regeneration

# Callus culture studies


A) Explant


B) Callus

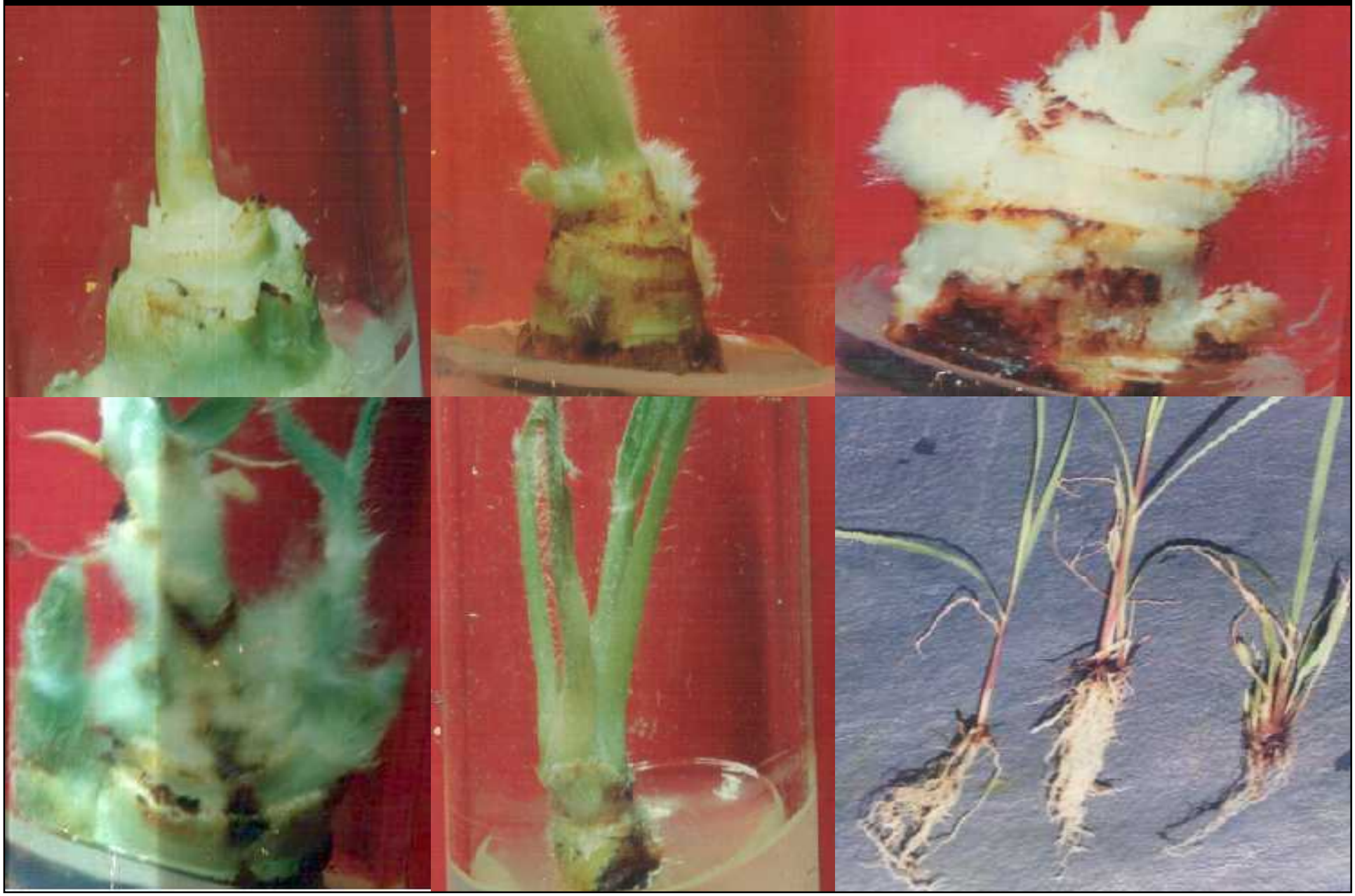

C) Regeneration

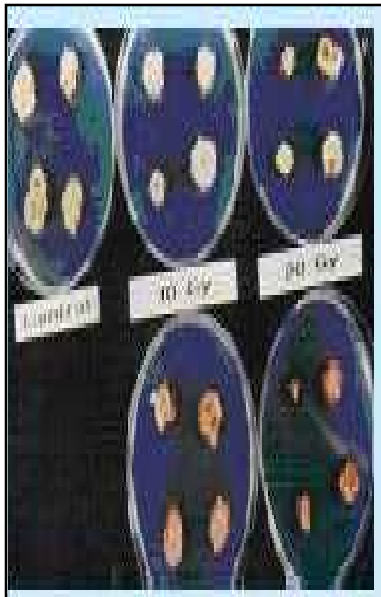

D) Rooted plantlets


E) Plantlets in earthen pot


F)Plants in the field

# MICROPROPAGATION IN SUGARCANE





Explant of sugarcane

Callus induction type A

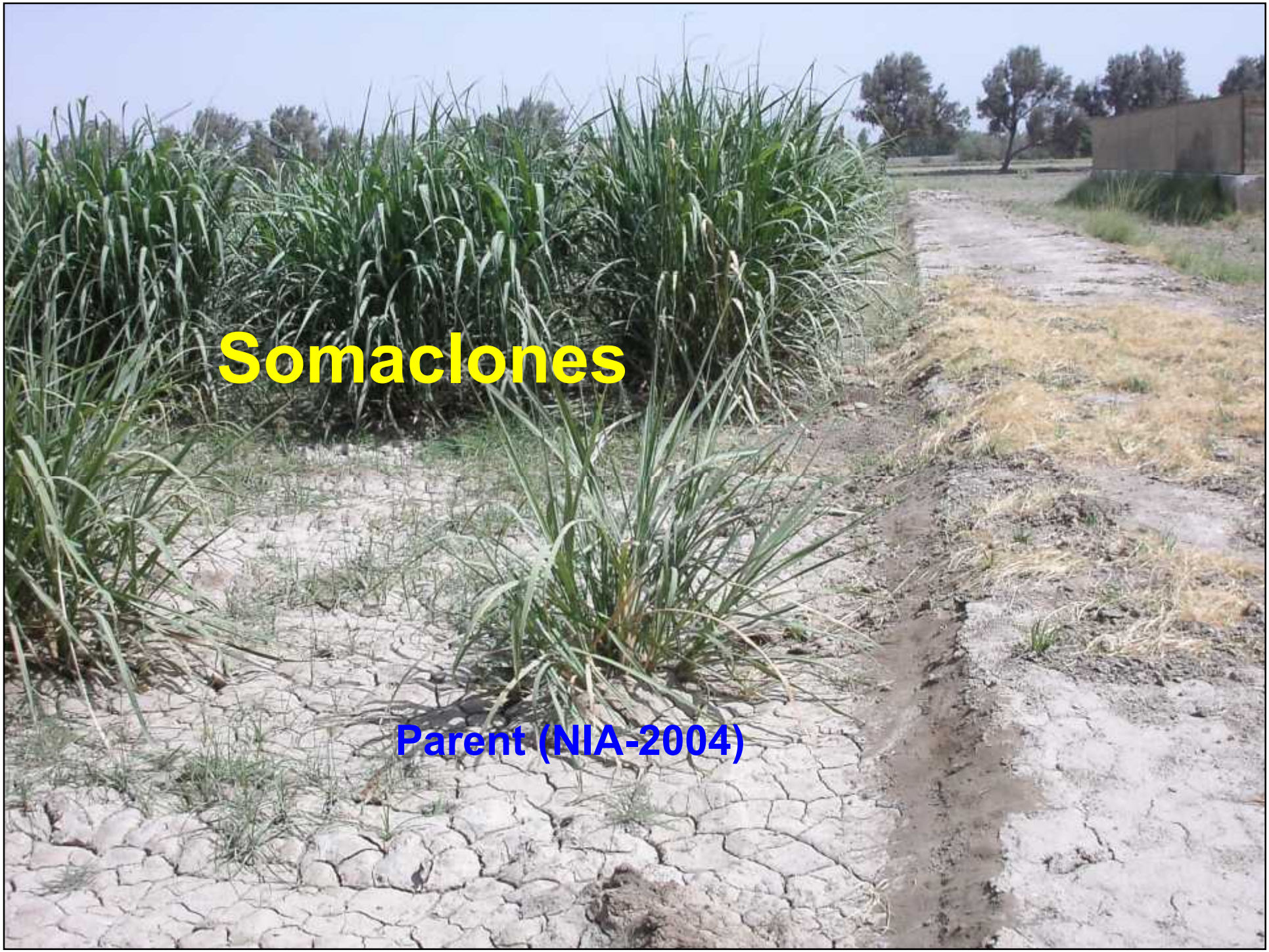
# *In vitro* mutagenesis studies

Non-regenerable callus Type B


Parent
Somaclone

# Plantlets regenerated through callus culture

# Plantlets regenerated through in vitro mutagenesis


Somaclones
Parent (NIA-2004)

# Plantlets Regenerated Through Direct Regeneration Method

# Variability observed in the mutants of CPF - 237

| Characters | Parent | Mutants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 5.11 | 4.50-8.97 | -11.93 to 75.53 |
| Cane length (cm) | 325 | 240-290 | -26.50 to -10.77 |
| Cane thickness (cm) | 2.60 | 2.33-2.63 | -10.38 to 1.55 |
| Cane weight (kg) | 0.82 | 0.77-0.97 | -6.09 to 18.29 |
| Cane yield (t/ ha) | 192.8 | 139.3-207 | -27.75 to 7.36 |
| CCS % | 10.63 | 12.55-13.07 | 18.06 to 22.95 |
| Fiber % | 12.68 | 12.90-12.91 | 1.73 to 1.81 |
| Sugar yield (t/ha) | 20.50 | 17.76-27.08 | -13.36 to 32.09 |

# Variability observed in the somaclone of NIA96-792

| Characters | Parent | Mutants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 4.00 | 4.00-8.94 | 0 to 123.5 |
| Cane length (cm) | 201 | 254-316 | 26.36 to 57.21 |
| Cane thickness (cm) | 2.32 | 2.46-2.67 | 6.03 to 15.08 |
| Cane weight (kg) | 0.67 | 0.76-0.84 | 13.43 to 25.35 |
| Cane yield (t/ ha) | 120 | 135-202 | 12.5 to 68.33 |
| CCS % | 14.87 | 12.93-14.29 | -13.04 to -3.90 |
| Fiber % | 11.32 | 11.29-12.81 | -0.26 to 13.16 |
| Sugar yield (t/ha) | 17.84 | 17.52-28.86 | -1.79 to 61.77 |

# Variability observed in the somaclones of CP67-1026

| Characters | Parent | Mutants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 3.60 | 4.25-8.40 | 18.05 to 133.33 |
| Cane length (cm) | 290.0 | 185-318 | -36.20 to 9.65 |
| Cane thickness (cm) | 3.20 | 3.10-3.33 | -3.12 to 4.06 |
| Cane weight (kg) | 0.98 | 0.64-1.07 | -34.69 to 9.18 |
| Cane yield (t/ ha) | 156.8 | 130-217 | -17.09 to 38.39 |
| CCS % | 13.18 | 9.63-14.65 | -26.93 to 11.15 |
| Fiber % | 12.58 | 12.43-13.87 | -1.19 to 10.25 |
| Sugar yield (t/ha) | 20.67 | 12.55-31.84 | -39.28 to 54.03 |

# DIRECT REGENERATION IN SUGARCANE







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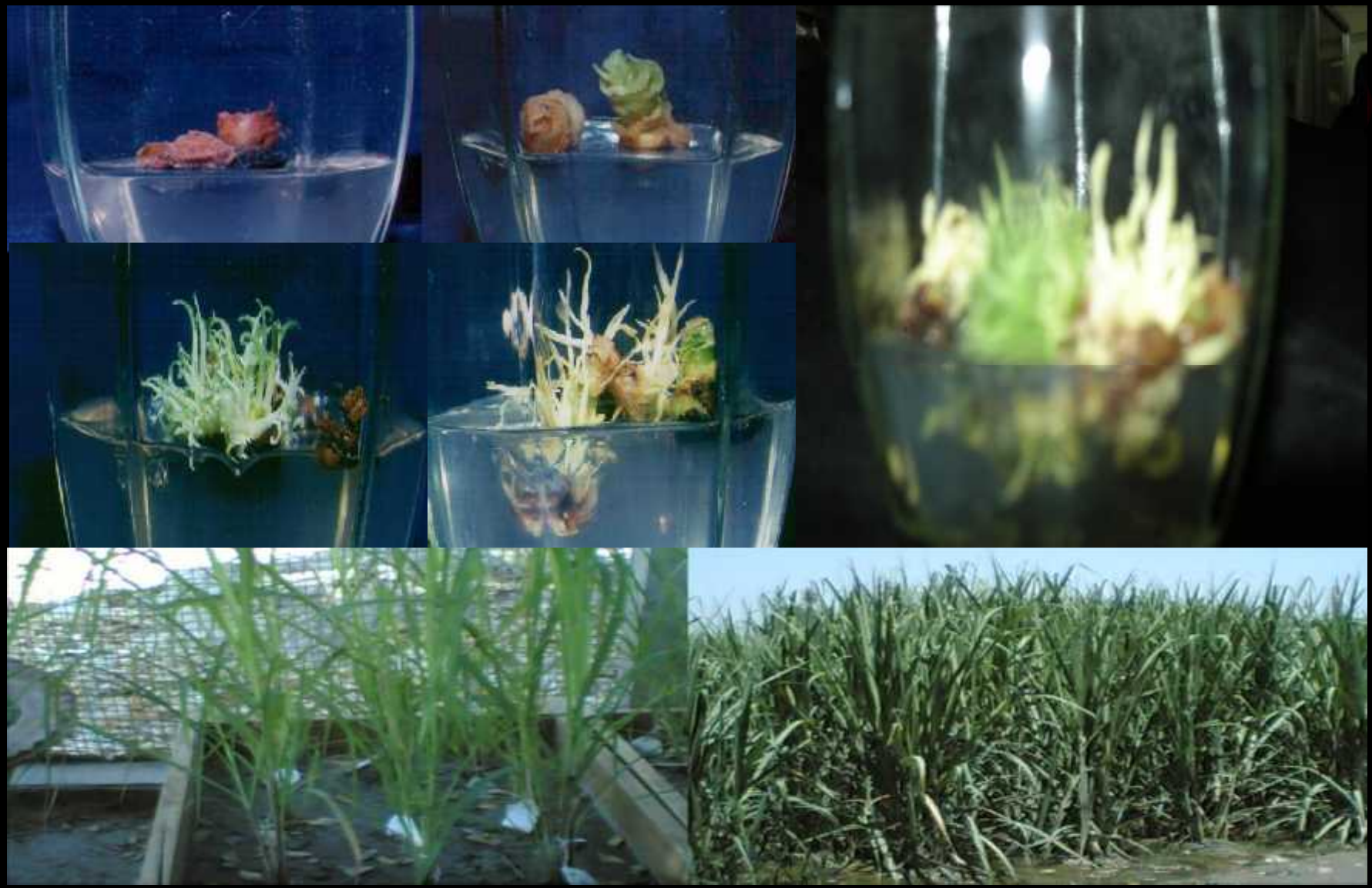


## Variability observed in the direct regenerants of Thatta-10

| Characters | Parent (Th-10) | Variants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 6.88 | 4.50-8.88 | -34.59 to 29.07 |
| Cane length (cm) | 252 | 240-276 | -4.76 to 9.52 |
| Cane thickness (cm) | 2.50 | 2.33-2.60 | -6.8 to 4.00 |
| Cane weight (kg) | 0.90 | 0.78-0.98 | -13.33 to 8.88 |
| Cane yield (t/ ha) | 169 | 146-203 | -13.60 to 20.11 |
| CCS % | 12.30 | 12.48-13.06 | 1.46 to 6.17 |
| Fiber % | 12.95 | 12.91-12.78 | -0.31 to -.1.31 |
| Sugar yield (t/ha) | 20.78 | 18.96-26.54 | -8.76 to 27.72 |

## Variability observed in the direct regenerants of SPSG-26

| Characters | Parent (SPSG-26) | Variants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 4.00 | 4.00-9.00 | 0 to 125 |
| Cane length (cm) | 201 | 256-315 | 27.36 to 56.72 |
| Cane thickness (cm) | 2.32 | 2.35-2.58 | 1.29 to 11.20 |
| Cane weight (kg) | 0.67 | 0.65-0.83 | -2.98 to 23.88 |
| Cane yield (t/ ha) | 120 | 129-200 | 7.50 to 66.66 |
| CCS % | 14.87 | 12.79-14.15 | -13.98 to -4.84 |
| Fiber % | 11.32 | 11.19-12.68 | -1.15 to 12.01 |
| Sugar yield (t/ha) | 17.84 | 16.59-28.33 | -7.00 to 58.80 |

## Variability observed in the direct regenerants of L-116

| Characters | Parent (L116) | Variants (range) | % of variation |
|---|---|---|---|
| Stalk/stool (no.) | 3.60 | 4.16-8.00 | 15.55 to 122.22 |
| Cane length (cm) | 290.0 | 175-313 | -39.65 to 7.93 |
| Cane thickness (cm) | 3.20 | 2.93- 3.23 | -8.43 to 0.93 |
| Cane weight (kg) | 0.98 | 0.74-1.05 | -24.49 to 7.14 |
| Cane yield (t/ ha) | 156.8 | 138-216 | -11.99 to 37.75 |
| Sucrose % | 17.17 | 12.62-19.05 | -26.49 to 10.95 |
| Fiber % | 12.58 | 12.37-13.35 | -1.67 to 6.12 |
| Sugar yield (t/ha) | 20.67 | 12.58-31.70 | -39.14 to 53.36 |

# SUGAR MILLS CO-ORDINATED TRIAL

**Mehran sugar Mills Farm, Tando Allahyar**

**Matiari Sugar Mills Farm, Matiari**

**Faran Sugar Mills Farm, Sheikh Berkio**

**Pangrio Sugar Mills Farm, Pangrio**

**Mirpurkhas Sugar Mills**

**Habib Sugar Mills, Nawabshah**

**Ranipur Sugar Mills**

Genotype Planted

NIA-2004

NIA-2010

NIA81-0819/P5

CPF-237/SPF-234

Thatta-10

Two years Pooled data of Sugar Mills

**Sugar Mills Coordinated trial**

| Clones / Locations | Stalk / stool (Nos) | Cane length (cm) | Cane girth (cm) | Weight/ stool (kg) | Cane yield (t/ha) | CCS % |
|---|---|---|---|---|---|---|
| NIA82-0819/P5 | 5.8ab | 276.6 a | 2.57 c | 5.67 b | 136.67 a | 16.14 a |
| NIA-2010 | 5.3bc | 230.8 b | 2.38 d | 5.23 b | 137.0a | 14.27 b |
| NIA-2004 | 5.0c | 215.0 c | 2.43 d | 4.55 c | 125.25 ab | 15.56 b |
| CPF-237 | 6.4a | 226.6b c | 3.03 a | 5.31 b | 109.50 b | 14.48 b |
| Thatta-10 | 4.8c | 214.1 c | 2.75 b | 6.68 a | 118.83 ab | 13.67 c |


NIA-2004

# NIA-2011

# NIA-2010

# Visit of sugarcane Expert from Sri lanka and AARI Faisalabad

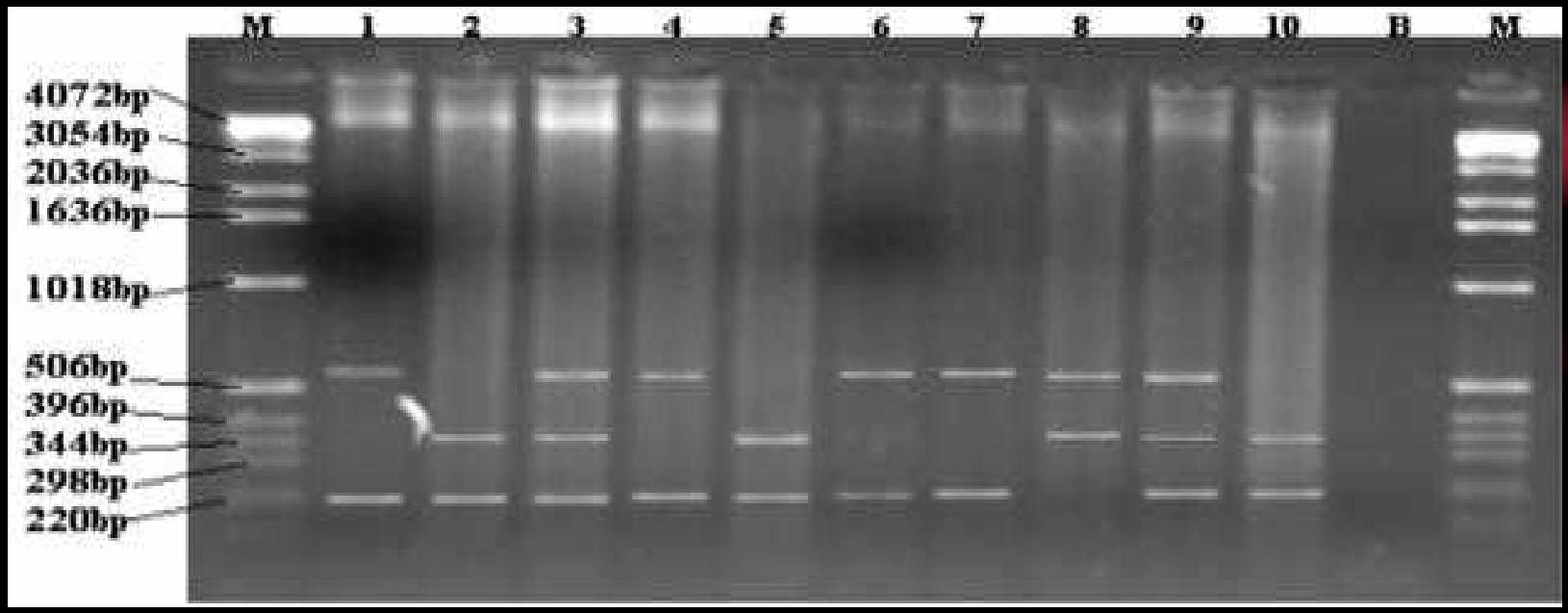



**Figure 1. TRAP profile of sugarcane genotype using surose synthase; M=DNA marker, 1= AEC82-1026, 2= GT-11, 3= AEC92-105, 4= AEC71-2011, 5= Thatta-10, 6= AEC82-223, 7= AEC81-0819, 8= NIA-2004,9= AEC86-328, 10= L116, B= Blank**

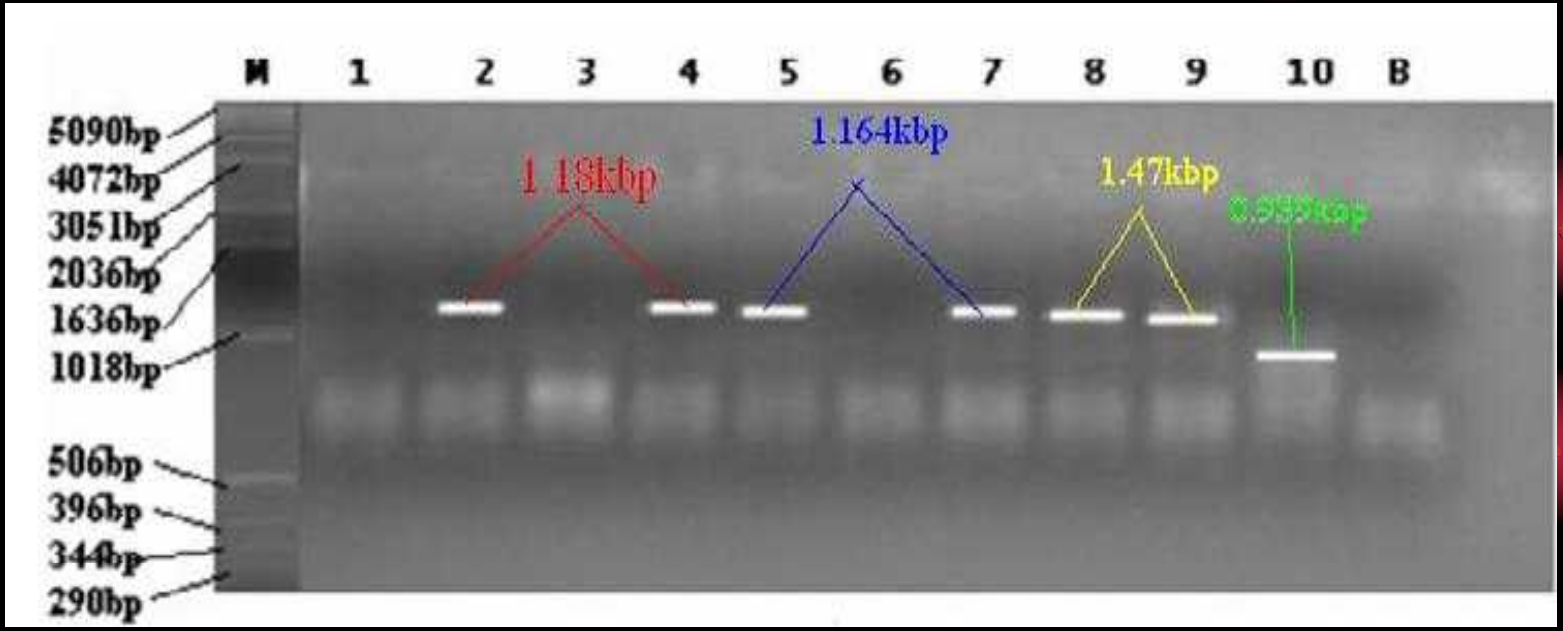
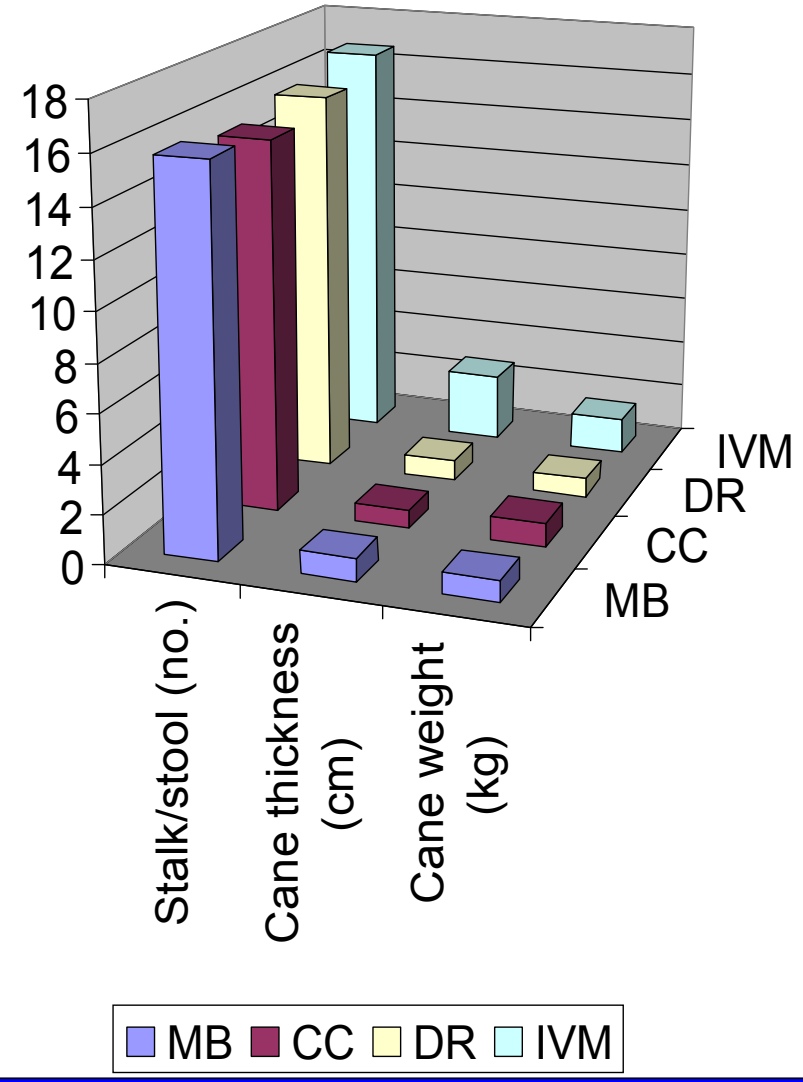
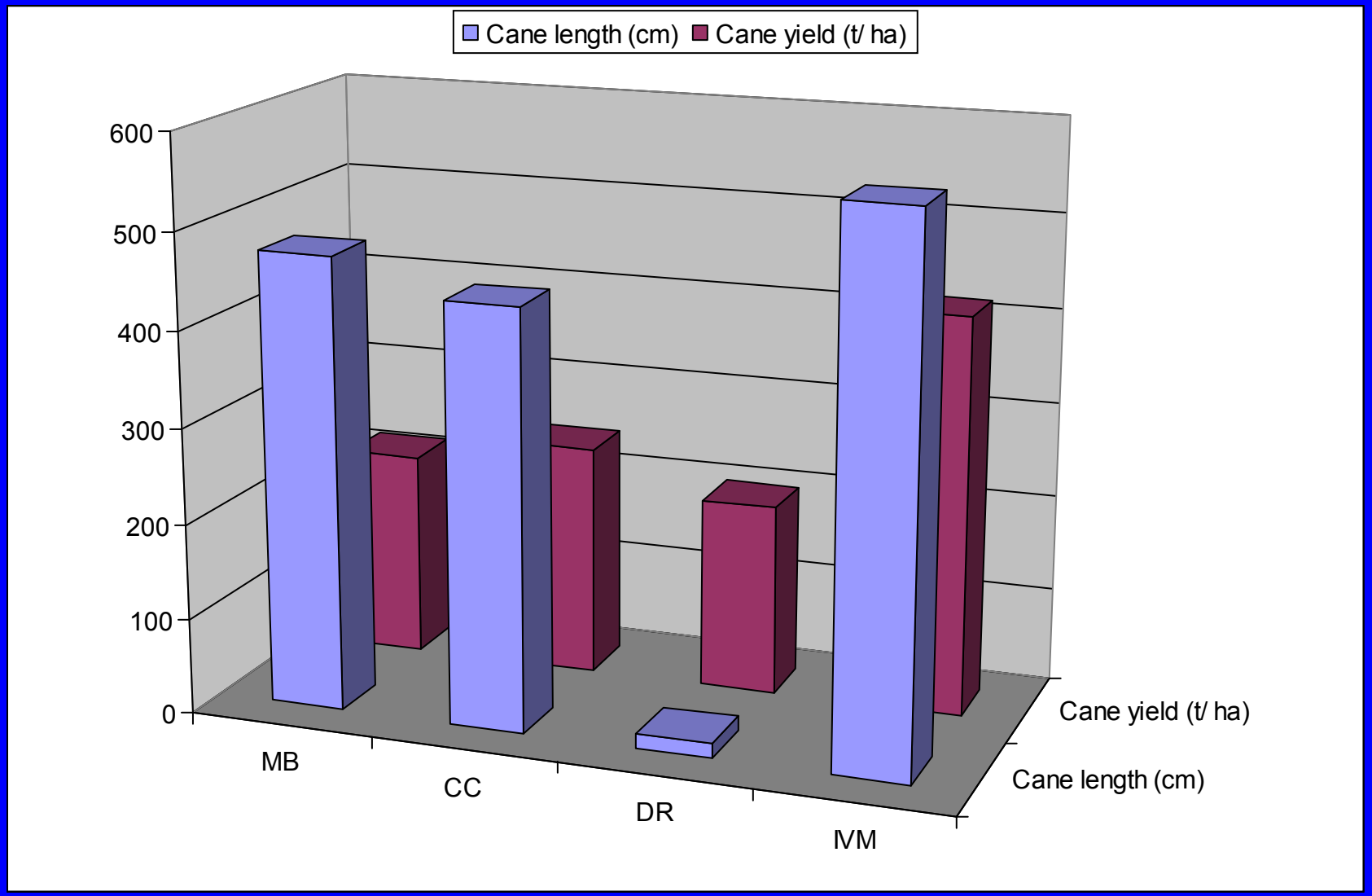



**Figure 2. STS profile of sugarcane genotype using DREB sequence; M=DNA marker, 1= AEC82-1026, 2= GT-11, 3= AEC92-105, 4= AEC71-2011, 5= Thatta-10, 6= AEC82-223, 7= AEC81-0819, 8= NIA-2004, 9= AEC86-328, 10= L116, B= Blank**

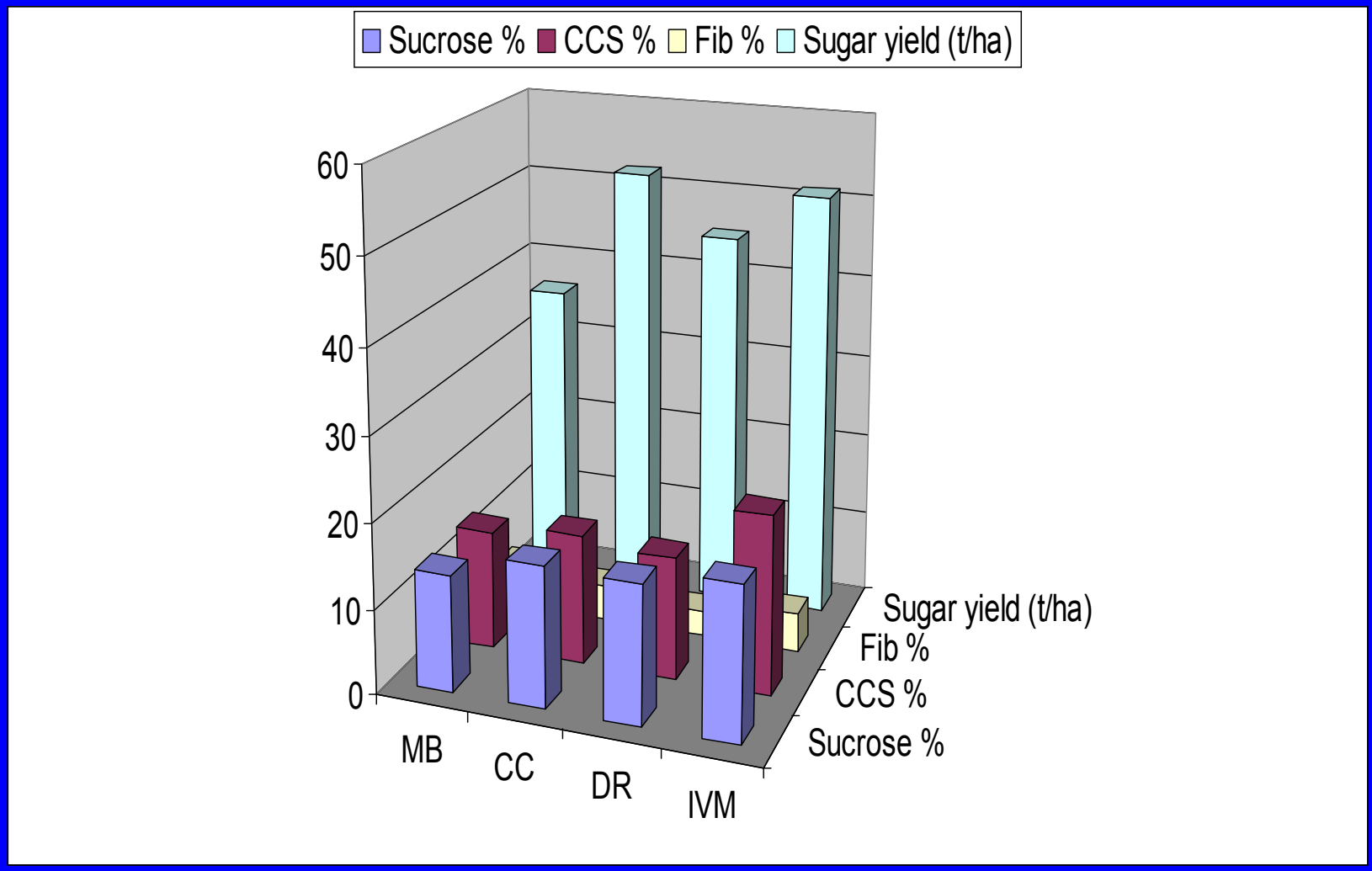
**Genetic variability in sugarcane developed through biotechnological techniques**



# Genetic variability in sugarcane developed through biotechnological techniques



**Genetic variability in sugarcane developed through biotechnological techniques**



| Accession number | Status |
|---|---|
| NIA-2013 | Sugar Mills coordinated Trial |
| NIA-2012 | Sugar Mills coordinated Trial |
| NIA-2014 | Sugar Mills coordinated trial |
| NIA-03/2011 | Will be advanced to zonal trial next year |
| BL4-P70 (somaclone) | Sugar Mills coordinated trial |
| 15 promising clones are in different stages of evaluation | In different stages of evaluation |

# Thanks for patience listening
# Your Questions Please