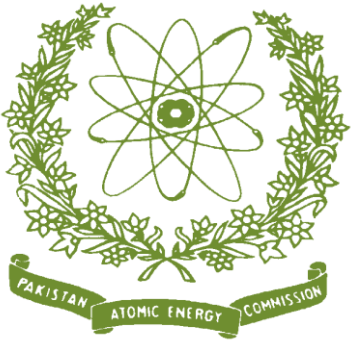


**IN THE NAME OF ALLAH THE MOST  
BENEFICIENT AND MERCIFUL**





# **Tissue culture assisted breeding of sugarcane**

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# Introduction

- Considering the environmental conditions of Pakistan where sugarcane breeding is constrained due to the production of non viable fuzz (seed), somaclonal variation can prove to be a useful tool for crop improvement.
- A true understanding of this phenomenon would allow its utilization as a tool for crop improvement programmes.

# Objectives

- To induce genetic variability through *in vitro* mutagenesis.
- To develop high yielding mutants for better sugar content.
- To develop early maturing varieties.



- **Materials and Methods**

- **Three sugarcane clones viz.**

- **NIASGS-571, NIA-0819 and NIA-2143**

## **EXPLANT**

- **Leaf primordia with meristematic tissue**
- **70% Absolute alcohol for one minute**
- **10% Sodium hypochlorite for 20 minutes**
- **One month old callus was irradiated with four different doses of gamma radiation (10, 20, 30 and 40Gy)**



# Callus induction:

**MS + 4.00 mg l<sup>-1</sup> 2, 4-D**



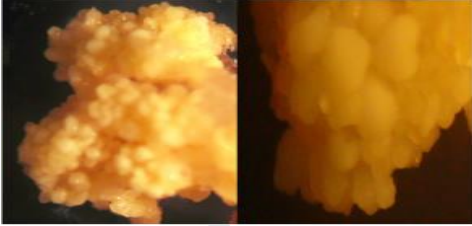
## **REGENERATION MEDIUM:**

**MS + 2.00 mg l<sup>-1</sup> IBA + 2.00 mg l<sup>-1</sup> IAA + 2.00 mg l<sup>-1</sup> Kinetin  
+ 4% sugar.**

## **ROOTING MEDIUM:**

- **1/2 MS + without hormones**
- **1/2 MS + 2.00 mg l<sup>-1</sup> IBA + 4% sugar**
- **1/2 MS + 1.00 mg l<sup>-1</sup> IBA + 6% sugar**

### Callus Induction & Proliferation



### Regeneration



### Multiplication & Elongation



Immature leaf rolls from field grown cultivar of sugarcane genotypes NIASGS-571, NIA-0819 and NIA-2143 were used for callus induction. Explants were cultured on Murashige and Skoog medium supplemented with 4mg/l of 2,4-D. Embryogenic callus was proliferated on the same medium. Cultures were incubated at  $25 \pm 2^\circ\text{C}$  in dark.

The callus then regenerated in MS medium containing growth regulators and incubated at  $25 \pm 2^\circ\text{C}$  under a photoperiod of 16h light and 8h dark.

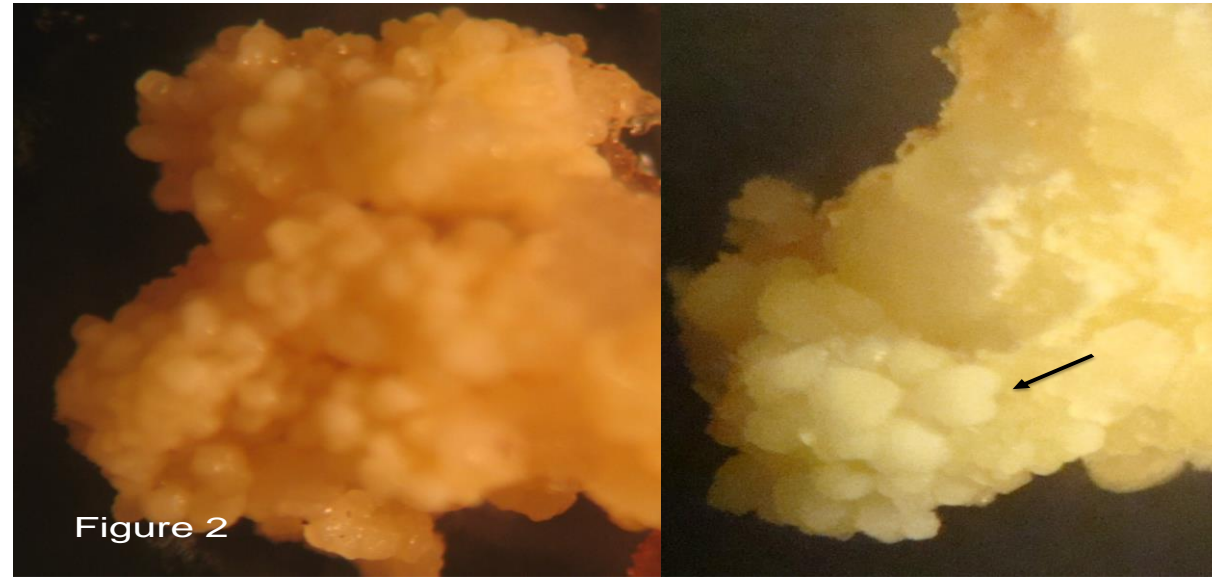
The regenerated plantlets were multiplied by continuous sub-culturing in the regeneration medium and were allowed to elongate up to 5-7 inches in length.



# Tissue Culture Observations

- 1. Callus induction after gamma radiation doses**
- 2 . After one month callus weight (g)**
- 3 . Proliferation of callus (g)**
- 4 . Regeneration of plantlets**
- 5 . Number of chlorophyll mutants**
- 6 . Number of plantlets**
- 7 . Root induction**

# *In Vitro Culture in Sugarcane*



**Table. 1. Effect of different gamma radiation doses on callus induction in sugarcane genotypes**

<b>Gamma radiation doses</b>	<b>Callus induction after gamma radiation doses</b>	<b>After one month callus weight bottle<sup>-1</sup> (g)</b>	<b>Callus proliferation</b>
<b>NIASGS- 571</b>			
<b>Control</b>	1.89 cd	1.16 de	2.26 d
<b>10Gy</b>	2.00 b	1.25 b	2.37 c
<b>20Gy</b>	1.94 bc	1.38 a	2.66 a
<b>30Gy</b>	1.64 g	1.18 cd	2.24 de
<b>40Gy</b>	1.48 hi	1.07 f-h	2.11 g
<b>NIA-0819</b>			
<b>Control</b>	1.72 f	1.06 f-h	2.13 fg
<b>10Gy</b>	1.89 cd	1.09 e-g	2.23 de
<b>20Gy</b>	2.11a	1.23 bc	2.49 b
<b>30Gy</b>	1.50 h	1.10 ef	2.21 de
<b>40Gy</b>	1.41 ij	1.11 ij	2.01 hi
<b>NIA- 2143</b>			
<b>Control</b>	1.63 g	0.97 ij	2.08 gh
<b>10Gy</b>	1.80 e	1.03 g-i	2.18 ef
<b>20Gy</b>	1.86 de	1.15 de	2.43 bc
<b>30Gy</b>	1.40 j	1.02 hi	2.13 fg
<b>40Gy</b>	1.32 k	0.94 j	1.95 i

**Table. 2 Effect of different gamma radiation doses on plant regeneration, chlorophyll mutants, and number of plantlets**

Gamma radiation doses	Plant regeneration	Chlorophyll mutants	Number of plantlets
<b>NIASGS- 571</b>			
Control	220 cd	10.00 fg	121 d
10Gy	300 b	14.60 cd	129 b
20Gy	320 a	16.20 c	134. a
30Gy	180 d-f	19.60 b	118 ef
40Gy	100 g-i	22.80 a	113 gh
<b>NIA- 0819</b>			
Control	140 e-h	7.00 h	114 g
10Gy	220 cd	8.20 gh	120 de
20Gy	280 c	11.20 ef	124. c
30Gy	160 d-g	13.00 de	112 gh
40Gy	80 hi	15.80 c	111 h
<b>NIA-2143</b>			
Control	80 hi	11.60	117 f
10Gy	140 e-h	6.60 h	124 c
20Gy	200 de	8.20 gh	129 b
30Gy	120 f-h	10.00 fg	114.g
40Gy	40 i	5.80 h	109 i

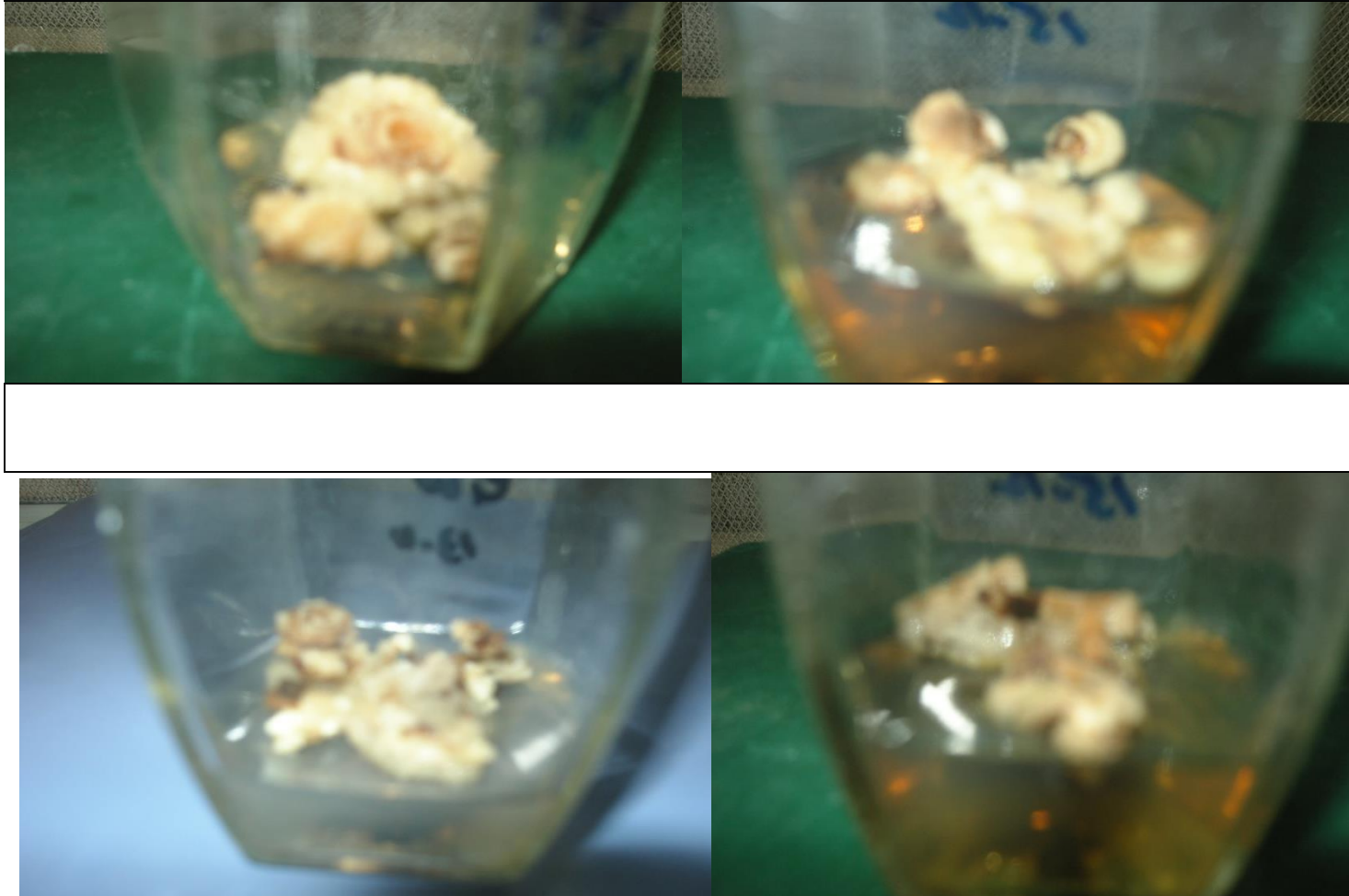
## Effect of different concentrations of indole-3-butyric acid on root induction in sugarcane

Concentrations	NIASGS-571	NIA-0819	NIA-2143
$\frac{1}{2}$ MS	+	+	-
$\frac{1}{2}$ MS + 2.00 IBA mg L <sup>-1</sup> + 4 % sucrose	++++	+++	++
$\frac{1}{2}$ MS + 1.00 IBA mg L <sup>-1</sup> + 6 % sucrose	++	++	+

-No root, + weak root, ++ better root, +++ good rooting, ++++ excellent rooting

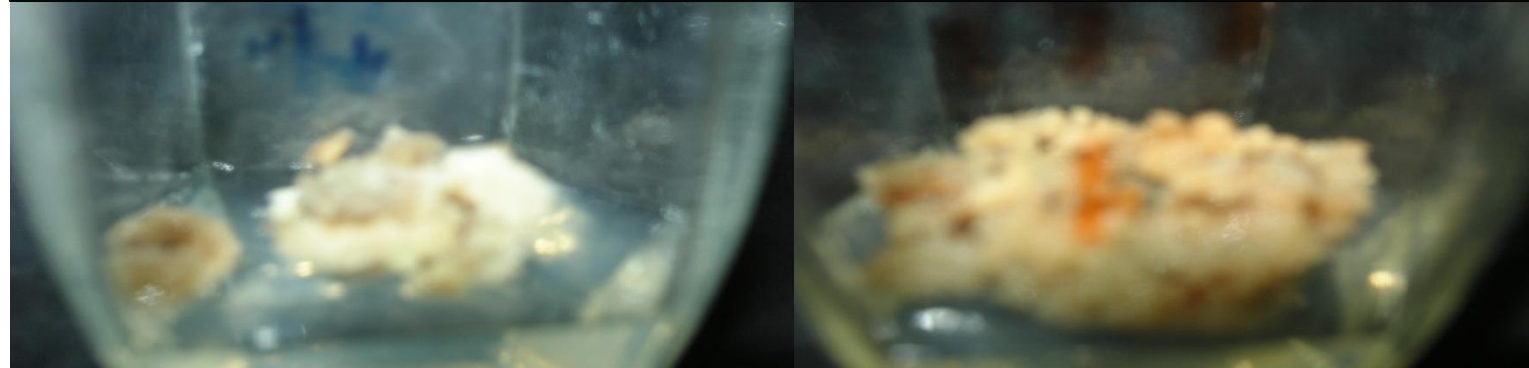


# Callus before irradiation



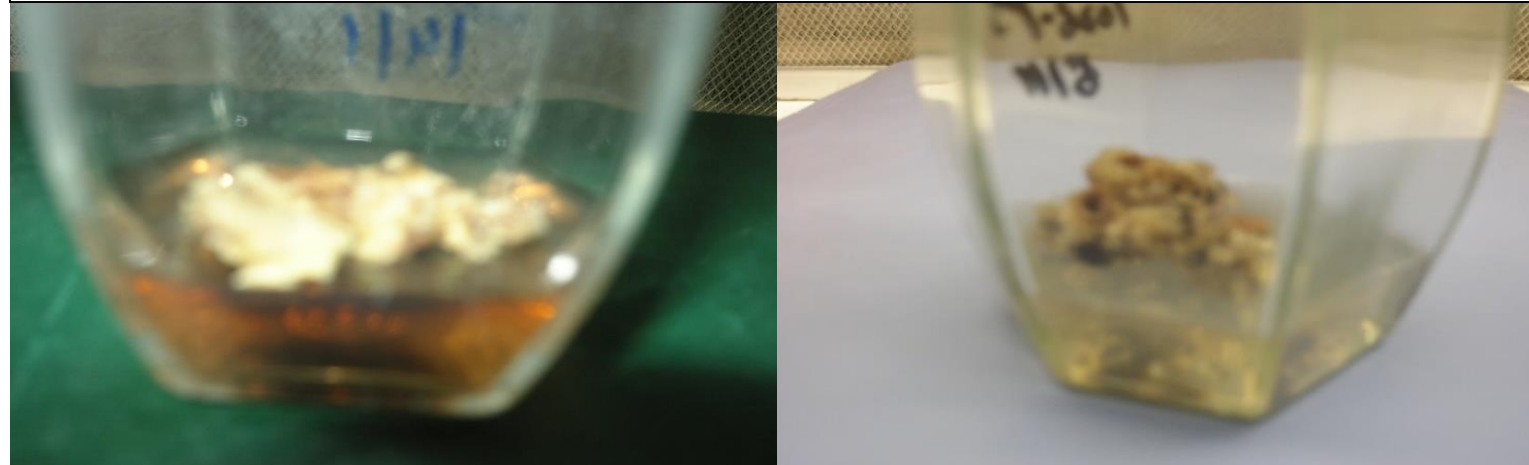
**Callus (before irradiation): Dry nodular compact and highly regeneration callus**

# Callus after one month of irradiation



10GY

20 GY



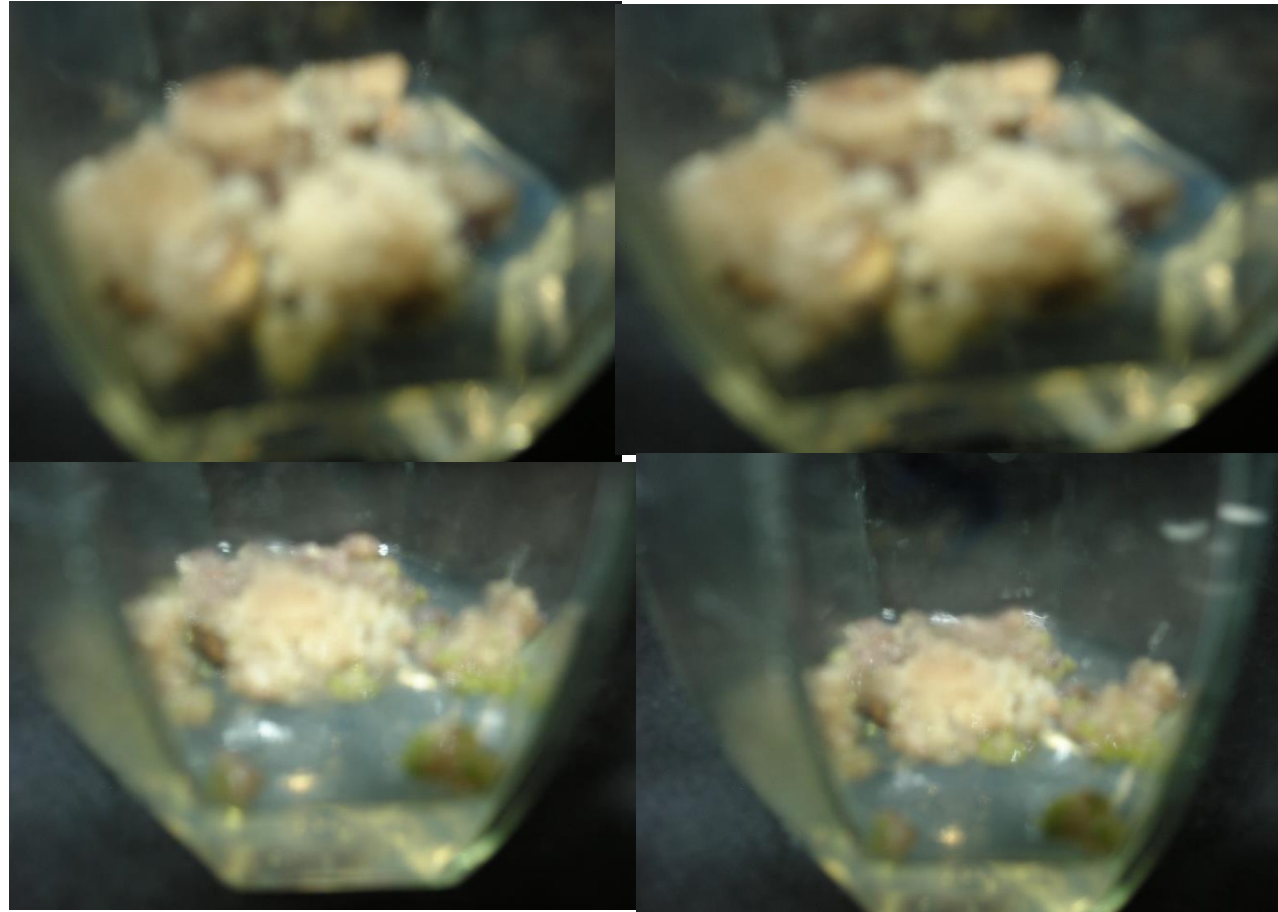
30GY

40 GY

**Morphologically well developed yellowish white type of callus, compact and dry nodular, capable of plant regeneration.**

**A friable non –regeneration callus globular non compact after one month gamma irradiation.**

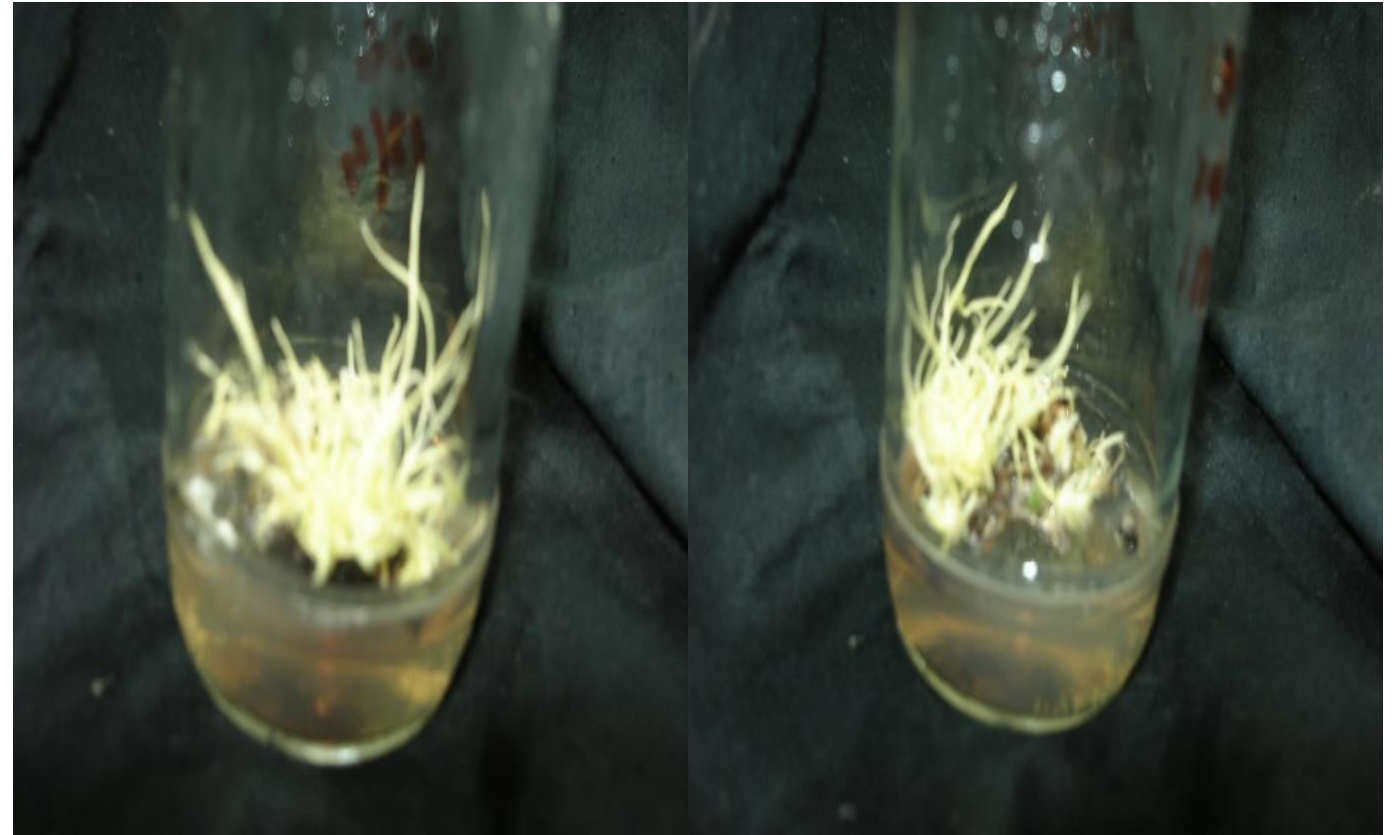
## Callus proliferation after irradiation



**After 6 weeks of the irradiated callus proliferation under 10, 20, 30, and 40 Gy of gamma radiation**

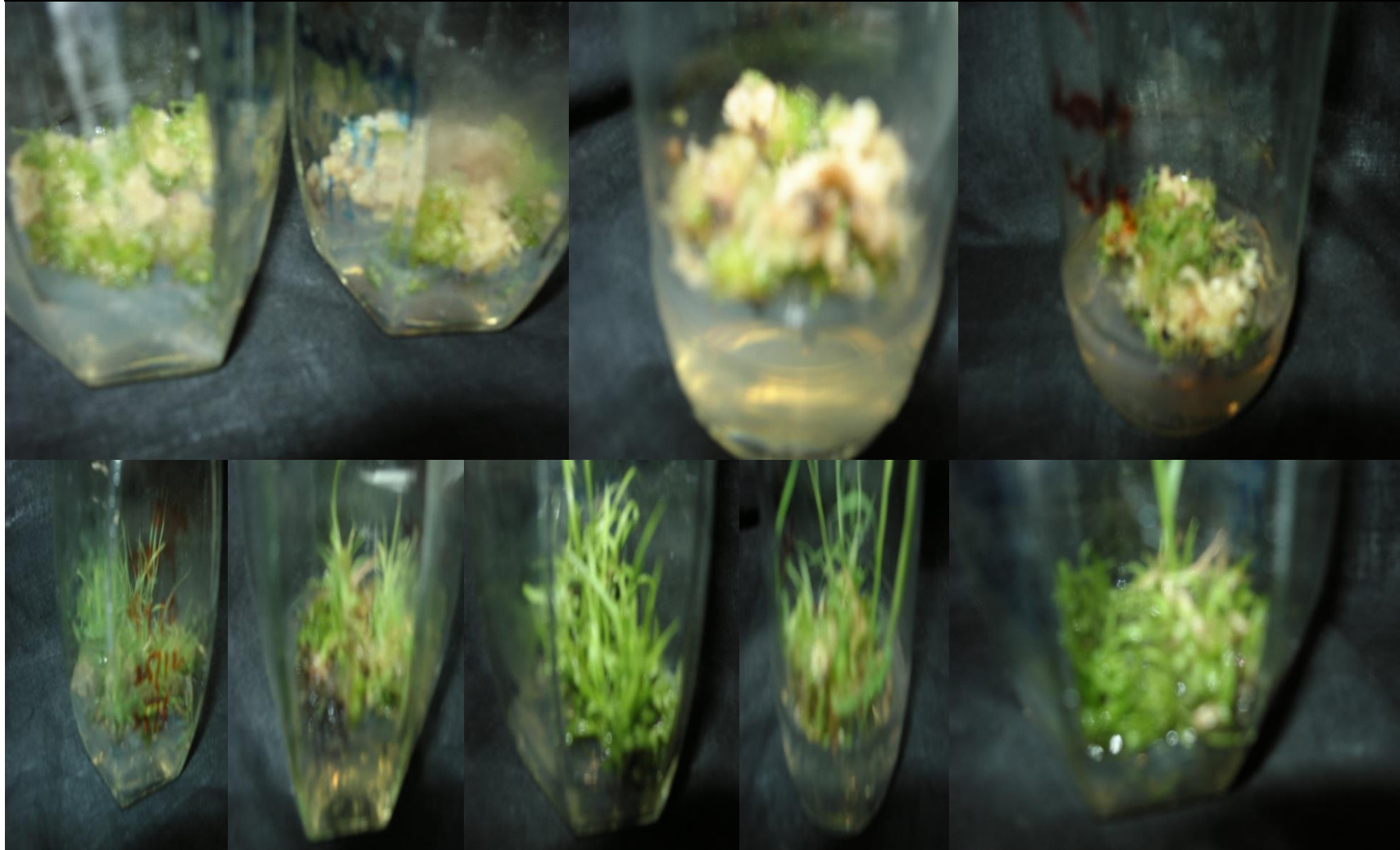


# Chlorophyll mutants



**Chlorophyll variants.**

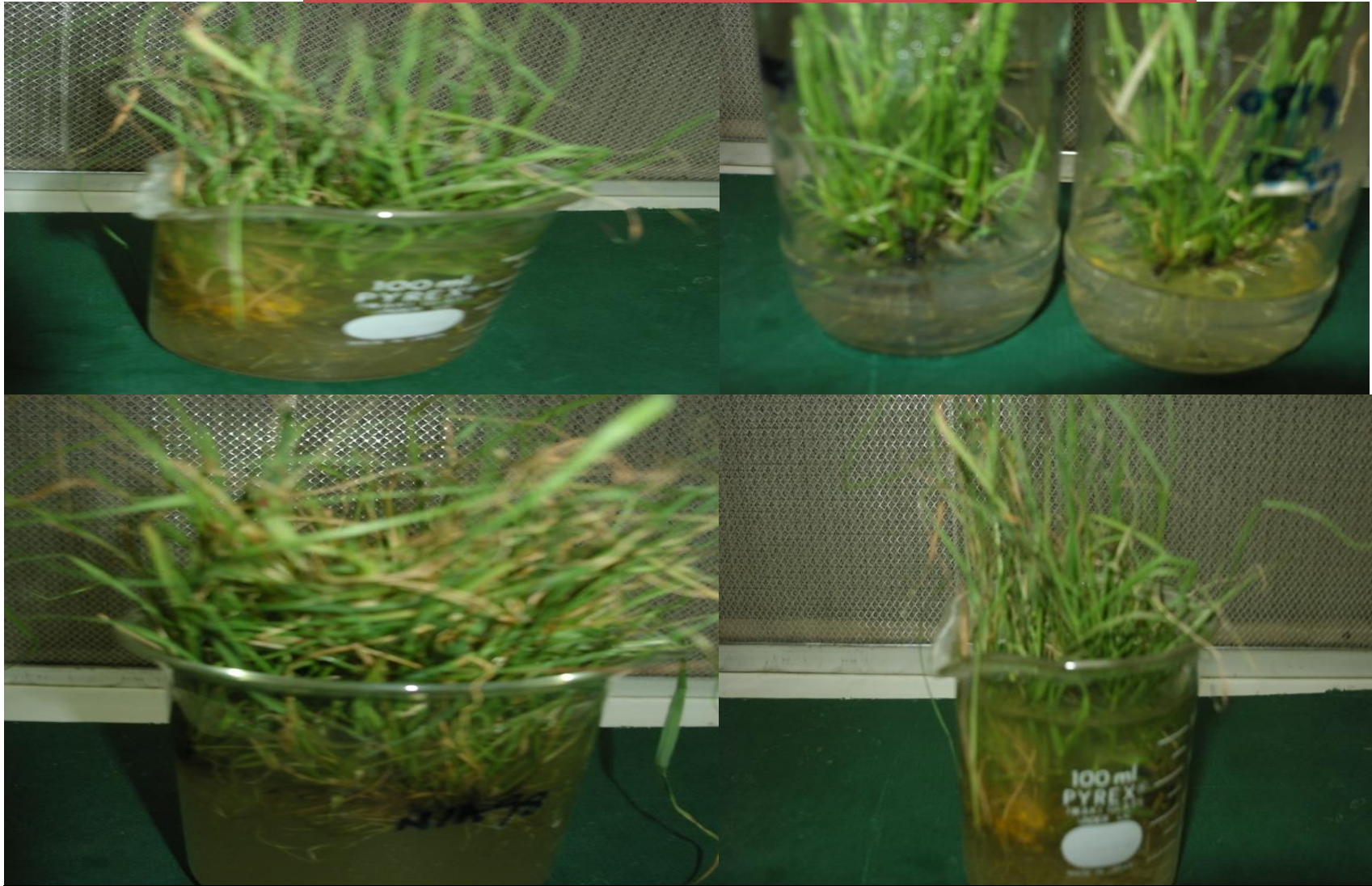
# Regeneration



Regeneration from irradiated callus after 12 weeks of growth in 5<sup>th</sup> subculture on MS medium containing 2.00 mg L<sup>-1</sup> IBA + 2.00 mg L<sup>-1</sup> IAA + 2.00 mg L<sup>-1</sup> Kin



# Rooting plantlets



**Root formation on MS medium containing 2.00 mg L<sup>-1</sup> IBA. The plantlets with well developed shoots and roots.**



# Plantlets in the earthen pots

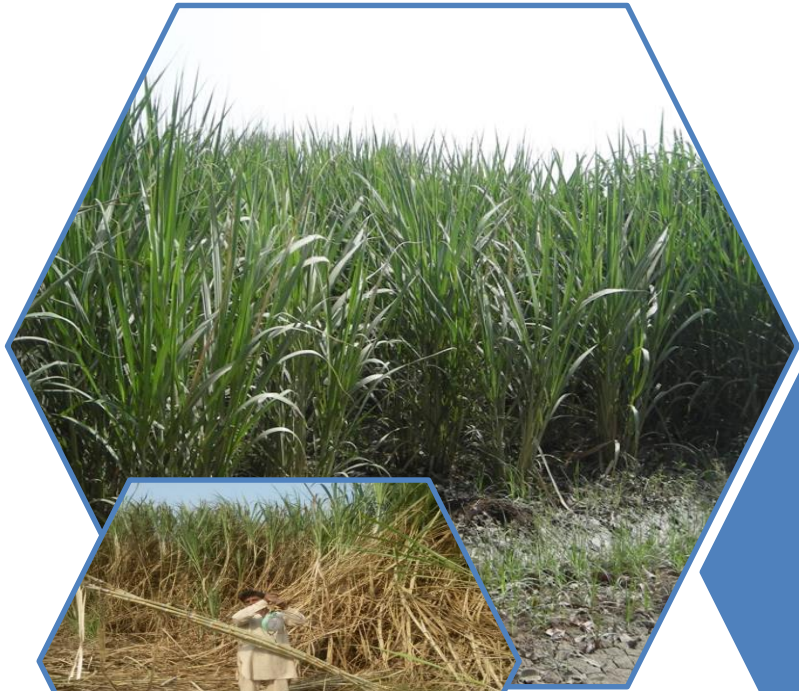




# Transplantation in the field







# Field Evaluation

# Field Evaluation

- Randomized complete block design with three replications.
- Row to row and plant to plant distance: 1 m.
- Different quality related and quantitative traits recorded in November to February.
- Quantitative observations included plant height, number of tillers, stool weight, cane diameter, number and length of internodes, and cane yield.
- The quality-related parameters included Brix, sucrose and sugar yield.



Field



Transplanted plantlets in the field



**TABLE 3. Effect of different gamma radiation doses on quantitative parameters through *in vitro* mutagenesis in sugarcane**

Varieties	Gamma radiation doses (Gy)	Plant height (cm)	Number of tiller plant <sup>-1</sup>	Stool weight (kg)
NIASGS-571	0	319.83d	9.00 b	9.00 a-c
	10	337.50b	9ab	9.63 b
	20	323.00c	9ab	10.00ab
	30	317.00d	8b	8.66 bc
	40	299.00e	9bc	9.13 a-c
NIA-0819	0	324.00c	9.00 b	9.00 a-c
	10	290.83e	9.50 ab	9.50 ab
	20	364.33a	10.16 a	11.46 a
	30	247.83g	8.00 cd	8.00 cd
	40	237.50h	8.00 cd	8.00 cd
NIA-2143	0	247.50g	6.66 e	6.66 e
	10	246.83g	7.00 de	7.00 de
	20	299.50f	6.33 de	7.33 de
	30	245.17h	5.33 e	6.33 e
	40	223.00i	5.00 f	5.00 f

**TABLE . 4. Effect of different gamma radiation doses on quantitative parameters through *in vitro* mutagenesis in sugarcane**

Varieties	Gamma radiation doses (Gy)	Cane diameter (cm)	Number of internodes	Length of internodes (cm)
NIASGS-571	0	2.03 d	25.66 b-d	18.33 cd
	10	2.30 ab	28.33ab	21.00 a
	20	2.46 a	27.33 a-c	20.00 ab
	30	2.10 cd	29.00 ab	19.66 a-c
	40	2.10 cd	25.66 b-d	19.00 bc
NIA-0819	0	2.16 b-d	24.33 c-e	17.00 de
	10	2.26 a-d	28.33 ab	21.00 a
	20	2.36 a-c	29.66 a	21.00 a
	30	2.13 b-d	26.00 b-d	19.36 a-c
	40	2.36 a-c	24.00 c-e	18.70 bc
NIA-2143	0	2.13a- d	20.33 f	14.00 gh
	10	2.30 b-d	22.00 ef	16.00 ef
	20	2.03 d	22.00 ef	15.00 fg
	30	2.40 ab	22.00 ef	13.33 h

**Table 5. Effect of different gamma radiation doses on quality-related characters in sugarcane**

Varieties	Gamma radiation doses (Gy)	Brix (%)	Sucrose (%)	Sugar yield (t ha <sup>-1</sup> )	Cane yield (t ha <sup>-1</sup> )
NIASGS-571	0	16.83g	11.41 b-d	6.10b-d	90.00 a-c
	10	16.25 g	12.76 ab	7.42 b	100.00a
	20	18.18d	11.13b-d	7.53 ab	96.33ab
	30	16.60fg	12.70bc	7.28 ab	91.33 a-c
	40	17.78cf	11.94 b-d	6.34 a-c	80.67 bc
NIA-0819	0	16.08 g	12.00 b-d	6.73 a-c	90.00 a-c
	10	17.20 c-f	10.84 d	6.56 a-c	95.00 ab
	20	16.78 g	12.42 bc	7.21 ab	101.67 a
	30	15.86 g	11.39 b-d	5.15 c-d	80.00cd
	40	16.60fg	11.04 cd	6.41 a-c	70.00 d
NIA-2143	0	18.07 e	11.38 b-d	5.22 ef	66.67 e
	10	18.61 c	12.34 b-d	6.67 c-e	70.00 de
	20	18.09 e	12.46 b-d	6.34 d-f	73.33 de
	30	19.61 a	14.43 a	8.42 a	63.33 e
	40	19.32 ab	12.54 a-c	6.63 f	56.00 f

# Conclusion

- **Maximum callus induction was observed in NIASGS-571 at 20Gy and 10Gy, and minimum callus was recorded in NIA-2143 at 40Gy.**
- **The maximum plantlets were regenerated at 20Gy, and minimum number of plantlets regenerated in NIA-2143 40Gy.**
- **The frequency of chlorophyll variants was highest in NIASGS-571 and NIA-2143 at 40Gy.**
- **Maximum rooting was observed in NIASGS-571, followed by NIA- 0819 on media containing MS<sup>1/2</sup> + 2.00 mg l<sup>-1</sup> IBA + 4 % sucrose.**
- **The induced mutagenesis of sugarcane genotypes in the study resulted in significant differences among the mutants.**
- **20Gy treated plants of NIA-0819 produced highest cane yield, whereas NIA-2143 (30Gy) recorded the highest sugar yields.**
- **The gamma radiation doses of 30Gy and 40Gy showed negative effect on the cane yield in all varieties.**
- **As sugarcane is a vegetatively propagated crop and has ratooning ability, the agronomic data indicates that *in vitro* mutagenesis can be employed for sugarcane improvement.**



**THANKS**