INT. J. BIOL. BIOTECH., 22 (1): 63-68, 2025.

STUDYING VARIABILITY OF SUGARCANE THROUGH *IN VITRO* MUTAGENESIS

Bilqees Magsi, Shahla Karim Baloch*, Muharram Ali Qambrani, Zohra Fatima Memon, Allah Jurio Khaskheli, Sayyed Zakir Hussain Shah, Amaar Meghwar, Mohsin Khaskheli and Ali Ahmed Bhatti

Department of Biotechnology, Sindh Agriculture University, Tandojam, Pakistan
***Corresponding author's email:** balochshahla21@gmail.com

ABSTRACT

The present study was performed to investigate the variability in different varieties of sugarcane through in-vitro mutagenesis. Preliminary, it was observed that the relationships of varieties with dissimilar amounts found that the total callus weight (2.33) was found in variety NIA-2013 beneath Dicomba 2.00 mg/L concentrations and the least callus weight (0.87 g) in variety SPS-234 beneath 2, 4-D 2.00 mg/l concentrations. The results of different concentrations indicated that the highest number of shoots (43.07) was observed beneath the concentration of MS + IBA 2.00 mg/L + IAA 2.00 mg/L+ Kint 2.00 mg/L sucrose 20 g/L and the lowest number of shoots (33.00) were seen beneath the concentration of MS + TDZ 3.00 mg/L+ BAP 2.00 mg/L + Kint 2.00 mg/L sucrose 20 g/L. The results of varieties and different amounts indicated that the highest number of shoots were recorded (45.7) in NIA-2014 beneath the concentration of MS + IBA 2.00 mg/L + IAA 2.00 mg/L + Kint 2.00 mg/L sucrose 20 g/L. The minimum number were recorded (30.66) in variety SPS-234 under the concentration of MS + TDZ 3.00 mg/L + BAP 2.00 mg/L + Kint 2.00 mg/L sucrose 20 g/L. The results of various amounts indicated that a maximum number of roots (18. 99) were recorded beneath the amount of MS½ + IBA 1.00 mg/L^{+} sucrose 30 g/L and a minimum number of roots beneath the concentration of MS½ + NAA 2.00 mg/L + sucrose 20 g/L were (16.59) noted. The results of varieties and different amounts indicated that the maximum number of roots were achieved (19.98) in variety NIA-2014 beneath the concentration of MS½ + IBA 1.00 mg/L^{+} sucrose 30 g/L. The results of varieties and the different amounts indicated that a minimum number of roots were achieved (15.87) in variety SPS-234 beneath the concentration of MS½ + NAA 2.00 mg/L + sucrose 20 g/L.

Key-words: *in vitro*, callus, phytohormones, mutagenesis.

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is the most efficient crop of high economic value for bioethanol production and renewable energy. Due to its excellent biomass yield, perennial growth, and the buildup of fermentable sugars in cane of internodes. Sugarcane is grown successfully in hot plain areas, and because the cane maturation cycle lasts for 12 to 18 months, sugarcane growers cannot avoid annual drought seasons (Gentile *et al*., 2013). Nevertheless, plant breeding is the foundation of our advanced civilization. Only a small proportion of the world's approximately 200,000 plant species have survived the rigorous scrutiny of the domestication process due to increased human demand for superior characteristics and productivity (Chrispeels and Sadava, 2003).

Despite the importance of sugarcane, conventional seed multiplication and propagation through cuttings is slow, poses pest and disease transmission risk s, and takes years before a new cultivar is released and commercially cultivated. Furthermore, the time spent is regarded as an economic loss, necessitating a significant expenditure in upgrading elite propagative planting materials from the few accessible parent stocks. Each abiotic stress has a significant detrimental impact on the tillering and main growth phases, resulting in a significant decrease in annual cane production and sugar content (Reis *et al*., 2014).

In vitro procedures, which are part of biotechnology, provide a lot of benefits for mutant breeding. They may hasten mutation breeding by increasing somaclonal variety and clonal replication of beneficial mutants. Tissue culture techniques with mutation induction can be utilised to speed up or improve the efficiency of breeding programmes in order to obtain new germplasm diversity (Jain, 2010). Physical means such as gamma rays and ultraviolet radiations can cause random genetic alterations. Chemical mutagens can also be used in tandem with high-activity growth regulators like 2,4-D to induce mutations. Chemical mutagens are the most effective and potent mutagens, causing a high frequency of gene mutations and a low frequency of chromosome abnormalities in plants. Plants tolerant to a range of stresses have been obtained by selecting somaclonal variations created with induced mutagenesis, followed by in vitro plant regeneration (Rai *et al*., 2011).

Furthermore mutagenesis to be positive, mutually a great output and effective stress assortment system that recognize potential genetically replicas exhibiting required phenotypes. Stress-tolerant clones can be acknowledged

invitro by the uses of chemicals like as glucose, (PEG), sucrose, and mannitol (Snyman *et al*., 2016). Substances used as osmotica in biological systems are normally inert, non-metabolisable, non-ionic, polymers that liquefy in water and transforms the osmotic potential of nutrient solutions in a precise manner (Blum, 2004). PEG like that, it is an inert non-ionic high molecular weight non-penetrating osmoticum that confirmed as water stress creating agent beneath *in vitro* conditions for various species (Wani *et al*., 2010). Huge molecular weight PEG (4000 to 10,000) drops the water potential of solutions not using cells or being phytotoxic (Hasson *et al*., 2004) (Masoabi *et al.,* 2017). So present studies objectives were too induce genetic variability through *in vitro* mutagenesis and mutation breeding in the genotypes, effect of phytohormones and Gamma irradiation on shoot elongation and effect of phytohormones on rooting.

MATERIALS AND METHODS

Collection of plant materials:

Explants were collected from 6 months of age fields produced plants from NIA Tando Jam's Experimental Farm three varieties (SPF-234, NIA-2013, and NIA-2014) were taken by extracting the leaf coat of sugarcane (*Saccharum officinarum* L.) immature meristems shoot tip by detaching the leaf sheath.

Surface Sterilization of explants:

All obtained explants were washed in consecutively tap water, then sterilized with 70 percent ethanol or a 100 percent sodium hypochlorite (NaOCl) solution, and then cleaned again completely with sterile distilled water in a laminar airflow chamber.

Preparation of nutrient media:

Murashige and Skoog's (1962) MS-basal medium comprising (macro, micro, and vitamin components) were employed with growth regulator supplementation in varying configurations. The pH of the medium was optimized to 5.8 before autoclaving for 20 minutes at 121°C.

Culture conditions:

Experiment specifies that the culture bottles be maintained in the growth/culture chamber at a temperature of 252 °C and for the specified photoperiod.

Composition of Callus induction media:

- M1 = MS + 2, 4-D 2.00 mg/L + Sugar 20 g/L
- M2 = MS + Piclogarm 2.00 mg/L + Sugar 20 g/L
- M3 = MS + Dicomba 2.00 mg/L + Sugar 20 g/L

Configuration of shoot induction media:

- SI = MS + IBA 2.00 mg/L + IAA 2.00 mg/L + Kint 2.00 mg/L + sucrose 20 g/L
- SII= MS + TDZ 3.00 mg/L + BAP 2.00 mg/L + Kint 2.00 mg/L + sucrose 20 g/L

Composition of root induction media:

- RI= MS½ + NAA 2.00 mg/L + sucrose 20 g/L
- RII= MS½ + IBA 1.00 mg/L + sucrose 30 g/L

Experimental design:

Completely Randomized Development (CRD) was used to design the experimental technique. Sugarcane growing was done using the shoot tip standard methods. Murashige and Skooge (MS, 1962) basal medium was used to cultivate the sterilized explants.

Statistical assessment:

The experimental data were analyzed over software Statistix (SWX), Version 8.1 (Analytical Software 2005). An Additional smallest substantial variance (LSD) test useful was to experiment with the level of significance amongst dissimilar means (Gomez and Gomez, 1984).

RESULTS

Callus weight (g) after one month:

Statistical assessment of variance showed that callus weight after one month for different varieties, various amounts with interactions were highly important at 5 percent probability level; accessible in Table 1, Figurers 1 to 4. Outcomes of varieties for the highest callus mass were detected (1.51 g) in variety NIA-2014, followed by (1.50 g) in variety NIA-2013, and the lowest weight of callus was achieved (1.47 g) in variety SPS-234. The outcomes of the diverse amounts showed that the highest callus weight was accomplished (2.31 g) with the concentration of MS + Dicomba 2.00 mg/L + Sugar 20 g/L, followed by (1.26 g) with Piclogarm 2.00 mg/L and the lowest callus weight was observed (0.91 g) beneath the amount of 2, 4-D 2.00 mg/L. The interactions of varieties with dissimilar amounts directed that the highest callus weight was analyzed (2.33) in variety NIA-2013 beneath the amount of Dicomba 2.00mg/L, followed by (2.29 g) in variety NIA-2014 with the amount of Dicomba 2.00 mg/l and the lowest callus weight was noted (0.87 g) in variety SPS-234 beneath the amount of 2, 4-D 2.00 mg/L.

Table 1. Effect of different phytohormones on callus weight after one month in sugarcane varieties.

Media Concentrations	**Varieties**			**Mean**
	SPS-234	**NIA-2013**	**NIA-2014**	
M1	0.87	0.89	0.98	**0.91 c**
M2	1.24	1.29	1.27	**1.26 b**
M3	2.31	2.33	2.29	**2.31 a**
Mean	**1.47**	**1.50**	**1.51**	

Grand Mean = 1.4967 **LSD (5%) = 0.0771**
CV= 2.58 **SD= 0.629782**

- M1 = MS + 2, 4-D 2.00 mg/L + Sugar 20 g/L
- M2 = MS + Piclogarm 2.00 mg/L + Sugar 20 g/L
- M3 = MS+ Dicomba 2.00 mg/L + Sugar20 g/L

CV= Cumulative Variance
LSD (0.05) = Least Significant Differences of means.
SD= Standard Deviation

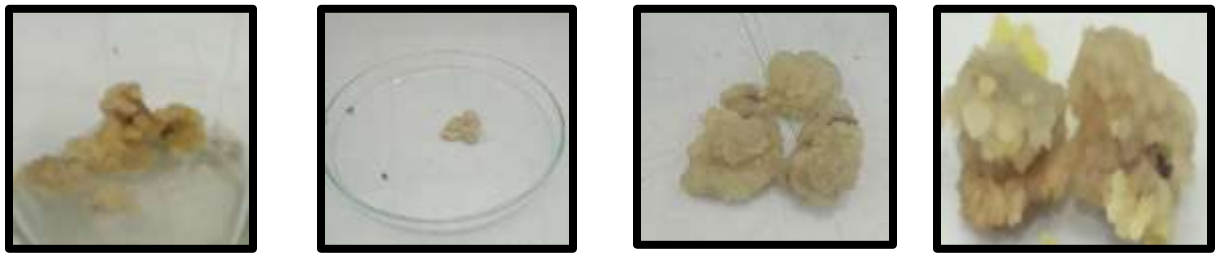

Plate: Effects of different phytohormones on callus formation in sugarcane varieties. 1. Callus formation in SPS-234. 2. Callus in the petri plate. 3. Callus formation in NIA-2013. 4. Callus formation in NIA-2014.

.

Total number of shoot bottles

The statistical assessment of modification specified that varieties concentration were highly significant, while their interactions were non-significant at 5 % possibility level, and data are obtainable in Table 2, Plate from 5 to 7. The results of varieties indicated that the highest number of shoots were recorded (40.85) in variety NIA-2014 and the lowest number of shoots was observed (35.49) in variety SPS-234. The results of different concentrations indicated that the highest number of shoots (43.07) was detected beneath the concentrations of MS + IBA 2.00 mg/L + IAA 2.00 mg/L + Kint 2.00 mg/L sucrose 20 g/L and the lowest number of shoots (33.00) were seen beneath the concentration of MS + TDZ 3.00 mg/L + BAP 2.00 mg/L + Kint 2.00 mg/L sucrose 20 g/L. The results of varieties and different amounts indicated that the highest number of shoots were recorded (45.7) in NIA-2014 beneath the concentration of MS + IBA 2.00 mg/L + IAA 2.00 mg/L+ Kint 2.00 mg/L sucrose 20 g/L and the minimum number

of shoots were accomplished (30.66) in variety SPS-234 beneath the concentration of MS + TDZ 3.00 mg/L + BAP 2.00 mg/L+ Kint 2.00 mg/L sucrose 20 g/L.

Plate: Effects of various phytohormones on number of shoots in sugarcane varieties. 4. Number of shoots in SPS-234. 6 Number of shoots in NIA-2013. 7. Number of shoots in NIA-2014.

Table 2. Effects of distinct phytohormones on total number of shoot bottles in sugarcane varieties.

Concentrations	**Varieties**			**Mean**
	SPS-234	**NIA-2013**	**NIA-2014**	
SI	40.33	43.20	45.70	**43.07 a**
SII	30.66	32.33	36.01	**33.00 b**
Mean	**35.49**	**37.76**	**40.85**	
Grand Mean = 7.3933			**LSD (5%) = 6.1486**	
CV= 5.69			**SD= 6.02884207**	

- **SI = MS+ IBA 2.00mg/l+ IAA 2.00mg/l+ Kint 2.00mg/lsucrose20g/l,**
- **SII= MS+ TDZ 3.00mg/l+ BAP 2.00mg/l+ Kint 2.00 mg/l sucrose 20 g/l**

CV= Cumulative Variance; LSD (0.05) = Least Significant Differences of means; SD= Standard Deviation

Total number of roots bottles:

The statistical assessment of adjustment directed that varieties, amounts were highly significant, and their interactions were significant at 5% probability level the data are presented Table 3, F. The results of varieties indicated maximum number of roots were recorded (18.82) in variety NIA-2014. The results of various amounts indicated that a maximum number of roots (18.99) were recorded beneath the amount of MS½ + IBA 1.00mg/l^{+} sucrose 30g/l and minimum number of roots at par (16.59) were recorded beneath the concentration of MS½ + NAA 2.00 mg/L+ sucrose 20g/L. The results of varieties and different amounts indicatedthat a maximum number of roots were achieved (19.98) in variety NIA-2014 beneath the concentration of MS½ + IBA 1.00 mg/L^{+} sucrose 30 g/L. The lowest number of roots were recorded (15.87) in variety SPS-234 beneath the amount of MS½ + NAA 2.00 mg/L + sucrose 20 g/L.

Table 3. Effects of different plant hormones on the total number of root bottles in sugarcane varieties.

Concentrations	**Varieties**			**Mean**
	SPS-234	**NIA-2013**	**NIA-2014**	
RI	15.87	16.23	17.67	**16.59 b**
RII	18.33	18.67	19.98	**18.99 a**
Mean	**17.10**	**17.45**	**18.82**	
Grand Mean = 17.792			**LSD (5%) = 2.0692**	
CV= 5.13			**SD= 1.548966**	

- RI= MS½ + NAA 2.00 mg/l + sucrose 20 g/l
- RII= MS½ + IBA 1.00 mg/l + sucrose 30 g/l

CV= Cumulative Variance; LSD (0.05) = Least Significant Differences of means; SD= Standard Deviation.

DISCUSSION

In this study, sugarcane cultivars NIA-2014, NIA-2013, and SPS-234 were used to determine the optimal concentration and mix of plant growth hormones, as well as their performance for in-vitro mutagenesis and callus culture. *In vitro* mutations are frequently the most reliable advancement for the effective use of various biotechnological techniques for product enhancement. There are various data available on sugarcane recovery and rapid augmentation by callus culture.

The results of varieties for highest callus mass were detected The outcomes of diverse amounts showed that the highest callus weight was accomplished (2.31 g) with the concentration of MS + Dicomba 2.00 mg/l+ Sugar 20 g/L. The relationships of varieties with dissimilar amounts found that the total callus weight (2.33) was found in variety NIA-2013 beneath Dicomba 2.00 mg/L concentrations, and the least callus weight (0.87 g) in variety SPS-234 beneath 2, 4-D 2.00 mg/L concentrations. According to current research, several sugarcane varieties are more susceptible to somaclonal variation than others, and in vitro instability is likely a result of the genotype-culture medium interaction (Zucchi *et al*. 2002). The results of different concentrations indicated that the highest number of shoots (43.07) was observed beneath the concentration of MS + IBA 2.00 mg/L+ IAA 2.00 mg/L+ Kint 2.00 mg/L sucrose 20 g/l and the lowest number of shoots (33.00) were seen beneath the concentration of MS + TDZ 3.00 mg/L+ BAP 2.00 mg/L+ Kint 2.00 mg/L sucrose 20g/L. The results of varieties and different amount directed that highest number of shoots were recorded (45.7) in NIA-2014 beneath the meditation of MS + IBA 2.00 mg/L+ IAA 2.00 mg/L + Kint 2.00 mg/L sucrose 20g/L and minimum number of shoots were accomplished (30.66) in variety SPS-234 beneath the meditation of MS + TDZ 3.00mg/L + BAP 2.00mg/L + Kint 2.00mg/lsucrose 20g/L.

However, this effect in sugarcane may be overridden by the genetic redundancy of having more than two copies of the genome, and therefore variability is not expressed phenotypically (Patade and Suprasanna, 2009). The acclimatization of sugarcane grown in vitro is often successful. Upwards of 85% of plants produced from somatic embryos survived being transferred to a greenhouse (Snyman *et al*. 2009). The results of varieties indicated maximum number of roots were recorded (18.82) in variety NIA-2014. The results of various amount indicated that maximum number of roots (18.99) were recorded beneath the amount of MS½ + IBA 1.00mg/L + sucrose 30g/L and minimum number of roots at par (16.59) were recorded beneath the concentration of MS½ + NAA 2.00mg/L + sucrose 20g/L. The results of varieties and different amount indicated that maximum number of roots were achieved (19.98) in variety NIA-2014 beneath the concentration of MS½ + IBA 1.00mg/L + sucrose 30g/L. The lowest numbers of roots were recorded (15.87) in variety SPS-234 beneath the amount of MS½ + NAA 2.00mg/l+ sucrose20g/l. The potential of in vitro culture techniques to swiftly generate vast numbers of plants has been harnessed for commercial purposes in the sugar industry around the world. When an epidemic of orange rust occurred in Australia in 2000, the in vitro SmartsettTM sugarcane propagation technique was used to substitute disease-resistant cultivars (Snyman *et al*. 2009).

REFERENCES

Blum, A. (2004). *Use of PEG to induce and control plant water deficit in experimental hydroponics culture*. http://www.plantstress.com/methods/PEG.htm. Accessed March 2015.

Chawade, A., P. Sikora and M. Br¨autigametal (2010). Development and characterization of an oat Tilling-population and identification of mutations in lignin and beta-glucan biosynthesis genes. *BMC Plant Bio*., 10 (86): 00-00.

Chrispeels, M.J. and D. E. Sadava (2003). *Plants, genes, and crop biotechnology*. Jones and Bartlett publishers.

Gentile, A., T. H. Ferreira, R. S. Mattos, L. I. Dias, A. A. Hoshino, M. S. Carneiro, G. M. Souza, T. Calsa, M. Nogueira and L. Endres (2013). Effects of drought on the microtranscriptome of field-grown sugarcane plants. *Planta*, 237: 783–798.

Hasson, N.S., L.D. Shaaban, A.H. El-Sayed and E.E. Seleem (2004). In vitro selection for water stress tolerant callus line of Heliathus annus L Cv Myak. *International Journal of Agricultural Biology*, 6: 13–18.

Jain, S. M. (2010). Mutagenesis in crop improvement under the climate change. *Romanian Biotechnol Lett*., 15(2): 88-106.

Masoabi, M., J. Lloyd, J. Kossmann and C. van der Vyver (2017). Ethyl Methanesulfonate Mutagenesis and In Vitro Polyethylene Glycol Selection for Drought Tolerance in Sugarcane (*Saccharum* spp.). DOI 10.1007/s12355-017-0524-8. ISSN 0972-1525.

Mahfouz M. M., A. Piatek and C. J. Stewart. (2014). Genome engineering via TALENs and CRISPR/Cas9 systems: challenges and perspectives. *Plant Biotechnol J*., 12: 1006–1014. https ://doi.org/10.1111/ pbi.12256

Patade, V. Y., and P. Suprasanna (2009). An *in vitro* radiation induced mutagenesis-selection system for salinity tolerance in sugarcane. *Sugar Tech.*, 11(3): 246-251.

Rai M. K., R. K. Kalia., R. Singh, M. P. Gangola and A. K. Dhawan (2011) Developing stress tolerant plants through in vitro selection—an overview of the recent progress. *Environ Exp Biol*., 71: 89–98.

Reis, R.R., A. D. B. B. Cunha, P. K. Martins, M. T. B. Martins, J. C. Alekcevetch, A. Ô. Chalfun-Júnior, A. C. Andrade, A. P. Ribeiro, F. Qin and J. Mizoi (2014). Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. *Plant Sci*., 221–222, 59–68.

Snyman S. J., G. M. Meyer, M. Banasiak, T. L. Nicholson, V. T. Antwerpen, P. Naidoo and J. D. Erasmus (2009). Micropropagation of sugarcane via NovaCane®: preliminary steps in commercial application. *Sugar Cane Int*., 27(6): 245–247;

Snyman, S.J., P. Mhlanga and M.P. Watt (2016). Rapid screening of sugarcane plantlets for *in vitro* mannitol-induced stress. *Sugar Tech*., 18: 437–440.

Wani, S.H., P.A. Sofi, S.S. Gosal and N.B. Singh (2010). In vitro screening of rice (Oryza sativa L.) callus for drought tolerance. *Communications in Biometry and Crop Science*, 5: 108–115.

Zhu G. Y., J. M. Kinet and S. Lutts (2004). Characterization of rice (*Oryza sativa*) F3 populations selected for salt resistance and relationships between yield related parameters and physiological properties. *Aust. Jour. Exp. Agronomy*, 44: 333-342.

Zucchi M. I., H. Arizono, V. A. Morais, M. H. P. Fungaro and M. L. C. Vieira (2002). Genetic instability of sugarcane plants derived from meristem cultures. *Genet Mol Biol.*, 25(1): 91–96.

(*Accepted for publication November 2024*)