International Journal of Plant & Soil Science

34(22): 725-733, 2022; Article no.IJPSS.90845
ISSN: 2320-7035

Drought Stress in Sugarcane: *In-vitro* Mutagenesis and Selection of Polyethylene Glycol (PEG) Tolerant Callus Lines

Athira Aji [a#], R. Gnanam [b*¥], P. Meenakshisundaram [c‡], M. K. Kalarani [d†] and C. Appunu [eφ]

[a] Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[b] Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[c] Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[d] Department of Crop Management, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India.
[e] Division of Crop Improvement Sugarcane Breeding Institute, (ICAR), Coimbatore, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i2231428

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/90845

Original Research Article

Received 06 June 2022
Accepted 12 August 2022
Published 17 August 2022

ABSTRACT

Drought is one of the key abiotic factors causing a loss in sugarcane output, which is one of the world's thirstiest crops. A mutant sugarcane callus line was used to create an *in vitro* selection system for drought resistance. The objective of this study was to obtain putative sugarcane mutant callus lines that had been chemically altered with sodium azide at various concentrations (0, 0.5, 1.0, and 1.5%). These lines were subsequently screened using high molecular weight PEG 6000

[#] M.Sc. Scholar;
[¥] Professor and Head;
[‡] Assistant Professor;
[†] Director;
[φ] Senior Scientist;
**Corresponding author: E-mail: gnanamtnau@gmail.com, gnanam.r@tnau.ac.in;*

as a selection agent at various concentrations (0, 5, 10, 15, 20, 25, and 30%). The experiment demonstrated a decline in the proportion of live callus and sensitivity index with increasing mutation concentration and PEG dose, with little to no live callus at the maximum mutation and PEG concentration. Biochemical investigation of the survived callus for proline accumulation and salicylic acid content revealed an elevated level of up to 20 percent PEG and 1.0 percent mutagen, followed by a quick drop as PEG and mutagen concentrations increased. The findings can be used to create drought-tolerant sugarcane lines and putative mutants, as well as to screen them *in vitro*.

Keywords: Sugarcane; callus; sodium azide; mutation; PEG; selection; proline; salicylic acid.

1. INTRODUCTION

India is one of the leading producers of sugarcane in the world, with a total area of more than 5 million hectares. Sugarcane (*Saccharum officinarum* L.) is a significant agricultural crop that is known for producing sugar and other by-products like ethanol throughout the world [1]. *S. officinarum* L. is a primitive wild species native to India that has spread widely in tropical and subtropical regions. The subtropical area of India comprises the states of Bihar, Haryana, Orissa, Punjab, Rajasthan, and Uttar Pradesh, and the tropical region, which includes the states of Andhra Pradesh, Gujarat, Karnataka, Kerala, and Tamil Nadu.

Sugarcane, a C_4 plant, has a one-year life cycle with four growth stages: germination, tillering, grand growth, and maturity. This suggests that it experiences all of the seasonal weather changes throughout the year [2]. At every stage of the crop's life cycle, abiotic stresses have the potential to hinder growth and development. Drought is a serious problem for sugarcane, affecting productivity primarily through morpho-physiological effects that restrict growth and photosynthesis and are to blame for reductions in biomass and cane yield (Zhao and Li, 2015). Because different sugarcane genotypes respond to drought stress differently, it is even more crucial to develop drought-tolerant genotypes.

Drought-tolerant sugarcane cultivars can be developed by traditional and novel approaches *viz.*, hybridization, mutation, *in vitro* breeding, genetic engineering, or a combination of the aforementioned goals [3,4,5]. Sugarcane presents difficulties for the conventional hybridization method due to its complex genomes, low fertility, and protracted selection cycle, but most importantly due to its high polyploidy level. Plant tissue culture is thought to be a quick and efficient approach to crop development [6]. Innovative cell culture uses callus culture to treat cultured cells as separate selection units rather than the complete plant. This method involves selecting tolerance cell lines from a dedifferentiated mass of cells (callus), subjecting the callus to appropriate selection pressure, and then regenerating tolerant plants.

Mutation induction can increase genetic diversity, and when combined with *in vitro* or *in vivo* selection, has resulted in new genotypes that are tolerant to drought, salt, aluminium, pests, and diseases in a variety of crops [7]. Introducing physical and chemical mutations in plants are two of the many approaches for producing mutants, with physical mutagens such as x-ray radiation or gamma rays and chemical mutagens such as sodium azide, colchicine, and EMS. Sodium azide was found to be one of the potent chemical mutagens which create point mutations [8]. In an experiment to determine sodium azide's impact on sugarcane callus, Mahmud et al. [9] observed that calli treated with sodium azide had higher plantlet regeneration. Callus cells are meristematic, making them more susceptible to radiation and mutagenic exposure than mature cells. It was viable to choose mutants during the in-vitro selection procedure by using particular selective agents. Similar research was done on sugarcane by Hartati et al. [10], who concluded that the right mutation dosage would produce mutants that could withstand drought.

The in-vitro selection of drought-tolerant cultivars uses an osmotic chemical as a selective agent that can mimic the effects of field drought. The most often utilized osmotic substance to alleviate drought stress is polyethylene glycol (PEG). PEG with a large molecular weight (6000–8000) has been used for a very long time as an inert, non–ionic, non–penetrating osmoticum to lower the water potential of nutritional solutions without being absorbed by or harmful to plants. Abbas et al*.* [11] employed PEG to produce drought stress and examined the biochemical properties of sugarcane, discovering that drought stress-induced alterations in sugarcane are reversible at the cellular level. The current study's goal is to identify sodium azide mutant callus lines that are

PEG tolerant, profile them based on proline and salicylic acid content, and compare them to non-mutated callus lines in the variety Co 86032.

2. MATERIALS AND METHODS

2.1 Callus Induction

Sugarcane variety, Co-86032 was utilized in the current study. The ICAR-Sugarcane Breeding Institute, Coimbatore experimental field provided the sugarcane leaf sheath explants, which were taken from field-grown sugarcane 8 to 10 months old. The explants were rinsed thoroughly under running tap water for 10 minutes, followed by Bavistin (Carbendazim 50% WP) 0.1% for 10 minutes, and then washed with sterile distilled water before being transported to a laminar airflow cabinet. The immature leaf sheath explants were first treated with ethanol (70%) for a minute, then with ice-cold mercuric chloride ($HgCl_2$, 0.1 percent (w/v)) for another 5 minutes, and finally thoroughly washed with ice-cold sterile water for 3 to 5 times. Murashige and Skoog's (1962) medium supplemented with 30 g/l sucrose, 8 g/l agar, 3 mg/L 2, 4-D, 100 mg Myo-inositol, and 10% coconut water was used for callus induction. After being adjusted to a pH of 5.8, the medium was autoclaved at 120°C and 15 lb pressure for 20 minutes. Cultures were kept at 26± 1°C with a 16/8hour light-dark cycle. The calli were again sub-cultured into the medium of the same composition after two weeks of development.

2.2 *In vitro* Chemical Mutagenesis

Mutation in general aims to broaden sugarcane's genetic diversity. The chemical mutagen sodium azide (NaN_3) will induce the preferential generation of point mutation of AT to GC. Calli obtained from the best 2, 4-D concentration (3 mg/L) were subjected to various concentrations of sodium azide mutagen (0, 0.5, 1.0 and 1.5 mg/L). The callus obtained were cultured on MS media supplemented with different concentrations of sodium azide for 5 days. After the mutation period, the mutated callus was transferred to MS media containing just 3 mg/L 2,4-D. Further, the callus was maintained by frequent and periodic sub-culture every 2 weeks. The observations recorded were the percentage of live callus and sensitivity index (SI) that has been worked out as follows:

> Sensitivity index (SI) = (Percentage of live callus on PEG media / Percentage of live callus on non-PEG media) X 100 % (eq.1)

2.3 *In vitro* Selection

In vitro selection was performed utilizing a drought-selecting chemical, PEG 6000, by culturing the mutated callus on MS media infused with PEG 6000. For the preparation of PEG-infused media, PEG at different concentrations (0, 5, 10, 15, 20, 25, and 30 w/v) was dissolved in sterile water and sterilized using a 0.22 µm microporous membrane [12] which was poured on MS medium. PEG-infused agar medium (50 mL) was poured into sterile bottles and kept for 2 days, allowing PEG-6000 to fully penetrate the solid medium after which the PEG was discarded and subsequently used for imparting drought stress. Twenty-five non-mutated and mutated calli were transferred to the bottles with different concentrations of PEG 6000 to initiate selection shock. This concentration of PEG was chosen based on the report that at 20 % PEG, the growth of the callus decreased considerably, and at 30% PEG, there was complete death of the callus occurred [13]. After subcultures for 40 to 45 days, most of the calli became dark brown as the concentration of both mutagen and PEG increased except few which remained light in color. Calli, which were actively growing at this stage, were considered as mutated PEG tolerant and further used for characterization. A biochemical examination of a 30-day-old mutant callus was conducted. Three replications were used for each experiment, and the average results were reported.

2.4 Estimation of Proline

Callus (500 mg) was homogenized in aqueous sulfosalicylic acid at a concentration of 3 percent (w/v) and centrifuged at 1,000 g for 10 min. The filtered homogenate was combined in equal parts with acetic acid and acid ninhydrin, and the reaction was carried out at 100°C for one hour before being stopped on an ice bath. Four ml of toluene was added to the reaction mixture and thoroughly stirred before extraction. After being removed from the aqueous phase, the toluene-containing chromophore was warmed to room temperature [14]. Using toluene as a blank, the absorbance of the proline-ninhydrin product was measured at 520 nm. The proline content was represented as μg^{-1} FW.

2.5 Estimation of Salicylic Acid (SA)

One gram of fresh callus was thoroughly mixed with ethanol to form a homogenate, and then the mixture was centrifuged at 10,000 g for ten

minutes. Then the supernatant was collected and placed on ice. For the measurement of the amount of SA present in the sample, the respective sample and standard solution were taken and each add 1% ferric chloride and sterile water to make the volume up to 10 ml. Finally, the absorbance of the violet-colored complex was measured using a UV-VIS spectrophotometer at 523 nm and SA content was represented as mg/10 ml.

2.6 Statistical Analysis

The experimental design used was a completely randomized design with the first factor as the concentration of PEG 6000 (0, 5, 10, 15, 20, 25, and 30%) and the second factor was the concentration of sodium azide (0, 0.5, 1.0, and 1.5%) Each treatment consisted of 3 replications, and each replicate consisted of 25 calluses. Data were analyzed using WASP 1.0 program. The values are mean ± SE for three samples in each group at a significance level of 1% and 5%.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Mutation and Callus Growth

Mutation with sodium azide in combination with PEG at varied concentrations has caused a significant effect on the growth and development of callus, which is indicated by the color changes. The damage that occurred in calli due to the mutagen sodium azide from 0.5 to 1.5% at varying levels of PEG concentrations was indicated by the decrease in the percentage of live callus, which is evident in Table 1. For PEG concentrations ranging from 0 to 25%, the proportion of living callus reduced from 96 to 12% at a 0.5% mutation dose, while it decreased from 94 to 6% for the same concentration of PEG. However, at 1.5% mutagen dosage, callus growth was only found at 5% PEG and there was no live callus at all other concentrations. Increases in mutation and PEG concentration cause changes in the percentage of live calluses. Both mutant and non-mutant callus lines suffer damage as a result of the PEG treatment.

Increased PEG dosage at the same mutation level produced more harm than an increase in mutation dose at the same PEG level, as demonstrated by the proportion of living callus.

The sensitivity index (SI) indicates the growth inhibition as a result of PEG-infused media. Non-mutated callus showed higher growth inhibition than the mutated callus lines as indicated in Table 2. The SI values for callus lines that had not undergone mutations ranged from 99.49% at 0% PEG to 34.51% at 25% PEG concentration. For 0.5% mutation, the SI value ranged from 97.46 to 12.18, while at 1.0% mutation, the range was 95.43 to 6.091%. However, observable live

Table 1. Effect of PEG and mutation at varying concentrations on the percentage of live callus

PEG Concentration(w/v)	Mutation percentage (%)			
	0	**0.5**	**1.0**	**1.5**
0	98	96	94	92
5	96	94	84	76
10	86	72	56	0
15	76	56	46	0
20	56	30	16	0
25	34	12	6	0
30	0	0	0	0

Table 2. Effect of PEG and mutation at varying concentrations on the sensitivity index of live callus

PEG Concentration (w/v)	Mutation percentage (%)			
	0	**0.5**	**1.0**	**1.5**
0	99.49	97.46	95.43	93.40
5	97.46	95.43	85.27	77.15
10	87.30	73.09	56.85	0
15	77.15	56.85	46.70	0
20	56.85	30.45	16.42	0
25	34.51	12.18	6.091	0
30	0	0	0	0

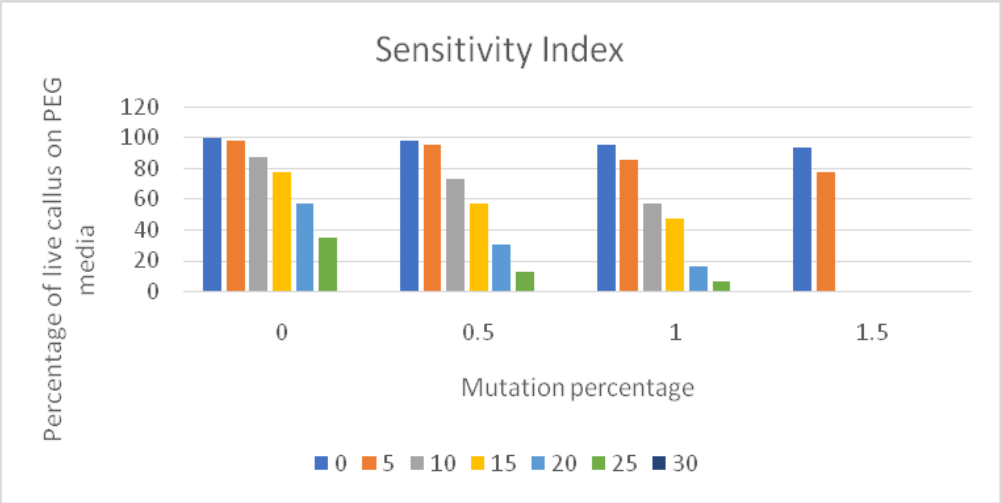

Fig. 1. Representation of SI based on different PEG concentrations at various mutation doses

callus was only discovered at 5% PEG for 1.5% mutation. Regardless of the mutagen levels, 30% PEG dosage was shown to have zero SI since there was no living callus line.

3.2 Selection of Mutated PEG Tolerant Callus

Callus lines obtained after mutation with different concentrations of sodium azide (0, 0.5, 1.0, 1.5%) were transferred to different concentrations (0, 5, 10, 15, 20, 25, and 30%) PEG-infused media. It was noticed that with increasing concentration of PEG resulted in a progressive reduction of live callus as well discoloration of the callus from yellowish white to dark brown color as well as death of callus at 30% PEG (Fig. 2). However, there were some patches of live callus seen surviving up to 25% of PEG with varied mutation doses. Those callus lines were considered mutant PEG tolerant callus lines. Those patches of callus line surviving were sub-cultured and maintained for further analysis and study. While screening for PEG-tolerant callus lines for their experiment, Rao and Jabeen [15] discovered comparable outcomes. It was also observed by Kumar et al. [16] and Aazami et al. [17] that the addition of PEG to the medium produces osmotic stress and decreases the water potential which negatively affects growth.

3.3 Proline Content

Proline content was analyzed for non-mutated and mutated callus at different concentrations of PEG supplemented media and it was found that proline levels were higher in mutated calli than for the non-mutated ones (Table 3). Mutant calli retained larger amounts of proline than the non-mutated ones at any given PEG concentrations. Proline content increased with an increasing concentration of PEG up to 20%, and then there was a rapid fall in proline content at 25% and no proline content was observed at 30%. Apart from the build-up of proline to different concentrations of PEG, there was a significant difference in the proline build-up with varied concentrations of mutagens. As the mutation percentage increases from 0.5 to 1.0, there was an increase in the proline content but as the mutagen concentration increased to 1.5% the growth of calli as well as the proline accumulation decreased.

This describes how proline build-up helps calluses survive and grow when there is a drought. According to Kumar et al. [18], proline levels in sugarcane increased during drought stress. Similar trends in proline build-up of PEG-tolerant calli were discovered by Rao and Jabeen [15]. According to Shah et al. (2012), the 20% PEG-selected calli of rice had a 17-fold higher proline concentration than the non-selected calli. The primary understanding of proline accumulation under water deprivation is as an osmotic agent [19]. The mutated callus exhibited osmotic adjustment in response to PEG stress through the production of proline better than the non-mutated callus.

3.4 Salicylic Acid Content

Comparatively to the non-mutated callus line, mutated callus lines accumulated more salicylic acid. There was also a rise in SA levels with an increase in mutation percentage and PEG

concentration, but this increase was abruptly reversed at higher mutation concentrations at 1.5% and 25% of PEG concentration, respectively, and there was no detectable SA level at 30% of PEG (Table 4). However, at any given concentration of PEG, mutated callus showed higher levels of SA when compared to non-mutated callus lines.

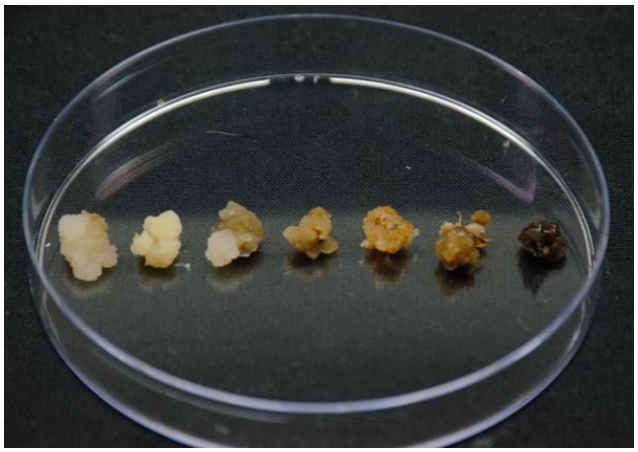

Fig. 2. Callus after treatment with different concentration of PEG (0, 5, 10, 15, 20, 25, and 30%)

Table 3. Effect of PEG on proline content of mutated and non-mutated callus at different concentrations of PEG and mutation

PEG Concentration	Mutation Percentage (%)			
%	0	0.5	1.0	1.5
0	2.095±0.08^{e}	5.73±0.07^{e}	5.94±0.09	5.85±0.02
5	5.793±0.02^{d}	7.17±0.02^{d}	8.71±0.33	7.83±0.07
10	7.821±0.04^{c}	10.775±0.14^{c}	12.53±0.29	12.10±0.05
15	10.179±0.15^{b}	13.58±0.41^{b}	14.59±0.38	14.34±0.11
20	12.101±0.12^{a}	18.35±0.43^{a}	19.26±0.17	18.86±0.11
25	0.8323±0.01^{f}	5.905±0.04^{f}	6.76±0.07	6.15±0.04
30	ND	ND	ND	ND

Treatments found Significant at 1% and 5% levels of significance

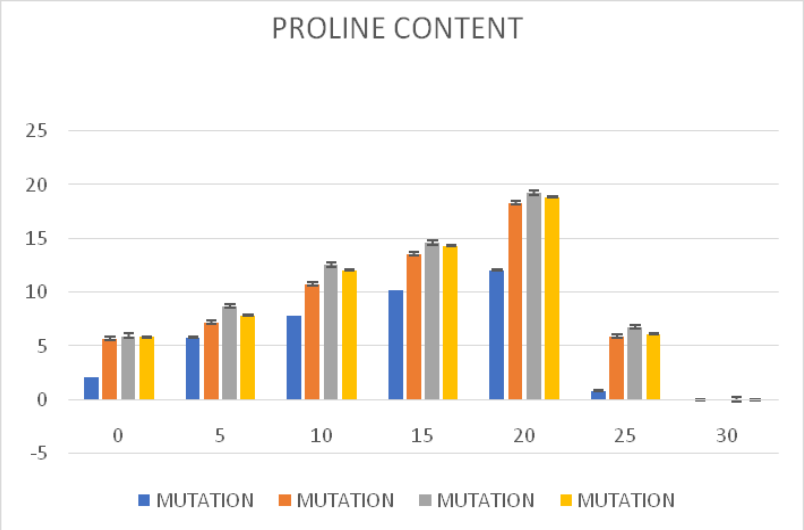

Fig. 3. Representation of effect of PEG and subsequent changes in proline content in mutated and non-mutated callus

Table 4. Effect of PEG on salicylic acid content of mutated and non-mutated callus at different concentrations of PEG and mutation

PEGConcentraion (%)	Mutation Percentage (%)			
	0	0.5	1.0	1.5
0	2.769±0.13	8.512±0.13^{e}	8.512±0.13	8.512±0.13
5	3.665±0.13	12.045±0.2^{d}	12.045±0.2	12.045±0.2
10	4.477±0.02	14.519±0.22^{c}	14.519±0.22	14.519±0.22
15	5.448±0.07	17.758±0.16^{b}	17.758±0.16	17.758±0.16
20	6.48±0.05	19.024±0.1^{a}	19.024±0.1	19.024±0.1
25	1.503±0.15	2.976±0.12^{f}	2.976±0.12	2.976±0.12
30	ND	ND	ND	ND

3.5 treatments found significant at 1% and 5% Levels of Significance

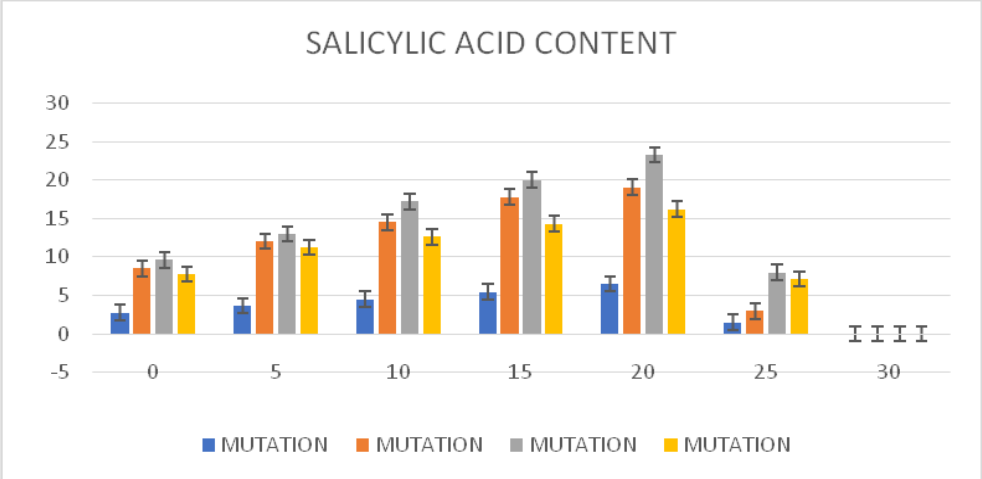

Fig. 4. Representation of effect of PEG and subsequent changes in SA content in mutated and non-mutated callus

Salicylic acid was found to be increasing the leaf area of sugarcane to alleviate the drought stress imposed [20]. Tripathi et al. [21] also found similar trends in salicylic acid content in sugarcane. Lower levels of salicylic acid at a higher concentration of PEG in non-mutated callus may be due to the necrosis of the callus in response to PEG and higher mutagen concentration [22-24].

4. CONCLUSION

According to the findings of this study, there was an increase in drought tolerance when the mutation dose reached 1.0 percent, as well as an increase in PEG concentration up to 20 percent PEG for the sugarcane variety Co-86032. As a result, it is possible to create sugarcane callus lines that are resistant to drought by inducing mutations and then screening the resulting offspring in PEG-infused media. It is supported by biochemical examination of proline and salicylic acid content, which demonstrates that mutated calli have more activity during drought stress than non-mutated callus lines and are responsible for superior development under stressful conditions. It can be concluded that the interaction of callus selection media containing PEG-6000 generated osmotic stress and mutation dose has a substantial effect on callus morphology, callus growth rate, survival, and regeneration and should be taken into account when screening drought tolerant sugarcane callus lines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Misra V, Solomon S, Mall AK, Prajapati CP, Hashem A, Abd Allah EF, Ansari MI.

Morphological assessment of water-stressed sugarcane: A comparison of the waterlogged and drought-affected crop. Saudi Journal of Biological Sciences. 2020b;27(5):1228–1236.
2. Shrivastava AK, Srivastava TK, Srivastava AK, Misra V, Srivastava S, Singh VK, Shukla SP. Climate change-induced abiotic stresses affect sugarcane and its mitigation. ICAR-Indian Institute of Sugarcane Research, Lucknow. 2016;108.
3. Masoabi M, Lloyd J, Kossmann J, Van der Vyver C. Ethyl methanesulfonate mutagenesis and *in vitro* polyethylene glycol selection for drought tolerance in sugarcane (*Saccharum* spp.). Sugar Tech. 2018;20(1):50-59.
4. Hapsoro Dwi, Titik Inayah, Yusnita Yusnita. Plant Regeneration of sugarcane (*Saccharum officinarum* L) calli in vitro and its response to gamma irradiation. J. ISSAAS. 2018;24(1):58-66.
5. Reis, Rafaela Ribeiro, Bárbara Andrade Dias Brito da Cunha, Polyana Kelly Martins, Maria Thereza Bazzo Martins, Jean Carlos Alekcevetch, Antônio Chalfun-Júnior, et al. Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. Plant Science. 2014;221:59-68.
6. De Filippis LF. Crop improvement through tissue culture. Improvement of Crops in the Era of Climatic Changes. 2014;289-346.
7. Jain S Mohan. Mutagenesis in crop improvement under the climate change. Romanian Biotechnological Letters. 2010; 15(2):88-106.
8. Khan, Salim, Fahad Al-Qurainy, Firoz Anwar. Sodium azide: A chemical mutagen for enhancement of agronomic traits of crop plants. Environ. We Int. J. Sci. Tech. 2009;4:1-21.
9. Mahmud Kuasha, Nasiruddin Khondoker, Hassan Lutful. Effects of sodium azide on callus in Sugarcane. 2016;1683-1691.
10. Hartati RS, Suhesti S, Wulandari S, Ardana IK, Yunita R. *In-vitro* selection of sugarcane (*Saccharum officinarum* L.) putative mutant for drought stress. In IOP Conference Series: Earth and Environmental Science, IOP Publishing. 2021;653(1):012135.
11. Abbas SR, Ahmad SD, Sabir SM, Shah AH. Detection of drought tolerant sugarcane genotypes (*Saccharum officinarum*) using lipid peroxidation, antioxidant activity, glycine-betaine, and proline contents. Journal of Soil Science and Plant Nutrition. 2014;14(1):233-243.
12. Osmolovskaya, Natalia, Julia Shumilina, Ahyoung Kim, Anna Didio, Tatiana Grishina, Tatiana Bilova, Olga A Keltsieva, et al. Methodology of drought stress research: Experimental setup and physiological characterization. International Journal of Molecular Sciences. 2018; 19(12):4089.
13. Jabeen FTZ. Selection of drought tolerant cell lines in sugarcane (*Saccharum offcinarum* Linn.) through cell and tissue culture technique. Ph.D. thesis submitted to Gulbarga University Gulbarga Karnataka India; 2007.
14. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973;39:205–207.
15. Rao, Srinath, Jabeen Ftz. *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (*Saccharum officinarum* L.). Physiology and Molecular Biology of Plants. 2013;19(2):261-268.
16. Kumar RR, Karjol K, Naik GR. Effect of polyethylene glycol induced water stress on physiological and biochemical responses in Pigeon pea (*Cajanus cajan* L. Millsp.). RRST-Plant Physiol. 2011;3:148–152.
17. Aazami MA, Torabi M, Jalili E. *In vitro* response of promising tomato genotypes for tolerance to osmotic stress. African Journal of Biotechnology. 2010;9(26): 4014–4017.
18. Kumar, Devendra, Nisha Malik, Rakesh Singh Sengar. Physio-biochemical insights into sugarcane genotypes under water stress. Biological Rhythm Research. 2021; 52(1):92-115.
19. Handa S, Handa AK, Hasegawa PM, Bressan RA. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiology. 1986;80:938–945.
20. Khodary SEA. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. International Journal of Agricultural Biology. 2004;6(1):5-8.
21. Tripathi P, Chandra A, Prakash J. Physio- biochemical assessment and expression analysis of genes associated with drought tolerance in sugarcane

(*Saccharum* spp. hybrids) exposed to GA_3 at the grand growth stage. Plant Biology. 2019;21(1):45-53.

22. Hassan NS, Shaaban LD, El-Sayed AH, Seleem EE. *In vitro* Selection for Water Stress Tolerant Callus Line of *Helianthus annus* L Cv Myak. International Journal of Agricultural Biology; 2004.
23. Silva MA, da Silva JAG, Enciso J, Sharma V, Jifon J. Yield components as indicators of drought tolerance of sugarcane. Scientia Agricola. 2008;65:620–627.
24. Weksanthia Napa, Tanapon Chaisan, Wannasiri Wannarat, Songyos Chotchutima, Peeranuch Jompuk. Mutagenesis and Identification of Sugarcane Mutants Using Survival on Polyethylene Glycol and Leaf Damage under Managed Water Stress. International Journal of Agronomy; 2021.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/90845