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Original Research Article

Analysis and Comparison of $NaNo_2$ Induced Mutagenesis for Draught Tolerance among Sugarcane Varieties Co- 86032, Co M265 and Co VSI 8005

Anita Dhobale[1*], Laxmikantdama[1] and Amol Shinde[2]

[1]Department of Biotechnology, D.B.F. Dayanand College of Arts and Science, Solapur, Maharashtra, India
[2]Department of Biotechnology, Lokmangal Agricultural Biotechnology College, Wadala, Solapur, Maharashtra, India
**Corresponding author*

A B S T R A C T

The present investigation was focused on $NaNO_2$induced mutagenesis for drought tolerance among three different sugarcane varieties i.e. Co- 86032, CoM265 and Co VSI 8005. $NaNO_2$ as mutagenic agent was used in this experiment. It is observed that this chemical has mutagenic properties. $NaNO_2$ was mixed in the culture medium in the proportion of 1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l. The actively growing callus tissues were separated from the explants after successful completion of 30 days and further treated with chemical mutagens. Ten samples of each selected varieties could grow under controlled conditions for next two weeks. Molecular analysis has been performed for all the samples with RAPD markers. Polymorphism has been observed in the culture at 2 mg/l concentration of $NaNO_2$. This concentration was assumed to get maximum percentage of mutagenesis and used for further selection process. The Various physiological Characteristics have been studied in normal and somaclonal plant samples of Co-8603, CoM265 and Co VSI 8005, and the observations have been recorded, particularly normal and somaclonal plant samples of sugarcane variety Co8603 grown in *in-vitro* condition, 75% increased RWC % at 0.75g/l PEG treatment and 55% survival rate has been observed. Similarly, in CoM265 sugarcane variety, 74% RWC has been recorded as highest rate at 0.75g/l PEG treatment compare to other PEG concentrations. In Co VSI 8005 Sugarcane variety, it has been observed that 86% RWC has been observed for 0.75g/l of PEG treatment.

Keywords

Sugarcane varieties, NaNO2, Induced mutagenesis

Introduction

Tissue and cell culture are considered an important breeding tool for crop improvement. In vegetative propagated species, mutagenesis combined with *in vitro* culture techniques may be the only method to improve existing cultivars. Tissue culture and mutagenesis were applied to increase variation in sugarcane by irradiation of dormant buds or cuttings and subsequent *in vitro* development of plants (Siddiqui and Javed, 1982).

It has been reported that *in vitro* irradiation and other mutagenic agents increase variation and the level of stress tolerance among regenerants (Chaudhary *et al.*, 1994).

Tissue culture derived plants show variation termed as somaclonal variation. *In vitro* culture in addition with mutation technique offer several advantages to overcome some of the problems of conventional breeding (Jain *et al.,* 1998).

Drought is the major environmental stress and greatly affect the plant productivity. Breeding for drought tolerance is difficult so the intervention of mutagenesis and tissue culture can greatly facilitate the selection and isolation of useful tolerant lines.

In this study, the selection of drought tolerant lines in popular sugarcane varieties, Co 86032, Co M 265 and Co VSI 8005 is done. *In vitro* mutagenesis was employed (Patade *et al.,* 2006).

Tissue culture when coupled with mutation breeding would become an important and valuable tool in the hands of plant breeders to create genetic variability for the selection of new genotypes with improved agronomic characteristics (Imtiaz, 2009).

In vitro explants have been becoming a useful target for chemical mutagenesis. *In vitro* systems may offer several advantages such as the availability of more standardized conditions and the possibility to prevent or restrict the formation of chimeras, successful EMS mutagenesis of banana shoot tip explants is an example of this. (Jankowicz-Cieslak *et al.,* 2012), and callus tissue of rice (Serrat *et al.,* 2014), wheat (Simonson *et al.,* 1991) and sugarcane (Purnaman Singh and Hutami, 2016) have been reported. Callus tissue was induced from bahiagrass seeds treated with sodium azide (Kannan *et al.,* 2015). A large mutant population of 19630 plants was regenerated from these calli via somatic embryogenesis. A superior mutant line with improved traits was later identified in multi-locational field trials.

Materials and Methods

Explant Preparation and Mutagens Employed

Healthy young meristem was collected by removing the leaf sheath from field grown plants of sugarcane (*Saccharum officinarum L*., cultivar, Co -86032, Co -M 265 and Co-VSI 8005) maintained in the Lokmangal College nursery and brought to the laboratory. This young meristem was cut into thin smaller pieces of 1.0 to1.5 cm length. The explants were washed thoroughly under running tap water for 20-30 minutes followed by Bavistin 0.2% for 10 minutes and then washed with sterile distilled water and transferred to laminar air flow cabinet. The young meristem explants were treated with 70 % alcohol for 30 seconds to one minute, followed by another treatment in 0.1% (w/v) mercuric chloride ($HgCl_2$) for another 5 minutes. Finally, the young meristem cuttings were washed thoroughly 3 to 5 times with sterile distilled water before the inoculating the sterilized nutrient agar media pre-packed in culture bottles. Such 50 bottles of above three varieties were prepared. All the above operations were performed under aseptic conditions in laminar airflow cabinet. The sterilized semisolid basal MS medium supplemented with different concentrations and combinations of different plant growth regulators was inoculated with young explants.

The pH of the medium was adjusted to 5.8 before gelling with Agar (6g/l bacteriological grade, Qualigens, India) and prior to autoclaving for 20 minutes at120 °C and at 15 lbs psi pressure. Sugar was added at the concentration of 30 gm/l. Molten medium of 20 ml was dispensed into the culture bottle and plugged with sterile cap sealed with wrapper. All the cultures were

incubated in a growth room with a 16h photoperiod. The light was cool, white, fluorescent light 30µmol $m^{-2}s^{-1}$) and the temperature was maintained at 25 ± 30 °C with 70-80% relative humidity in the culture room. Each treatment consisted of 10 replicates and repeated thrice.

Callus Induction

For callus induction 5 different concentrations of 2, 4-dichlorophenoxy acetic acid (i.e.1.0, 1.5, 2.0, 2.5, 3.0 mg/l) in MS medium along with control (0 mg/l) were used. All concentrations of auxins and hormones were kept constant. For callus induction, culture bottles were kept at 20, 25 and 30°C in complete dark condition with 16/8 h light/dark photoperiod with 140µmol $m^{-2}s^{-1}$ light from cool, white, fluorescent lamps. Parameters i.e., Average no of days for callus induction, callus morphology, percentage of calli induction and dry weight of the calli were studied when the calli were of 2 weeks old. The induced callus was sub cultured and maintained for 6 weeks.

Mutagenesis Induction

Many factors can influence the outcome of chemical mutagenesis including the characteristics of the target plant material, the dose of the chemical applied, the physio-chemical properties of the chemical mutagen, the nature of the mutagenic solution (e.g. pH), the controlled conditions of the laboratory (e.g. temperature) as well as the growing conditions *(in vitro,* etc.) of the plant before and after the mutagenic treatment.

A new approach of using $NaNO_2$ as mutagenic agent was used in this experiment. It is reported that this chemical has mutagenic properties. $NaNO_2$ was mixed in the culture medium in the proportion of 1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l. After one month of culture, the actively growing callus tissues were separated from the explants and treated with chemical mutagens. Ten samples of each selected varieties could grow under controlled conditions for next two weeks. Molecular analysis was done for all the samples with RAPD markers. Polymorphism was seen in the culture at 2 mg/l concentration of $NaNO_2$. This concentration was assumed to get maximum percentage of mutagenesis and used for further selection process. The explants survived in healthy condition in above experiment were used for further somaclonal variation. The induced callus was sub cultured and maintained for 45 days. Then it was used for applying selection pressure in the form of different quantities of PEG.

Selection for Drought Stress Tolerance

Development of drought stress tolerant plants by using tissue culture technique was reported in wide range of plant species. Through *in vitro* selection technique, better control of culture environment can be achieved. Selection agents like PEG (Polyethylene Glycol) can be used at growth inhibitory levels in callus, cell, or protoplast culture to obtain drought tolerance.

PEG Treatment for Drought Tolerance

PEG was used to stimulate water stress in plants. PEG of high molecular weight is a non-penetrating inert osmoticum. It lowers the water potential of nutrient solutions without being taken up or being phytotoxic. Polyethylene glycol 6000 was used to evaluate resistance to drought at germination stage by creating somaclonal variation in three different cultivars of sugarcane. After the mutagenesis treatment, plants with greater vigor were selected and transferred

to culture bottles with approximately 40 ml of medium, in which the different PEG concentrations were applied. The experiment was maintained for 45 days in the plant growth chamber at a temperature of 27 ± 2°C with a photoperiod of 16 hours, light intensity of 50 µmolm-2 s-1 provided by cold white light (Manuela Maria, 2017). The PEG was added in the media at different concentration, 0.75mg/l, 1.5mg/l, 2.25mg/l and 3 mg/l in addition to a control treatment (T0) without the addition of PEG. Three sub culturing were done at the interval of 45 days for same concentrations in the selected varieties of sugarcane in ten multiples. After third subculturing the plants could grow in shooting and rooting media and used for further analysis.

Analysis

Then the *in vitro* plantlets were analysed for following physiological and molecular characters to screen for the desired variations resulting into a stable somaclone which can be further propagated and grown.

Physiological Characteristics

Photosynthetic rate

Serial harvest technique was used to monitor dry mass where several plants were harvested, dried to constant weight, and weighed. This was repeated over the duration of the experiment. The sugarcane plants after completion of rooting phase was dried, weighed and recorded how much mass they had accumulated. An accurate measure of the surplus photosynthesis over and above the respiration that has taken place was found. Taking several plants replicate measurements, an average was found. This method was used to determine variations in the photosynthetic rates of the plants studied.

Relative Water Content

It estimates the current water content of the sampled leaf tissue, relative to the maximal water content it can hold at full turgidity. Normal values of RWC range between 98% in fully turgid transpiring leaves to about 30-40% in severely desiccated and dying leaves, depending on plant species.4 to 6 samples (replications) were taken from a single treatment. Each sample representing different plant. Top-most fully expanded leaves were sampled. Large veins were avoided. Each sample was placed in a pre-weighed airtight vial. Vials were immediately placed in a freeze (around 10^0C-15^0C). Leaf sampling was done quickly.

In the Lab, vials were weighed to obtain leaf sample weight (W), after which the sample was immediately hydrated to full turgidity for 3-4hrs under normal room, light, and temperature. Leaf discs and small leaflets were hydrated by floating on de-ionized water in a closed Petri dish. After hydration, the samples were taken out of water and were well dried of any surface moisture quickly and lightly with filter/tissue paper and immediately weighed to obtain fully turgid weight (TW). Samples were then oven dried at 80^0C for 24hrs and weighed (after being cooled down in a desiccators) to determine dry weight (DW). All weighing is done to the nearest mg.

Calculation:
RWC (%) = [(W-DW) / (TW-DW)] x 100,

Where,

W – Sample fresh weight

TW – Sample turgid weight

DW – Sample dry weight.

No of Plantlets Alive (Survival Rate)

Three clumps were set in the media bottle while inoculation. Each clump generated average 2 to 6 tillers. While sub culturing and treating culture with selection pressure at multiplication stage, initially ten plantlets were placed in a culture bottle. No of alive plantlets were observed and noted for each treatment at the end of 45 days.

Plant Height and No of Leaves

At the end of each treatment, all the plants were segregated to measure and note the plant height and no of leaves of each sugarcane plant using 20 samples per replication and three replications.

Leaf Area

Each leaf was spread over millimetre graph paper and the outline of leaf was drawn. The leaf area of each leaf was measured using the graph paper. The observations were recorded and analysed statistically to obtain mean values with acceptable range of deviations.

Dry Weight

Since plants have a high composition of water and the level of water in a plant is depend on the amount of water in its environment (which is very difficult to control), using dry weight as a measure of plant growth tends to be more reliable.

Transpiration Rate

The respiration rate was calculated as the amount of water lost per unit leaf area in unit time. Water loss was calculated by the gravimetric method. Intact, bare-root plants were sealed in 50 ml vessels (one plant per vessel) through a rubber bung. Transpiration rate was determined by the rate of weight loss of the vessel during 1 and 3 h in control and treated plants, respectively (Aroca *et al.*, 2003).

Results and Discussion

In vitro culturing of sugarcane explants of cultivars, Co 86032, CoM 265, and Co VSI 8005 was done. Standard tissue culture technique was used. The M.S. media was used as basal media with slight modifications to obtain *in vitro* cultured plantlets in the above-mentioned cultivars.

Callus Induction

Initial 4-5 days of culture of young sugarcane leaf meristem tissue on callus induction medium exhibited swelling and within the next 2 weeks, callus formation was noticed. BAP (6-Benzylaminopurine, benzyl adenine) is used in the proportion of 1 mg/l to get maximum callus induction as per major studies reference. (Arjun and Srinath Rao, 2015). The details of the callus formed for different concentrations of 2, 4-D is explained in the table below.

Mutagenesis Treatment

The callus induced for all the three varieties in this experiment at the concentration of 2.5 mg/lit of 2,4-D was used for mutagenesis treatment. $NaNO_2$ was used as the mutagenic agent (Dhobale *et al.*, 2019).Treatment was given at four different concentrations of $NaNO_2$, 1mg/lit, 2mg/lit, 3mg/lit and 4mg/lit. This proportion of salt was added to the culture media bottles and above selected calli of all three sugarcane varieties were subculture in these bottles.

Regeneration started with the appearance of green dots of callus within a week on regeneration medium and generally produced normal stem and leaves in six

weeks. The genetic variability among the in vitro mutagenized and selected plants was analysed using RAPD molecular marker technique.

Effect of Peg on Physiological

Characteristics

The various Physiological characteristics are studied in normal and somaclonal plant samples grown in vitro and recorded as below.

Molecular analysis

DNA from 30 samples of somaclones obtained from sugarcane callus treated with different concentrations of PEG and $NaNO_2$ was isolated as per the method suggested by (Murray and Thompson, 1980).The quality of the DNA sample was checked by agarose gel electrophoresis. The results were noted.

Further the DNA samples were subjected to PCR amplification using SSR markers. The results obtained in the form of photograph of the gel were analysed and presented.

As shown in Fig.5 above, among the DNA samples amplified using SSR UGSM351, there are samples which have shown similarities and there are samples which has shown differences in the number of bands as well as the position of bands. There was no amplification in Lane B, C and D. Among the remaining samples the sample in Lane E has shown a completely different band profile as compared to all other samples.

This sample was of sugarcane cultivar CoM 265with PEG treatment at the concentration of 0.75 mg/lit. The absence of a band in Lane H which is present in all other samples of same type that is sugarcane cultivar Co VSI 8005 (treated with 0.75 gm/lit.) microshoots indicate variability in this sample. This band pattern is a useful clue indicating a pattern in variability detected by this particular marker.

The results indicate that the genetic variability occurred in two mutagenic treatments. By this result, assuming $NaNO_2$ as mutagenic agent, the regenerated plantlets are used in further experimentation of selection of salt and drought tolerant somaclones. The induced callus is sub cultured and maintained for 8 weeks. Then, it was used for applying selection pressure in the form of different quantities of PEG for inducing drought tolerance.

The callus appearance was found deteriorating as the concentration of PEG was increased. The drought stress tolerance mechanism was developing in the callus. The performance of drought stress tolerance was better in cultivar Co VSI 8005. The concentration of growth hormone BAP was kept same in all treatments changing only of PEG. No shoot generation was seen at the concentration of 3.00gm/lit of PEG. The no of shoots formed, and its length was reducing as the PEG concentration was increased in the culture media.

No roots were observed at the PEG concentration, 2.25 gm/lit, in cultivar, Co 86032 and Co M 265. Sugarcane cultivar Co VSI formed roots at 2.25 gm/lit of PEG concentration. The no of roots and its length was observed to be reduced as the PEG concentration was increased. Plants under water stress shows modifications in their metabolism to sustain water stress.

Table.1 Band Pattern

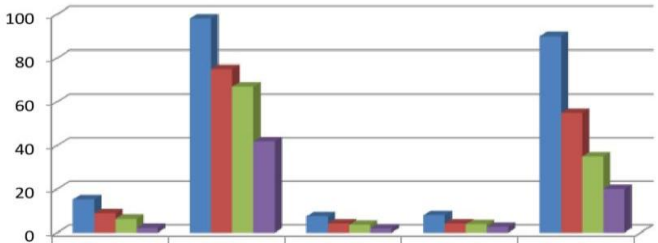
Lane	Sample Name
Lane A	**50 bp DNA Ladder**
Lane B	86032 (0.75 gm/lit, PEG)
Lane C	86032 (1.50 gm/lit, PEG)
Lane D	86032 (2.25 gm/lit, PEG)
Lane E	265 (0.75 gm/lit, PEG)
Lane F	265 (1.50 gm/lit, PEG)
Lane G	265 (2.25 gm/lit, PEG)
Lane H	8005 (0.75 gm/lit, PEG)
Lane I	8005 (1.50 gm/lit, PEG)
Lane J	8005 (2.25 gm/lit, PEG)

Table.2 Polymorphism analysis detected using SSR

Marker	M.W.(bp)	B	C	D	E	F	G	H	I	J
UGSM 351	550	-	-	-	-	-	-	+	-	-
UGSM 363	-	-	-	-	-	-	-	-	-	-
SGM 118	350	-	-	+	-	-	-	-	-	-

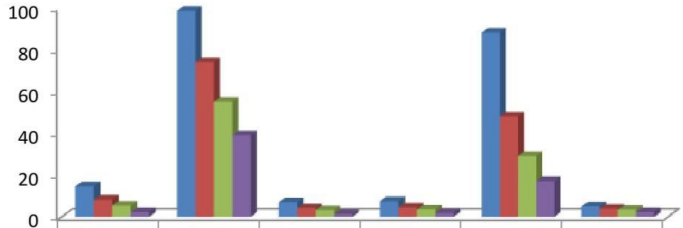
Fig.1 Callus Induction and regeneration in sugarcane samples under controlled conditionand samples treated with $NaNO_2$

Fig.2 Effect of PEG Treatment on all physiological characteristics of Co 86032



	Photosynthetic Rate, µmolm²/s	RWC, (%)	Leaf Area (cm²)	Plant height (cm)	Survival rate (%)
Control	15.33	98	7.5	8	90
0.75 g/l PEG treated	8.94	75	4.3	4.3	55
1.5 g/l PEG treated	6.37	67	3.7	4	35
2.25 g/l PEG treated	2.22	42	1.9	2.7	20

Fig.3 Effect of PEG Treatment on all physiological characteristics of CoM 265

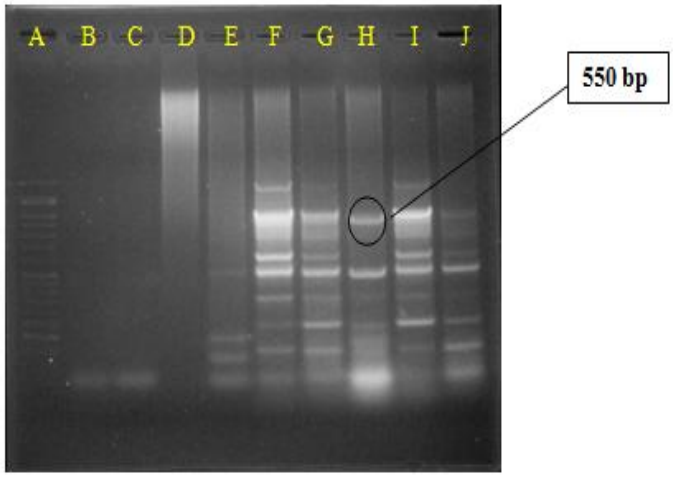


	Photosynthetic Rate	RWC	Leaf Area	Plant height	Survival rate (%)	Transpiration rate, (µg/cm²/s)
	CoM 265					
Control	14.67	98.5	7	7.5	88	5.02
0.75 g/l PEG treated	8.21	74	4.1	4.5	48	3.9
1.5 g/l PEG treated	5.37	55	3.2	3.5	29	3.4
2.25 g/l PEG treated	2.18	39	1.3	1.8	17	2.18

Fig.4 Effect of PEG Treatment on all physiological characteristics of Co VSI 8005

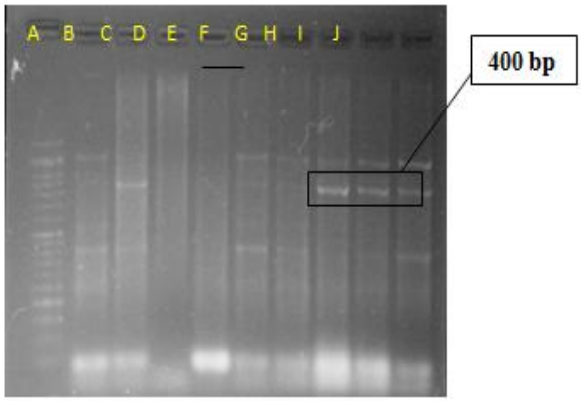
	Photosynthetic Rate	RWC	Leaf Area	No. of Leaves	Plant height	Survival rate (%)	Transpiration rate, (µg/cm²/s)
	Co VSI 8005						
Control	16	98	8.5	6	6	92	6.06
0.75 g/l PEG treated	11.78	86	5.5	5	4.8	58	2.06
1.5 g/l PEG treated	9.65	73	4.9	4	3.9	47	1.65
2.25 g/l PEG treated	6.55	45	2.4	2	2.2	24	1.47

Fig.5 Sugarcane DNA amplification using SSR UGSM 351 primer



Molecular Analysis using SSR UGSM 363 Primer

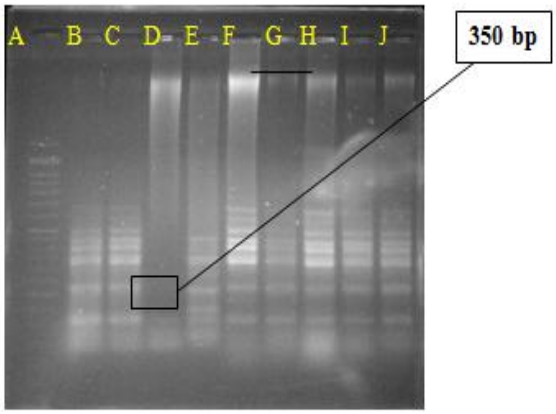
Fig.6 DNA amplification using SSR UGSM 363 primer



Callus regenerated and PEG treated sugarcane microshoots of Co 86032, CoM 265, Co VSI 8005 with varying concentrations

Molecular Analysis using SSR UGSM 118 Primer

Fig.7 DNA amplification using SSR UGSM 118 primer



Callus regenerated and PEG treated sugarcane microshoots of Co 86032, CoM 265, Co VSI 8005 with varying concentrations, As shown in figure 7.no polymorphism is detected with the help of this

Considering this stress, roots are the first which sense the drought conditions and signal to other tissues. As no callus was seen at 3.00 mg/lit concentration of PEG in Co 86032 and Co M 265 and very few calli were seen in Co VSI 8005, this culture bottles of these concentrations were not considered for further analysis. The shoot and root differentiation from the seen callus were not observed in Co VSI 8005.

Relative water content (RWC) estimates the current water content of the sampled leaf tissue relative the maximal water content it can hold at full turgidity. It was observed that when plant water stared to reduce the RWC decreased and triggered the decrease in CO2 uptake rate due to stomatal closure, by Thomas Buckly, (2005).

It resulted in decreased survival rate, plant height, no of leaves and leaf area. All these factors are decreased indicating the adoption of the plant to the stress. The plant survival rate was observed less in cultivar Co M 265 among all three under study. As the no of leaves and leaf area were observed more in the cultivar Co VSI 8005.

Somaclonal variation in combination with *in vitro* mutagenesis can be beneficial for isolation of drought tolerant lines in a short duration employing *in vitro* selection. Genetically stable somaclones, confirmed in field-testing can be used in a crop improvement program. The present study on RAPD analysis of genetic variation among the *in vitro* selected drought and salinity tolerant from NaNO2 treated callus cultures suggest that the variation can be detected at the stage of regeneration even before hardening in the green house. Salt NaNO2 is used as chemical mutagenic agent and it created polymorphism. The use proved effective.

Development of abiotic stress tolerant plants by using tissue culture technique was reported in wide range of plant species. Through *in vitro* selection technique, better control of culture environment can be achieved. Selection agents like PEG (Polyethylene Glycol) be used at growth inhibitory levels in callus, cell or protoplast culture to obtain drought tolerance. Drought is the major environmental stress that limits Sugarcane productivity worldwide. Sugarcane is known as most water consuming crop. In the agricultural economy of Maharashtra, it contributes more than 60 percent. Sugarcane belongs to the genus *Saccharum* L. of the tribe Andropogonae, family Poaceae. The increasing number of sugar factories results increasing area under sugarcane cultivation. During last ten years area under sugarcane has been increased by 70 per cent. Four per cent of total precipitation is diverted towards agriculture, 80 per cent of which is utilized for sugarcane only. The major crunch of water available has been utilized by this crop. This affects the cultivation of other basic grains, pulses, vegetables. If the water requirement for sugarcane reduces by developing drought tolerant variety then area under sugarcane can be increased with same available water. Along with water, the fertilizer requirement of this crop is more. Due to irrational use of chemical fertilizers for sugarcane; soil becomes saline. Reclamation of these soils leads to development of salt tolerant sugarcane variety. Use of Tissue Culture technology to find out somaclonal variants for draught tolerance in different varieties of sugarcane is the cheapest method. Drought is a major abiotic stresses which affects crop productivity. Somaclonal variation is defined as the genetic variability present among cultured somatic cells .*In vitro* somaclonal variation using different selection agents has been used for crop improvement.

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References

Altman A. (2003). Plant tissue culture to biotechnology: scientific revolutions, biotic stress tolerance and forestry. *In Vitro* Cell. Dev. Biol-Plant. 39:75-84.

Arjun and Srinath Rao.(2015). Callus induction and Organogenesis in Sugarcane (*Saccharum officinarum* L.) Var. 93v297. Int. Lett. of Nat. Sci. 48:14-22.

Anita Dhobale, Laxmikant Dama and Aruna Vinchurkar.(2019). Use of SSR and ISSR markers for detection of Sodium Nitrite induced mutagenic somaclones in sugarcane variety Co 86032. Rev. Res. 6(9):33-38.

Aroca R., Vernieri P., Irigoyen J., Sánchez-Díaz M.,Tognoni F. and Pardossi A. (2003). Involvement of abscisic acid in leaf and root of maize (*Zea mays* L.) in avoiding chilling-induced water stress. J. Plant Sci. 165(3):671-679.

Ather A., Khan S., Rehman A. and Nazir M.(2009). Optimization of the protocol for callus induction, regeneration and acclimatization of sugarcane. Pak. J. Bot. 41(2):815-820.

Barba R. C., Zamora A. B., Mallion A. K. and Linga C. K.(1978). Sugarcane tissue culture research. Pr. Int. Soc. Sugarcane Tech.16:1843–1863.

Bhatnagar-Mathur P., Vadez V. and Sharma K. K.(2008). Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. J. Plant Cell Rep.27:411-424.

Chaudhary M. T., Wainwright S. J., Merrett M. J. and Shah M. (1994). Salt tolerant of Lucerne (*Medicago media* Pers.) regenerated from salt selected suspension cultures. J.Plant Sci.98:97-102

Imtiaz A. K., Muhammad U. D., Nighat S., Shafqat Y., Sajida B., Saboohi R. and Abdullah K. (2009). Genetic variability In Sugarcane plantlets developed through *in vitro* mutagenesis. Pak. J. Bot. 41(1):153-166.

Jankowicz-Cieslak J., Huynh O.A., Brozynska M., Nakitandwe J. and Till B. J. (2012). Induction, rapid fixation and retention of mutations in vegetatively propagated banana. J. Plant Biotech. 10(9):1056–1066.

Jain S. M., Brar D. S. and Ahaloowalia B. S. (1998). Somaclonal variation and induced mutation for crop development. ISBN-978-90-481-4956-8.Published by Kluwer Academic Publishers. New York

Kannan B., Davila-Olivas N. H., Lomba P. and Altpeter F. (2015). *In vitro* chemical mutagenesis improves the turf quality of *Paspalum notatum* F.(bahia grass). Plant Cell Tiss. Org. 120(2):551–561.

Munns R. (2005). Genes and salt tolerance: bringing them together. New Phytologist. 167(3): 645–663.

Murray M. G., and Thompson W. F. (1980).Rapid isolation of higher weight DNA. Nucleic Acids Res.8(19):4321-4325.

Padmathilake K. R., Wickramaarachchi V. N., Anver M. A. and Bandara D. C. (2007). Biological and economical feasibility of growing Mint (Mentha sylvestris), Mustard (*Brassica integrifolia*) and Asamodagam (*Trachyspermum involucratum*) under hydroponics. Trop. Agri. Res. J.19:193-201.

Patade V. Y., Suprasanna P., Bapat V. A and Kulkarni U. G. (2006). Selection for abiotic (salinity and drought) stress tolerance and molecular characterization of tolerant lines in sugarcane. BARC newsletter. 273:5-6.

Thomas Buckley.(2005). The control of stomata by water balance. New Phytol. 168(2):275-292.