



# REVIEW OF RESEARCH

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## USE OF SSR AND ISSR MARKERS FOR DETECTION OF SODIUM NITRITE INDUCED MUTAGENIC SOMACLONES IN SUGARCANE VARIETY CO- 86032

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### ABSTRACT:

*Sugarcane is an important crop which is widely cultivated in Maharashtra and known as a cash crop. Different varieties of sugarcane have been recommended for different agro climatic regions and some varieties have been cultivated in large area by the farmers because of their favorable agronomic and economic traits. Sugarcane genome is a complex genome having considerable diversity. New varieties are continuously developed in sugarcane through breeding programmes. Tissue culture offers another dimension to explore diversity and variations in the sugarcane germplasm. Callus was induced using M.S. basal media and 2,4-D (1.0mg/lit). This callus was subcultured and transferred on shoot induction media containing 0.0mg/lit, 1.0mg/lit, 2.0mg/lit, 3.0mg/lit, 4.0mg/lit and 5.0mg/lit NaNO<sub>2</sub> respectively. The microshoots formed were then used for screening those showing heritable variation using selective ISSR and SSR primers. Out of 12 molecular markers (7SSR and 5 ISSR) tried for detecting somaclonal variation those giving amplification and polymorphism were used for further analysis. IISR18 and UGSM351 were found to be polymorphic. These primers can be used for isolation of NaNO<sub>2</sub> induced somaclonal variants.*



**KEYWORDS:** *Sugarcane, breeding programs, tissue culture, callus, markers, somaclonal variation.*

### INTRODUCTION:

Sugarcane (*Saccharum officinarum* L.) is a monocotyledonous perennial crop which belongs to the grass family (Poaceae), an economically important family that includes maize, wheat, rice, *Sorghum* and many forage crops. The diploid number of chromosome of this plant is 2n=80 (Anita *et al.*, 2000). Sugarcane is the main source of

sugar in Asia and Europe. This crop is grown primarily in the tropical and sub-tropical zones of the southern hemisphere. Sugarcane is the raw material for the production of white sugar, jaggery, khandsari, and alcohol etc.

#### Use of tissue culture in sugarcane micro propagation:-

Tissue culture protocols are available for most crop species,

although optimization is still required for many crops including sugarcane. Large-scale micro propagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market. In order to meet the demands, it is urgent to increase cane productivity in India. For this purpose, plant regeneration through tissue culture technique

is recently envisaged for improving the quality and production. The sugarcane productivity largely depends on the availability of quality seed material, varieties, soil health and farming practices. Development and release of a new variety of sugarcane for commercial cultivation requires about ten years which is a very long duration. Lack of rapid multiplication has been a serious problem in sugarcane breeding. Sugarcane micropropagation can be a solution to these problems. (Krishnamurthy, 1994). Khan et.al. (2009) has regenerated and studied the genetic heterogeneity.

#### **INDUCTION OF VARIATION USING CALLUS CULTURE IN SUGARCANE:**

Initial attempts to regenerate plants through *in vitro* techniques were made on sugarcane by Nickell (1964) and Heinz and Mee (1969). Callus culture of sugarcane has been successfully established using shoot apices, young leaves and young inflorescence as explants. Callus is now induced in a large number of sugarcane species indicating that, this phenomenon is not limiting. The plants, which are regenerated from the callus, are not true-to-type due to chromosomal aberration. Many new characters have been identified in these plants. If any character is found to be superior to mother plant, then the somaclone with this phenotype can be realized as a new variety. One important feature of callus culture is that we can alter one or few characters of the questioned genotypes, keeping the rest of genome intact. It is easy to create somaclonal variations in the tissues of sugarcane by *in vitro* callus culture. Somaclonal variation can be used for breeding new cultivars as (i) the variation can occur for agronomic traits (ii) variation occur at high frequency (iii) some variants may be novel and have not been obtained by conventional breeding (iv) in some cases, *in vitro* selection may allow isolation of genotypes tolerant to biotic and abiotic stresses (Faria *et al*, 2014).

#### **MUTAGENESIS:**

It is a process by which the genetic information of an organism is changed, resulting in a mutation. It may occur spontaneously in nature or as a result of exposure to mutagens. The use of induced mutation has played a key role in the improvement of different plant varieties (Ahloowalia *et al.*, 2004). It is also a driving force of evolution. Mutagenesis is the technique to modify only few characters in a cultivar without altering significantly the complete genomic makeup of an organism. In this work, sodium nitrite ( $\text{NaNO}_2$ ) is used as a mutagenic agent.

#### **DETECTION OF SOMACLONAL VARIATION USING MOLECULAR MARKERS:**

Somaclonal variation frequently occurs as a consequence of the repeated and rapid regeneration through vegetative propagation process in different plant species and it may induce the desired traits in plants. But, it is important to confirm the genetic stability and heritability of the induced traits. It is also important to detect the somaclonal variation at the earlier stage of plant growth to make it commercially viable and time saving factor for breeding and crop improvement programme. (Chuang *et al.*, 2009). Ali et.al. (2007), has studied the explants and media compositions for efficient somatic embryogenesis in sugarcane.

#### **USE OF SSR FOR DETECTION OF VARIATION:**

Among PCR-based marker systems, SSR is a powerful marker system due to its ubiquitous distribution across the entire genome, high polymorphism, co-dominant detection and repeatability. Molecular breeding scientists worldwide have successfully developed several SSR and ISSR markers in sugarcane. The sugarcane SSR markers were applied to paternity analysis, genetic diversity assessment, genetic linkage map construction, germplasm evaluation, variety identity testing and the segregation analysis in sugarcane polyploids (Pan *et al.*, 2014).

ISSRs are randomly distributed throughout the genome. They are multi-locus and useful for random sampling of the whole genome and provide multiple fragments. ISSR is a very potential DNA marker system in discriminating within and among population genetic variation without prior DNA sequence information especially for crops that are very difficult to classify/identify morphologically (Devarumath *et al.*, 2012).

The genetic variability in the callus of sugarcane variety Co-86032 and treatment of NaNO<sub>2</sub> is detected using selective SSR and ISSR markers.

#### **MATERIAL AND METHODS:-**

**Plant Material:** The ex-plant of Sugarcane variety Co-86032 was used for the research. *In vitro* cultured calli of sugarcane variety Co-86032 were developed from the explants and plantlets regenerated after the treatment with sodium nitrite were used.

#### **Methodology:-**

##### ***In vitro* propagation of sugarcane:-**

The explants of Co-86032 were obtained from their respective mother plants. They were surface sterilized and inoculated on standardized media. After sub culturing for one month, the explants were used for shoot multiplication. The multiple shoots developed were sub cultured for two to three cycles and these *in vitro* cultured explants were used as one of the experimental materials. Khan et.al (2006) have already studied the *effect* of sucrose and growth regulators on the micro propagation of sugarcane clones. .

In another experiment, the sugarcane explants were treated with 2, 4-D (1.0mg/lit) for callus formation. Four to six weeks old proliferating callus was used to regenerate sugarcane plants on M.S. media containing 0.0mg/lit (control) 1.0mg/lit, 2.0mg/lit,3.0mg/lit, 4.0mg/lit and 5.0mg/lit sodium nitrite respectively. The plantlets regenerated were used after three to four weeks of culturing for molecular analysis. Altaf et.al.(2004) have studied the effect of salt stress on the growth of sugarcane cultivars cp-77-400 and coj-84.

##### **Extraction of DNA from sugarcane plant samples:**

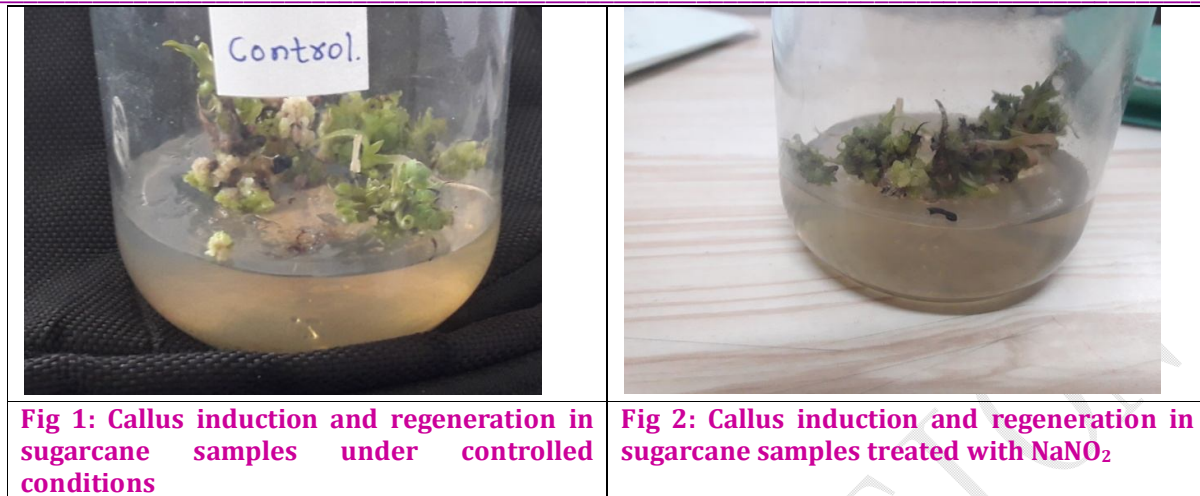
The total genomic DNA was extracted from leaf using the procedure described by Murray and Thompson (1980) with slight modifications. Paterson et.al. (1991) have explained the role of DNA markers in plant improvement. The isolated and purified DNA samples were labeled and kept in -20°C refrigerator until further use. Senapati et.al. (2012) have studied the genetic fidelity of *in vitro* grown plantlets of rose molecular markers. Singhet.al.(2009) evaluated the genetic fidelity of *in vitro*-propagated gerbera using DNA-based markers.

##### **ISSR and SSR analysis:**

The isolated and quantified DNA samples were used for molecular analysis. A set of selective SSR and ISSR primers were used to amplify and characterize the DNA samples using standardized PCR reaction conditions to obtain maximum and repeatable amplification results. Suprasanna et.,al. (2007) used RAPD markers for assessing culture induced variation in somatic embryogenesis derived plants of sugarcane. Almeida et.al. (2008), have studied the molecular characterization of the sugarcane cultivars obtained by ISSR markers. Chidambaram K., Sivasubramaniam K., (2017) have studied the Molecular characterization of sugarcane genotypes using SSR markers. Costa et.al. (2011) have worked on the assessment of genetic diversity in contrasting sugarcane varieties using inter-simple sequence repeat (ISSR) markers. Gaus et.al. (2008) have also studied Molecular characterization of the sugarcane cultivars by ISSR markers. Tiwari et.al and Sharma M. L. (2012) have studied the genetic stability in micro propagated sugarcane variety Cos 07250 through SSR markers.

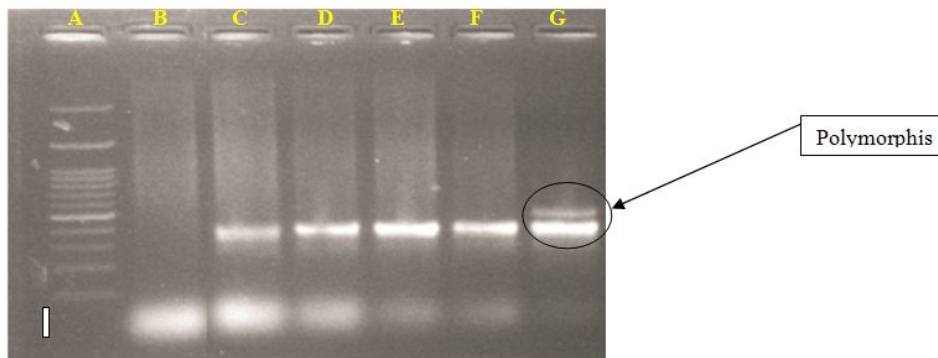
##### **Resolution of amplified product:**

The PCR product was analyzed using 1.5% agarose gel. The gel was stained with ethidium bromide and bands were observed and recorded using gel documentation system.



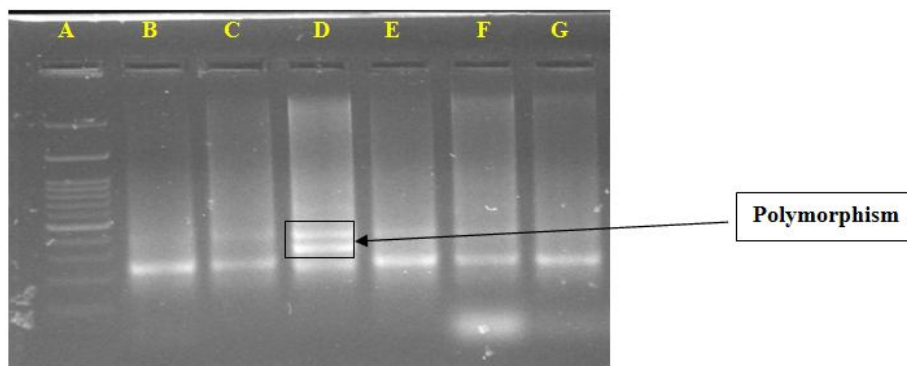
**RESULT AND DISCUSSION:**

The position of amplified band of ISSR18 is different in the samples treated with NaNO<sub>2</sub> and those without NaNO<sub>2</sub> treatment. Thus, NaNO<sub>2</sub> has shown its effect as a mutagenic agent in this case. The polymorphic effect is detected by ISSR 18 primer in the sample treated by 5mg/lit of NaNO<sub>2</sub>. This polymorphic primer will be used for detection and isolation of somaclones under different types of section pressure as a reliable tool for early detection of variants and their isolation. (Fig .3)



**Fig 3 : Sugarcane DNA amplification using ISSR 18 primer (Lane A : DNA ladder (100bp) , Lane B:0.0mg/lit, Lane C: 1.0 mg/lit, Lane D: 2.0 mg/lit., Lane E: 3.0 mg/lit, Lane F: 4.0 mg/lit, Lane G: 5.0 mg/lit NaNO<sub>2</sub>)**

UGSM351 was a SSR primer used for amplifying DNA from NaNO<sub>2</sub> treated and untreated somaclones .It has also indicated distinct polymorphism.



**Fig 4: Sugarcane DNA amplification using SSR UGSM 351 primer (Lane A : DNA ladder (100bp) , Lane B:0.0mg/lit, Lane C: 1.0 mg/lit, Lane D: 2.0 mg/lit, Lane E: 3.0 mg/lit, Lane F: 4.0 mg/lit, Lane G: 5.0 mg/lit NaNO<sub>2</sub>)**

Among the DNA samples amplified using SSR UGSM351, the polymorphic band is indicated by the arrow. This particular band is present in NaNO<sub>2</sub> treated samples at the concentration of 3.0 mg/lit, but absent in the sample without NaNO<sub>2</sub>. SSR UGSM351 is also found as an important primer to detect NaNO<sub>2</sub> induced mutagenesis in this study. This primer can also be useful to identify and isolate desired somaclonal variants at early stage. ( Fig 4)

#### CONCLUSION:

It is possible to identify the distinct polymorphic variations among the samples amplified by selective molecular markers. These markers can be used as an important tool to isolate and identify the mutant somaclones at very early stage of development. Thus saving time and material requires for selective isolation and introduction of desired traits in a plant like sugarcane.

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#### REFERENCES:

- Ahaloowalia, B. S. and Maluszynski, M. (2001). Induced mutations a new paradigm in plant breeding. *Euphytica* 118: 167-173.
- Ali A., Naz S. and Iqbal J. (2007). Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (*Saccharum officinarum*). *Pak J Bot* 39(6): 1961-1977.
- Almeida C. M., Lima S. E., Gaus S. L., Brito J., Donato V. M. and Silva V. M. (2008). Molecular characterization of the sugarcane cultivars obtained by ISSR markers. *Science Agrotec.*, 33: 1771-1776.
- Altaf H., Zafar I. K., Muhammad A., Muhammad H. R. and Muhammad S. A., (2004) Effect of salt stress on some growth attributes of sugarcane cultivars cp-77-400 and coj-84. *Int. Jr. of Agr. and Bio.* 68: 188-191.
- Anita P., Jain R. K., Schrawat A. R. and Punia A. (2000). Efficient and cost effective micropropagation of two early maturing varieties of sugarcane (*Saccharum spp.*). *Indian Sugar*. 50: 611-618.
- Chidambaram K. and Sivasubramaniam K. (2017). Molecular characterization of sugarcane genotypes using SSR markers. *Int. Jr. of Bot. and Res.* 7: 23-28.
- Chuang S. J., Chen C. L., Chen J. J., Chou W. Y. and Sung J. M. (2009). Detection of somaclonal variation in micro-propagated *Echinacea purpurea* using AFLP marker. *Sci. Hort.* 120: 121-126.

- Costa M. L., Lidiane L. Barbosa A, Alberto V. C., Luiz J. O. Tavares M., Maria B. M., Carvalho R. D. and Benko-Iseppon A. M. (2011). Assessment of genetic diversity in contrasting sugarcane varieties using inter-simple sequence repeat (ISSR) markers. *American Jr of Pla. Sci.*, **2**: 425-432.
- Devarumath R. M., Kalwade, S. B., Kwar P. G. and Sushir K. V. (2012). Assessment of genetic diversity in sugarcane germplasm using ISSR and SSR markers. *Sugar Tech*, **4**: 334-344.
- Faria S., Shabana L., Rehana L., Fazeela L., Tabassum S. A., Paras A., Uzma A. A. and Arman A. R. (2014). Callus Induction in Sugarcane. *J. Agric. Food. Tech.*, **4**(3): 8-11.
- Gaus S, ClebiaM, Sue E and Marcia, V. (2008). Molecular characterization of the sugarcane cultivars obtained by ISSR markers. *CIENC Agrotech.* **33**:1771-1776.
- Khan I.M., Dahot M. U., Nighat S., Yasmeen S., Bibi S., Raza G., Khatri A. and Naqvi M. H.(2009). Direct regeneration of sugarcane plantlets: a Tool to unravel genetic heterogeneity. *Pak. J. Bot.*, **41**(2): 797-814.
- Khan S. A., Rashid H., M. Chaudhary F., Chaudhry Z. and Afroz A. (2006). Effect of sucrose and growth regulators on the micropropagation of sugarcane clones. *Pak. J. Bot.*, **38**(4): 961-967.
- Krishnamurthy S., He X., Reyes Reyes M., Moore C. and Hampsey M.(1994).Ssu72 Is an RNA polymerase II CTD phosphatase. *Mol Cell* **14**(3):387-94, Journal Article | Research Support, U.S. Gov't, P.H.S.
- Murray M. G. and Thomon W. F. (1980). Rapid isolation of higher weight DNA. *Nucleic Acids Res.* **8**(19): 4321-4325.
- Murray M. G., and Thompson W. F. (1980). Rapid isolation of higher weight DNA. *Nucleic Acids Res.* **8**(19): 4321-4325.
- Nickell, L.G. 1964. Tissue and cell culture of sugarcane: Another research tool. Hawaii Planters Records, **57**: 223-229.
- Pan, Y.-B., Liu, P. and Que, Y. (2014). Independently segregating simple sequence repeats (SSR) alleles in polyploid sugarcane. *Sugar Tech*, **17**: 235-242.
- Paterson A. H, Tanskley S. D and Sorrels M. E.(1991). DNA markers in plant improvement. *Adv. Agron.* **46**: 40-90.
- Senapati S. K., Aparajita S. and Rout G. R. (2012). An assessment of genetic fidelity of in vitro grown plantlets of rose (*Rosa hybrida*) through molecular markers. *Afr. Jr. of Biotech.* **11**(100): 16532-16538.
- Singh K. P., Bhatia R., Sharma T. R. and Jhang T. (2009). Evaluation of the genetic fidelity of in vitro-propagated gerbera (*Gerbera jamesonii Bolus*) using DNA-based markers. *Pla. Cell Tissue and Org. Cul.*, **65**: 312-316.
- Suprasanna P, Desai N.S., Chowdhary R. S. and Bapat V. A. (2007). RAPD markers for assessing culture induced variation in somatic emryogenesis derived plants of sugarcane. *Sugar Tech* **9**: 284-289.
- Tiwari A. K., Mishra N., Tripathi S., Lal M., Singh R. K. and Sharma M. L. (2012). Assessment of genetic stability in micro propagated population of sugarcane variety cos 07250 through SSR markers. *Int. jr. of pl. res.* **24** (2): 75-81.



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