Plant Archives Vol. 25, No. 1, 2025 pp. 2513-2519 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.363

EXPRESSION OF SALINITY TOLERANT GENES IN SUGARCANE

Saddamhussain Aminbhai Shaikh[1]*, Anil A. Kale[2], Shahid Jafar Mulani[3] and Priya Uday Shinde[4]

[1]Department of Agricultural Botany, Shriram College of Agriculture, Paniv - 413113, Maharashtra, India.

[2]Department of Biochemistry, M.P.K.V., Rahuri, Maharashtra, India.

[3]Department of Post-Harvest Management and Technology at Shriram College of Agriculture, Paniv - 413 211, Maharashtra, India.

[4]Division of Post-Harvest Management, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha - 180 009, J&K, India.

*Corresponding author E-mail : 1992saddamshaikh@gmail.com

(Date of Receiving-16-01-2025; Date of Acceptance-17-04-2025)

ABSTRACT

Salinity stress is a critical abiotic factor limiting sugarcane productivity by adversely affecting plant growth, yield, and sugar accumulation. This study was conducted to analyze the expression of salinity-responsive genes in sugarcane genotypes subjected to varying levels of salt stress and to optimize annealing temperatures for gene-specific primers. Two contrasting cultivars, CoM-0265 (salt-tolerant) and CoC-671 (salt-sensitive), were exposed to different concentrations of NaCl, and electrical conductivity (EC) was measured to confirm stress induction. Visible stress symptoms such as chlorosis were more pronounced in CoC-671, indicating higher sensitivity. Genomic DNA was isolated from CoM-0265 using a modified CTAB method with added PVP and sodium sulphite to counteract polyphenolic interference. DNA quality and integrity were confirmed via spectrophotometry and agarose gel electrophoresis. Gradient PCR was carried out between 46°C and 60°C using primers specific to salinity-responsive genes such as NHX, SUT1, P5CS, PDH, CAT2 and β-actin. Specific amplification was observed with minor size discrepancies, likely due to amplification of non-coding regions in genomic DNA. Optimal annealing temperatures for each primer pair were identified between 54°C and 57°C and will be used in cDNA-based expression analysis. These results contribute to understanding salt tolerance mechanisms and aid in marker-assisted selection in sugarcane breeding.

Key words : Sugarcane, Salinity stress, Gene expression, Gradient PCR, Primer optimization.

Introduction

Sugarcane (*Saccharum* spp. hybrid), a highly productive C4 grass belonging to the family *Poaceae*, possesses a complex polyploid genome (2n = 100-130) arising from interspecific hybridization, predominantly involving *Saccharum officinarum* (2n = 80) and *S. spontaneum* (2n = 40-128) (Sreenivasan *et al.*, 1987; D'Hont *et al.*, 1996). Despite the agronomic advances in yield and sugar content, modern cultivars exhibit a narrow genetic base, which limits progress in conventional breeding programs, especially for complex traits like salinity tolerance (Jackson, 2005).

Salinity stress is a major abiotic factor restricting crop productivity globally. In India, around 5 million hectares of sugarcane cultivation are challenged by soil salinity and alkalinity, with over 9 million hectares of salt-affected land reported (Rao *et al.*, 2015). High salt concentrations cause both osmotic stress and ionic toxicity, disrupting nutrient uptake and triggering oxidative damage in plant tissues (Munns and Termaat, 1986; Flowers and Flowers, 2005). The resulting stress impairs various physiological and biochemical processes, ultimately reducing plant growth and productivity. In response to salinity, plants activate complex molecular and biochemical mechanisms, including ion homeostasis, osmotic regulation, and antioxidative defence. These responses are orchestrated through stress-responsive gene expression networks that regulate transcriptional, translational, and post-translational modifications. Notably, antioxidant enzymes such as

superoxide dismutase (SOD), catalase (CAT) and peroxidases play a key role in mitigating salt-induced oxidative stress (Pagariya *et al.*, 2012).

Gene expression profiling under salinity stress provides insights into the regulatory circuitry of salt tolerance. It enables identification of salt-responsive genes, which may be used as markers or targets in molecular breeding and transgenic approaches (Sairam and Tyagi, 2004; Yamaguchi and Blumwald, 2005). Recent studies have demonstrated that salt-tolerant sugarcane genotypes exhibit altered expression of genes involved in membrane transport, osmolyte biosynthesis (e.g., proline), and stress signalling pathways (Patade *et al.*, 2012; Zahra *et al.*, 2013). Furthermore, salinity stress induces the synthesis of specific proteins termed salt shock proteins (SSPs)which may contribute to stress adaptation (Igarashi *et al.*, 1997; Gomathi *et al*., 2013). Reverse transcription PCR (RT-PCR) has emerged as a sensitive tool for evaluating gene expression patterns in sugarcane under salt stress. It allows for the identification and quantification of transcripts associated with salinity responses. By comparing gene expression in salt-tolerant and salt-sensitive cultivars under different salt concentrations, key candidate genes can be identified for use in developing salinity-tolerant sugarcane varieties.

Therefore, the present investigation focuses on studying the expression of salinity-tolerant genes in sugarcane using gene-specific primers, with an emphasis on understanding the molecular responses to salt stress at the transcriptional level.

Materials and Methods

Material

The present investigation entitled "Expression of Salinity Tolerant Genes in Sugarcane" was conducted during 2015-16 at the State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Dist. Ahmednagar. Sugarcane cultivars identified as salt tolerant (CoM-0265) and salt sensitive (CoC-671) were obtained from the Central Sugarcane Research Station (CSRS), Padegaon. These were grown in earthen pots containing 10 kg of soil, with five NaCl treatment levels (0, 10 mM, 50 mM, 100 mM, and 150 mM) imposed after 3 months of growth. Each treatment was replicated thrice. For stress application, 2.5 L of respective NaCl solution was added to each pot and second leaf samples were harvested 24 hours post-treatment. Samples were snap frozen in liquid nitrogen and stored at -80°C for further analysis.

Table 1 : Salt tolerant and salt sensitive sugarcane varieties used for analysis.

S. no.	Salt Tolerant Variety	Salt Sensitive Variety
1	CoM-0265	CoC-671

Methods

Soil Analysis under different NaCl Concentrations

Electrical conductivity (EC) of the soil samples stressed with 10 mM, 50 mM, 100 mM and 150 mM NaCl, along with non-stressed controls was determined using the conductometric method as described by Jackson (1973). Soil was collected from the Post Graduate Institute Farm, MPKV, Rahuri, and used to grow sugarcane plants in pots. Five NaCl treatments (0, 10 mM, 50 mM, 100 mM, and 150 mM) were imposed in triplicates. For EC analysis, 20 g of soil was mixed with 50 mL of the respective NaCl solutions, and in the control, distilled water was added. The mixtures were stirred intermittently for 1 hour and allowed to settle for 30 minutes. The EC of the supernatant was then measured using a conductivity meter.

Growing of Plants and Imposition of Salinity Stress

Two-eyed sets of sugarcane cultivars, CoM-0265 (salt-tolerant) and CoC-671 (salt-sensitive), obtained from the Central Sugarcane Research Station, Padegaon, were washed and planted in earthen pots containing 10 kg of soil sourced from PGI, MPKV, Rahuri. Five treatments0, 10 mM, 50 mM, 100 mM, and 150 mM NaCl were applied with three replications each. Salinity stress was imposed three months after planting by irrigating each pot with 2.5 L of NaCl solution at the desired concentrations, while control plants received distilled water. After 24 hours of stress application, the second fully expanded leaf was sampled, immediately snap-frozen in liquid nitrogen, and stored at -80°C until further analysis.

Isolation and Purification of Genomic DNA

DNA Isolation from leaves

Genomic DNA was isolated from the leaf tissue of CoM-0265 using the CTAB extraction method as described by Aljanabi *et al*. (1999). Approximately 2 g of leaf tissue was cut into small pieces and ground into a fine powder using liquid nitrogen and a pre-chilled mortar and pestle. The powdered tissue was homogenized in 8 mL of chilled homogenization buffer (200 mM Tris-HCl, 50 mM EDTA, 2.2 M NaCl, 2% CTAB, 0.06% sodium sulfite, pH 8.0) and the homogenate was transferred into 50 mL falcon tubes. To each tube, 4 mL of 5% N-lauroylsarcosine, 4 mL of 10% PVP and 4 mL of 20% CTAB were added and mixed gently by inversion for 15 minutes. The mixture was incubated at 65°C for 1 hour

Table 2 : Composition of gradient PCR reaction mixture.

PCR Reaction Component	Stock concentration	Volume per Reaction (25 µL)
Taq DNA polymerase buffer B	10× (100 mM Tris-HCl, 500 mM KCl)	2.5 µL
MgCl,	25 mM	2.5 µL
dNTP mix	10 mM (2.5 mM each)	2 µL
Forward primer	10 pmol/µL	2 µL
Reverse primer	10 pmol/µL	2 µL
Taq DNA polymerase	3 U/µL	0.33 µL (1 U)
Template DNA	20 ng/µL	3 µL (60 ng)
Sterile distilled water	-	10.67 µL
Total Volume	-	**25 µL**

Table 3 : Temperature profile used in gradient PCR for salinity gene-specific primers.

Step	Temperature	Time	Cycle(s)
Initial Denaturation	95°C	5 min	1
Denaturation	94°C	1 min	40
Annealing	46-60°C	1 min	40
Extension	72°C	1 min	40
Final Extension	72°C	10 min	1
Final Hold	4°C	–	-

in a water bath, with periodic mixing every 15 minutes. After cooling to room temperature, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, and the mixture was centrifuged at 3000 × g for 10 minutes at 4°C. The aqueous phase was carefully collected, and DNA was precipitated by adding an equal volume of isopropanol along with 4 mL of 6 M NaCl. The mixture was vortexed gently and incubated overnight at -20°C. DNA was pelleted by centrifugation at 10,000 × g for 5 minutes, washed twice with 70% ice-cold ethanol, air dried and resuspended in 2 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

DNA Purification

Purification of genomic DNA was carried out by treating 1 mL of crude DNA extract with 25 µL of 20% SDS and 5 µL of proteinase K. The mixture was incubated at 37°C for 1 hour with gentle occasional mixing. Following incubation, 200 µL of 5 M potassium acetate was added, and the mixture was kept on ice for 20 minutes. The samples were centrifuged at 5000 rpm for 5 minutes at 4°C, and the clear supernatant was transferred to fresh tubes. DNA was re-precipitated by adding 400 µL of isopropanol and 40 µL of 5 M ammonium acetate, followed by incubation at -20°C for 1 hour. DNA was recovered by centrifugation at 5000 rpm for 5 minutes at 4°C, washed with 70% ice-cold ethanol, and air dried. The final purified DNA was dissolved in 100 µL of TE buffer for downstream analysis.

DNA Quantification and Purity analysis

The concentration and purity of the purified genomic DNA were determined using a UV-visible spectrophotometer (NanoDrop ND-1000, USA) by measuring the absorbance at 260 nm and 280 nm wavelengths. The purity was assessed by calculating the A260/A280 ratio. DNA integrity was further evaluated by agarose gel electrophoresis. A volume of 2 µL from each DNA extract was loaded onto a 0.8% (w/v) agarose gel containing 0.5 µg/mL ethidium bromide. Electrophoresis was carried out in 1× TBE buffer (prepared by diluting 10× TBE: 1 M Tris base, 830 mM boric acid, 10 mM EDTA, pH 8.0) using a Bio-Rad subcell electrophoresis unit (Model 96, USA) at 6 V/cm. Tracking dye comprising 1% (w/v) bromophenol blue, 20% (w/v) Ficoll, and 10 mM EDTA was used. DNA bands were visualized under UV light using a gel documentation unit (Fluor Chem™, Alpha Innotech, USA), and the intensity was compared to a calf thymus DNA standard to assess quantity and quality.

Gradient PCR for Optimization of Primer Annealing Temperature

Gradient PCR was conducted using a thermal cycler (Eppendorf Mastercycler Gradient, Germany) to determine optimal annealing temperatures for salinity-related gene primers. The PCR amplification was carried out in 0.2 mL thin-walled PCR tubes, with a total reaction volume of 25 µL. The composition of the reaction mixture is described in Table 2. Each PCR mixture was gently vortexed and briefly spun down before loading into the thermal cycler.

The gradient PCR conditions for primer optimization are shown in Table 3. Annealing temperatures ranged from 46°C to 60°C to identify the optimal conditions for each primer pair.

Primer Designing

Primers specific to salinity tolerance-related genes in sugarcane were designed based on sequences reported by Patade *et al.* (2012). The selected gene targets included NHX, SUT1, P5CS, PDH and CAT2, with β-actin used as the internal reference gene. The forward

Table 4 : Primers used for amplification of salinity tolerant genes in sugarcane.

S. no.	Gene	Primer	Sequence (5′ –3′)	Product Size (bp)
1	NHX	F	TGTCTTTGGGCTACTGACGA	201
		R	CTCGCTAGGAGCAAATGGAG	
2	SUT1	F	AGACGGAGGCCATTTATCCT	208
		R	GTGCTGGACCTTGCACAGTA	
3	P5CS	F	TCTTTATGGAGGGCCTGTTG	215
		R	CACTATCAACTTGGCGCAGA	
4	PDH	F	GCAGATCATCCCCTACCTCA	204
		R	CTTGTCCCAGCTTCACCTTC	
5	CAT2	F	ACTTCCCCTCCAGGTACGAC	242
		R	AGACCAGTTGGAGAGCCAGA	
6	β-actin	F	GCCAAGAACAGCTCCTCAGT	220
		R	GAGCACAATGTTGCCGTAGA	

and reverse primer sequences, along with the expected product sizes, are presented in Table 4.

Results and Discussion

Currently, salinity is one of the most important constraints affecting crop productivity worldwide and, largely, its magnitude and intensity are on the rising trend. It influences all stages of sugarcane growth and development and affects yield and sugar content. Thus, it becomes imperative to view mitigation of salinity induced damage in an integrated way to sustain productivity. Breeding, salinity tolerant sugarcane genotypes with an increased ability to survive and grow under salinity conditions is an important component of this strategy. Generally, plant tolerance is a measure of the plant's ability to survive and even to grow proactively in the presence of stress. Efforts have been made to develop salinity tolerant genotypes which, after being integrated into appropriate crop management programs, sustain sugarcane productivity. Normally, salinity tolerant sugarcane genotypes are selected depending upon certain well defined morphological and physio-biochemical characteristics related to tolerance under natural field conditions. Hybrid genotypes or clones possessing tolerance to salinity at a specific level are used in hybridization to develop tolerant varieties. Screening of genotypes for a particular trait by traditional methods is a time-consuming and relatively laborious process. The use of molecular markers in breeding programs, during recent years, has been proved as an efficient tool in this regard. Study of expression of salinity tolerant genes in various is also useful in developing more salinity tolerant genotypes for salinity.

Hence the goal of this investigation is to study the expression of salinity tolerant genes in sugarcane under different degrees of salinity stress conditions. Results obtained are presented under following headings.

Induction of Salinity stress

Sugarcane cultivars CoM-0265 (salt tolerant) and CoC-671 (salt sensitive) were grown in earthen pots containing 10kg soil. The stress was imposed with various concentration of the NaCl solution and corresponding EC was measured tabulated in Table 5 and shown in Plate 1. It was observed that after the induction of salinity stress leaves becomes yellowish. The intensity of the yellow colour of leaves increased with increase in stress. The control plants were found to be green. Between the two cultivars COC-671 was found to have more yellowish leaves than CoM-0265 cultivars.

Optimization of the annealing temperature of thegene specific primers

DNA extraction

Genomic DNA was isolated from the CoM-0265 salt-tolerant genotype of sugarcane. The quantity and quality of the DNA isolated from three-month-old plant material was found to be good and satisfactory as revealed by

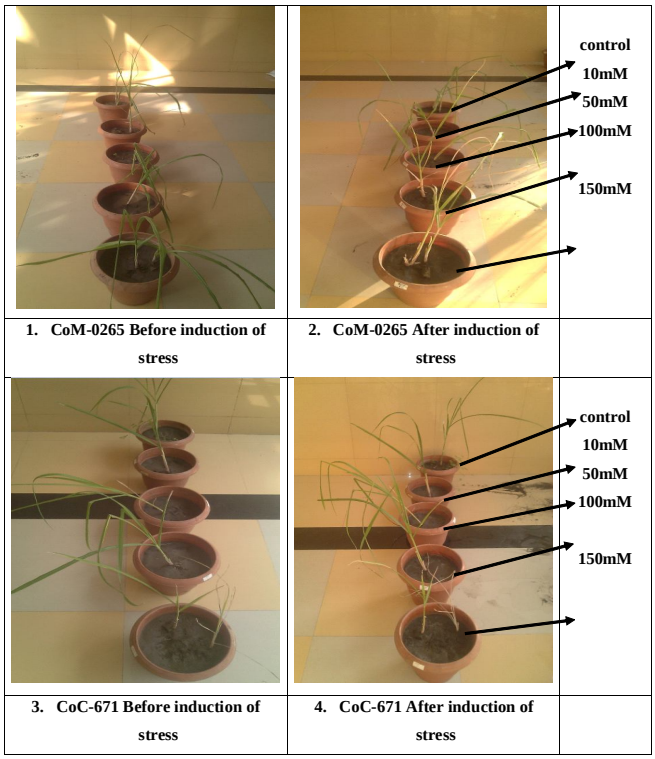

Plate 1 : Induction of salinity stress on 3-month-old sugarcane plant.

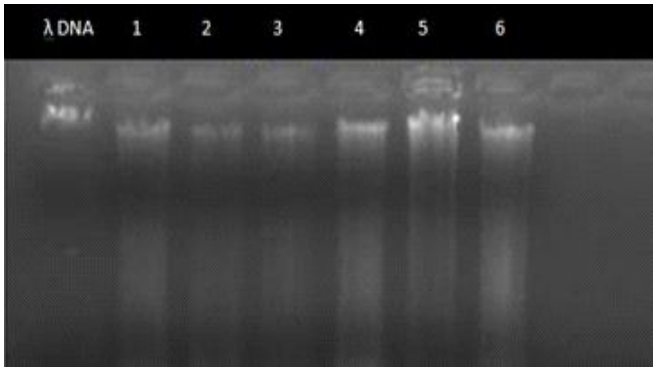

Plate 2 : Resolution of genomic DNA of sugarcane genotype.

Table 5 : EC reading at different NaCl concentration.

NaCl concentration	EC reading (ds m^{-1})
0	0.47
10 mM	1.01
50 mM	2.16
100 mM	4.11
150 mM	8.00

280/260nm absorption ratio, resolving on agarose gel (Plate 2) and amplification of DNA with salinity gene specific primers. The 280/260nm absorption ratio ranged from 1.8-2.0. The yield of DNA ranged from 400-600 ηg of DNA per 100 mg of tissue. Usually, plant tissues are ground in liquid nitrogen and extracted in buffer (Murray and Thomson, 1980). In the present study, CTAB was added to the homogenization buffer is a cationic detergent that precipitates DNA, leaving the neutral polysaccharides in solution during DNA isolation. Presence of polyphenolics in plant tissues, hamper DNA isolation, restriction, amplification and cloning reactions (Khanuja *et al.*, 1999; Kim *et al.*, 1997 and Porebski *et al.*, 1997). These impart brown colour and reduce the yield and purity of extracted DNA (Aljanbi *et al*., 1999, Guillemaut and Marechal-Drouard, 1992 and Katterman and Shattuck, 1993). In present investigation, PVP and sodium sulfite were also added to prevent the oxidation of phenolic compounds (Loomis, 1974). Use of PVP in extraction buffer has been reported earlier to remove polyphenolics (Kim *et al.*, 1997; Porebski *et al*., 1997). PVP binds to the proteins, especially the polyphenol oxidase, which oxidizes the phenolic compounds in the samples resulting in the formation of polyphenol/protein/ DNA complexes that is brown in colour, leads to degraded DNA. The DNA pellets were treated with NaCl, so that Na exchanges with the CTAB if any remained resulting in clean DNA and salt neutralizes the charge on the nucleic acid backbone, causing the DNA to become less hydrophilic and fall out of solution. The proteinase K was used during the purification of DNA since it removes the

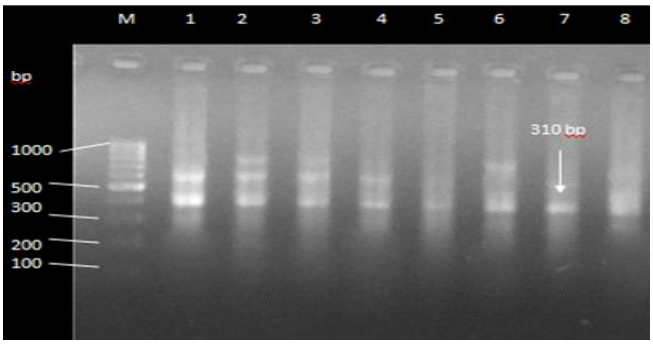

Plate 3 : Gradient PCR of *SUT1* gene of CoM-0265 cultivar of the sugarcaneDNA. Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C 2-47.3°C
3-50°C 4-51.3°C
5-54.8°C 6-56°C
7-57.3°C 8-60°C

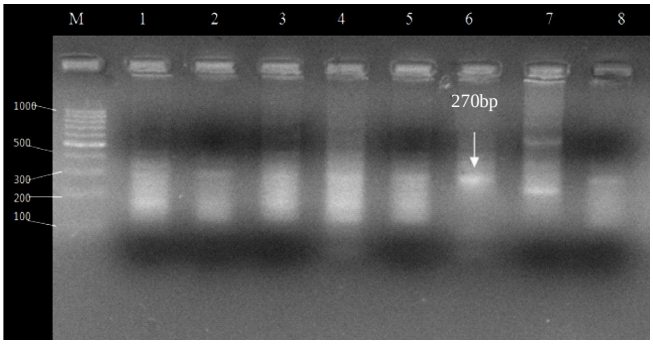

Plate 4 : Gradient PCR of P5CS gene of CoM-0265 cultivar of sugarcane DNA.Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C 2-50°C
3-51.3°C 4-54.8°C
5-56°C 6-57.6°C
7-58°C 8-60°C

proteins. For precipitation isopropanol was used because DNA is less soluble in isopropanol so it will fall out of solution faster and at a lower concentration (Sharma *et al.*, 2008). The ammonium acetate was used for purification since it removes the free nucleotides, proteins and precipitates the DNA (Triboush *et al*., 1998).

Gradient PCR for amplification of sugarcane genomic DNA using gene specific primers

The gradient PCR was performed within the range of 46-60°C for all the primers. The detailed sequence and their expected size were shown in Table 4. In the present investigation specific amplification of *SUT1* gene was observed at 57.3°C (Plate 3). The product size observed was 310bp; however expected size was 208bp. This may be due to the primers being designed gene-specific and the non-coding regions may also amplify along the coding regions present in genomic DNA. Therefore 57°C was used as the optimum annealing

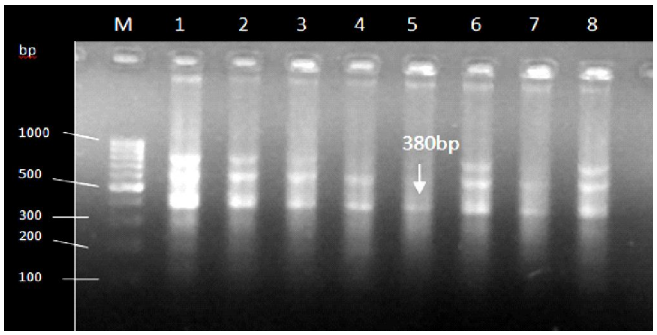

Plate 5 : Gradient PCR amplified product of *PDH* gene from CoM-0265 cultivar sugarcane DNA.Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C	2-47.3°C
3-50°C	4-51.3°C
5-54.8°C	6-56°C
7-57°C	8-60°C

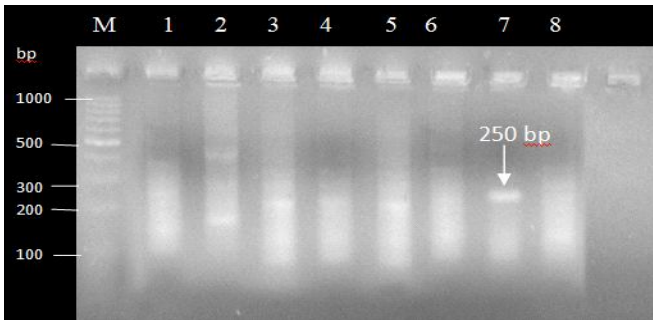

Plate 6 : Gradient PCR amplified product of beta-Actin gene from CoM-0265 cultivar sugarcane DNA.Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C	2-48.7°C
3-51.3°C	4-53.6°C
5-54.8°C	6-56°C
7-57.3°C	8-60°C

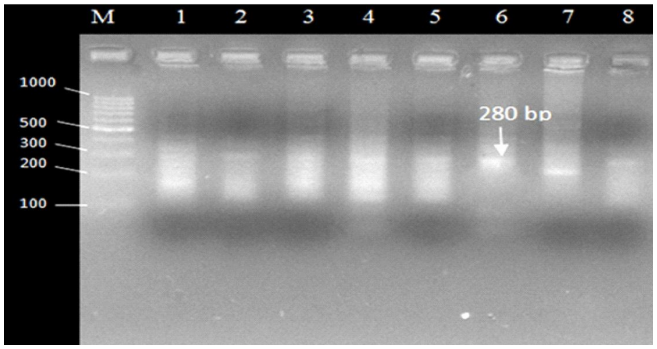

Plate 7 : Gradient PCR amplified product of CAT2 gene from CoM-0265 cultivar of sugarcane DNA.Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C	2-50°C
3-51.3°C	4-54.8°C
5-56°C	6-57.6°C
7-58°C	8-60°C

temperature for cDNA amplification.

Specific amplification of the *P5CS* gene was observed at 57.3°C (Plate 4). The product size observed was

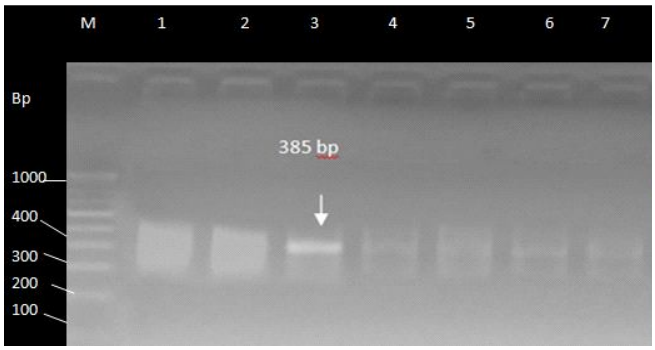

Plate 8 : Gradient PCR of *NHX* gene of CoM-0265 cultivar of the sugarcane DNA.Lane M= Molecular weight ladder (100bp).

Lane No 1-46°C	2-50°C
3-54.8°C	4-56°C
5-57.3°C	6-58.7°C
7-60°C	8-60°C

270bp, however expected size was 215bp. This may be due to primers were designed gene-specific and the non-coding regions may also be amplified along with the coding regions present in genomic DNA. Therefore, 57°C was used as the optimum annealing temperature for cDNA amplification. The specific amplification of *PDH* gene was observed at 54.8°C (Plate 5). The product size observed was 385bp, however expected size was 208bp. This may be due to the primers being designed gene-specific and the non-coding regions may also be amplified along the coding regions present in genomic DNA. Therefore 54°C was used as the optimum annealing temperature for cDNA amplification.

Specific amplification of the *Beta-actin*gene was observed at 57.3°C (Plate 6). The product size observed was 250bp, however expected size was 220bp. This may be due to primers were designed gene-specific and the non-coding regions may also be amplified along with the coding regions present in genomic DNA. Therefore, 57°C was used as the optimum annealing temperature for cDNA amplification. Specific amplification of the *CAT2* gene was observed at 57.3°C (Plate 7). The product size

Table 6 : Optimum annealing temperature confirmed from gradient PCR and used for cDNA amplification.

S. no.	Gene Specific Primer	Optimum annealing temperature selected from gradient PCR for cDNA amplification
1	*NHX*	55°C
2	*SUT1*	57°C
3	*P5CS*	57°C
4	*PDH*	54°C
5	*CAT2*	57°C
6	*Beta-Actin*	57°C

observed was 280bp, however expected size was 242bp. it may be due to the primers were designed gene-specific and the non-coding regions may also be amplified along the coding regions present in genomic DNA. Therefore, 57°C was used as the optimum annealing temperature for cDNA amplification.

Specific amplification of the *NHX* gene was observed at 54.8°C (Plate 8). The product size observed was 385bp, however expected size was 208bp. This may be due to the primers were designed gene-specific and the non-coding regions may also be amplified along with the coding regions present in genomic DNA. Therefore, 55°C was used as the optimum annealing temperature for cDNA amplification. Annealing temperature was optimized for different gene-specific primers using gradient PCR, which were further used for cDNA amplification. Optimum annealing temperatures selected for amplifying specific genes for cDNA as a template are shown in Table 6.

Acknowledgement

Author thanks to guide Anil A. Kale sir, Associate Professor of Biochemistry, MPKV, Rahuri, Maharashtra (India) and also faculty colleague V.H. Badake & S.J. Mulani sir. Also thankful to my seniors and juniors.

References

Aljanabi, S.M., Forget L. and Dookun A. (1999). An improved and rapid protocol for the isolation of polysaccharide and polyphenol-free sugarcane DNA. *Plant Mol. Biol.Rep*., **17**, 1-8.

D'Hont, A., Grivet L., Feldmann P., Glaszmann J.C., Rao S. and Berrding N. (1996). Characterization of the double Genome structure of modern Sugarcane cultivar (*Saccharum* spp.) by molecular cytogenetics. *Mole. Gen. Genet*., **250**, 405-413.

Flowers, T.J. and Flowers S.A. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agric.Water Manage*., **78**, 15-24.

Gomathi, R., Vasantha S., Shiyamala S. and Rakkiyappan P. (2013). Differential accumulation of salt induced proteins in contrasting sugarcane genotypes. *EJBS*, **6(1)**.

Igarashi, Y., Yoshiba Y., Sananda Y., Yamaguchishinozaki K., Wada K. and Shinozaki K.(1997). Characterization of the gene for delta (1) pyrroline-5-carboxylate synthetase and correlation between the expression of the gene and salt tolerance in (*Oryza sativa* L.). *Plant Mol. Biol*., **33**, 857-865.

Jackson, M.L. (1973). *Soil chemical analysis*. Prentice Hall of India Pvt. Ltd, New Delhi. pp. 498.

Katterman, F.R.H. and Shattuck V.I. (1983). An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. *Prep. Bioch*., **13**, 347-359.

Khanuja, S.P.S., Shasany A.K., Darokar M.P. and Kumar S. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Mol. Biol. Rep*., **17**, 1-7.

Kim, C.S., Lee C.H., Shin J.S., Chung Y.S. and Hyung N.I. (1997). A simple and rapid method for isolation of high-quality genomic DNA from fruit trees and conifers using PVP. *Nucl. Acids Res*., **25**, 1085-1086.

Loomis, W.D. (1974). Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. *Methods in Enzymology*, **31**, 528-544.

Munns, R. and Termaat A. (1986). Whole plant response to salinity. *Aust. J. Pl. Physiol.*, **13**, 143-160.

Murray, H.G. and Thomson W.F. (1980). Rapid isolation of plant DNA: A fast, inexpensive and reliable method. *Plant Mol. Biol. Rep*., **10**, 60-65.

Pagariya, M.C., Devarumath R.M. and Kawar P.G. (2012). Biochemical characterization and identification of differentially expressed candidate genes in salt stressed sugarcane. *J. Plant Sci.*, **184**, 1– 13.

Porebski, S., Bailey L.G. and Baum B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep*., **15(1)**, 8-15.

Rao,V.P., Sengar R.S., Singh S. and Sharma V. (2015). Molecular and metabolic perspectives of sugarcane under salinity stress pressure. *Prog. Agric.*, **15 (1)**, 77-84.

Sairam, R.K. and Tyagi A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci*., **86**, 407-421.

Sharma, K., Mishra A.K. and Misra R.S. (2008). A simple and efficient method for extraction of genomic DNA from tropical tuber crops. *Afr. J. Biotechnol*., **7(8)**, 1018-1022.

Sreenivasan, T.V., Ahloowalia B.S. and Heinz D.J. (1987). Cytogenetics. In:Sugarcane Improvement Through Breeding (ed. by Heinz, D.J.). *Elsevier Amsterdam*, **7**, 211-253.

Triboush, S.O., Danilenko N.G. and Davydenko O.G. (1998). A method for isolation of chloroplast DNA and mitochondrial DNA from sunflower. *Plant Mol. Biol. Rep*., **16**, 183–189.

Zahra, A.R.F., Costa D.M., De Costa W.A. and De J.M. (2013). Identification of differentially-expressed genes in response to salt stress in the salt-tolerant Sri Lankan rice variety At354. **41(2)**, 93-112.