Homeopathic Intervention in Salinity-Stressed *Pennisetum glaucum*: Role of Natrum Muriaticum

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Abstract: The present investigation, conducted between 2022 and 2025 at IIS (Deemed to be University), aimed to evaluate the efficacy of homeopathic remedies *Natrum muriaticum* 6CH and 12CH in alleviating saline stress induced by 100 mM NaCl in *Pennisetum glaucum* (L.) R. Br. under in vitro conditions. The remedies were incorporated into Murashige and Skoog medium supplemented with appropriate hormones BAP and 2,4-D for callus induction, and IAA with kinetin for regeneration. Seed germination, callus formation, and regeneration were initiated in 11, 10, and 3 days respectively under treatment with the remedies. Morphological parameters such as root-to-shoot ratio and callus biomass, along with biochemical markers including chlorophyll a, chlorophyll b, total chlorophyll, total phenolic content, and DPPH radical scavenging activity, showed significant improvement. Gene expression analysis through quantitative RT PCR revealed modulation of stress-responsive genes (*NAC21* and *APX*) with *ACTIN* as the reference, confirming upregulation under treatment. Statistical analysis validated the reliability of these findings. Overall, both potencies of *Natrum muriaticum* demonstrated efficiency in ameliorating NaCl-induced salt stress, thereby enhancing seed germination, callus formation, and regeneration in pearl millet.

Keywords: Homeopathic, Seed germination, Natrum muriaticum, Remedy, Saline stress, Callus, Media

Introduction

Plants under saline stress are affected morphologically as well as physiologically which pauses their overall growth thus reducing their biomass. According to FAO, Land and Nutrition Management service 2015, over 7% of world's land is affected by salinity. Salinity may occur due to different ions present in soil like Na^+, Cl^-, CO_3^{2-}, SO^{2-}_4 divalent

ions Ca^{2+} and Mg^{2+}. Soil of a region is considered saline when concentration of salt is 40mM or more[2,1]. Salinity in agricultural land reduces quality and overall yield of crop due to various primary and secondary factors. It results in generation of reactive oxygen species and free radicals that can damage cell components like proteins, membrane lipids, carbohydrates and DNA structures [16,15]. Agro homeopathy is a new field where homeopathic remedies at different potencies are applied on plants to overcome different stress conditions and diseases[17]. In the present study, experiments were conducted on *Pennisetum glaucum*(L.) R. Br. utilizing homeopathic remedies to overcome stress.

Materials and methods

Present study was carried out during the year 2022-24 at Department of Botany, IIS (Deemed to be University), Jaipur, Rajasthan. Seeds of *Pennisetum glaucum* (L.) R. Br. variety Raj 171 were collected from Rajasthan Agriculture Research Institute, Jaipur, Rajasthan, India. The homeopathic remedies supplied by Dr. Willmar Schwabe India Pt. Ltd. were provided by Vipassana Homeopathy. All the experiments were carried out under in vitro conditions. Plant tissue culture is sensitive towards contamination so a thorough process of sterilization was carried out before starting the experiments.

Sterilization of Explant: Seeds were sterilized using distilled water to remove any dust particles. To avoid any surface contamination tween -20 was used followed by three washes with distilled water to remove any traces of detergent. All subsequent procedures were conducted under Laminar Air Flow to minimize the risk of microbial contamination. Seeds pre-treated with Tween-20 were then subjected to surface sterilization using 70% ethanol for one minute, followed by three washes with autoclaved distilled water. To further ensure sterility, seeds were soaked in 0.1% mercuric chloride ($HgCl_2$) solution for three minutes. This was followed by multiple rinses with autoclaved distilled water to remove any traces of the sterilant used.

Viability testing of seeds: To evaluate seed viability, a total of 60 seeds were inoculated across 15 sterile culture flasks, with 4 seeds placed in each flask. The viability test was conducted using hormone-free Murashige and Skoog (MS) medium. Seed germination was monitored as the primary indicator of viability, with successful radicle emergence considered evidence of metabolic activity and seed health.

Identification of toxic level of saline stress induced by NaCl: To identify the threshold level of salinity stress affecting seed germination, seeds were inoculated on Murashige and Skoog (MS) medium supplemented with varying concentrations of NaCl: 50 mM, 100 mM, 150 mM, and 200mM. Each treatment was conducted in triplicate to ensure statistical reliability. Germination responses were closely monitored across all concentrations to assess the impact of increasing salinity induced by NaCl.

Homeopathic preparations: To alleviate the adverse effects of salinity stress induced by 100 mM NaCl, Natrum muriaticum was applied as a biotherapeutic intervention at homeopathic potencies of 6CH and 12CH. The objective was to assess its potential in enhancing stress tolerance during seed germination. To determine the optimal working concentration of the remedy, seeds were inoculated on Murashige and Skoog (MS) medium supplemented with NaCl at the identified toxic level (100 mM), along with graded concentrations of Natrum muriaticum ranging from 0.1% to 1.2% for both potencies. Germination performance under these conditions was carefully monitored to evaluate the efficacy of each treatment in mitigating salt-induced stress.

Callus induction and subculture

For seed germination Murashige and Skoog media was used without any supplementation. Further for callus induction control medium was augmented with BAP (0.50mg/L) and 2,4-D (0.49 mg/L). Callus formed was sub cultured on media supplemented with 0.2 mg/L of 2,4-D for control. For seed inoculated under stress, callus subculture was done with 2,4-D at 0.2 mg/L along with NaCl at 100mM concentration.

Regeneration from callus: After first subculture, 100 mg (fresh weight) of callus was inoculated on media supplemented with IAA and Kinetin at 1mg/L and 0.5mg/L respectively. For regeneration under saline stress (NaCl 100mM) and to overcome stress Natrum muriaticum 6 CH and 12 CH were added in media separately and results were observed.

Chlorophyll estimation [Arnon method,12]-Chlorophyll content was estimated by taking 2 g of plant leaves, to it 10 ml of 80 % of acetone was added and blended for 2 minutes. Centrifuged at 3000 rpm for 10 min. Supernatant was collected and made up to 25 ml using 80% acetone. Absorbance was recorded at 645 nm for chlorophyll a and 652 nm for chlorophyll b.

Calculations: Chlorophyll a- (mg/g FW) = $(12.7A_{663} - 2.69A_{645}) * x/1000 * n$
Chlorophyll b- (mg/g FW) = $(22.9A_{645} - 4.68A_{663}) * x/1000 * n$
Total Chlorophyll content (mg/g FW) = $(20.2A_{645} + 8.02A_{663}) * x/1000 * n$

Proline estimation [3,23]**:** Proline content was estimated following a modified acid ninhydrin method. A 0.5 g sample of fresh plant tissue was homogenized in 10 mL of 3% sulfosalicylic acid. The homogenate was filtered, and 2 mL of the filtrate was mixed with 2 mL of acid ninhydrin solution (prepared in glacial acetic acid). The mixture was incubated at 100 °C for 1 hour. After cooling to room temperature, 4 mL of toluene was added, and the solution was vigorously shaken for 15–20 seconds to extract the chromophore into the organic phase. The upper toluene layer was carefully separated,

and its absorbance was measured at 520 nm using a spectrophotometer against a toluene blank.
L-proline was used as the standard. A stock solution of 500 µg in 100 mL distilled water was prepared, and serial dilutions ranging from 10 µg/mL to 100 µg/mL were used to generate the standard curve. The highest recorded absorbance corresponded to the 50 µg/mL standard.

DPPH antioxidant assay [13]- 975 µL DPPH solution (0.002g in 100ml methanol) was taken. To it 25 µL sample extract was added. Incubated for 30 min. and absorbance was recorded at 515 nm using spectrophotometer. For blank instead of sample 25 µL of methanol was used. Ascorbic acid was used to prepare the standard curve (1mg/ml). Different dilutions were prepared to plot the curve from 20 µL to 100 µL concentration. The percent radical scavenging activity was calculated using the formula:
%RSA= Control OD - Sample OD/ Control OD* 100

Estimation of total phenolic content [25]. A total of 5 ml reaction mixture was prepared consisting of 0.05 ml of sample with 3.95 ml of distilled water followed by 0.25ml of Folin ciocalteau reagent. To above reaction mixture 20% Na_2CO_3 was added and absorbance was recorded at 760nm using spectrophotometer. Standard curve was prepared by preparing dilutions of Gallic acid (1mg/ml in methanol) at dilution of 15µg-50 µg/ml (R^2 = 0.9919). Total phenolic content is expressed in terms of Gallic acid equivalent (mg GAE/g of extract).

RNA extraction and RT-q PCR analysis For RNA extraction NucleoSpin RNA Plant kit was used. Up to 100 mg of plant tissue was ground under liquid nitrogen until a fine powder was obtained, followed by the addition of 500 µL Buffer PFL and 10–50 µL Buffer PFR in a 1.5 mL micro centrifuge tube. The powdered sample was immediately transferred into the Buffer PFL–PFR mixture to ensure thawing occurred only within the lysis buffer, and the tube was incubated for 5 minutes at 56 °C. After centrifugation at 14,000 × g for 1 minute to sediment cell debris, the clear supernatant was loaded onto a NucleoSpin RNA Plant Filter Column (green ring) placed in a Collection Tube and centrifuged for 1 minute at 14,000 × g. The flow-through was mixed with 500 µL Buffer PFB, incubated for 5 minutes at room temperature, and subsequently loaded onto a NucleoSpin RNA Plant Column (light blue ring) preassembled with a Collection Tube. A total of 650 µL of the sample was applied, centrifuged for 30 seconds at 14,000 × g, and the flow-through discarded, followed by loading of the residual sample (~200 µL) and centrifugation under the same conditions. The column was then washed sequentially with 500 µL Buffer PFW1 and two washes of 500 µL Buffer PFW2, each centrifuged for 1 minute at 14,000 × g, with collection tubes replaced as required. Finally, the column was inserted into a fresh 1.5 mL Collection Tube, and RNA was eluted by adding 50 µL RNase-free water, incubating for 1 minute at room temperature,

and centrifuging for 1 minute at 14,000 × g. The resulting RNA eluate was handled with care to avoid RNase contamination and stored at –20 °C for short-term or –70 °C for long-term preservation.

Real Time PCR Reaction Preparation

Name of the Reagent	1 RXN
2X One Step TB Green RT-PCR Buffer 4	10 µl
PrimeScript 1 step Enzyme Mix 2	0.8 µl
PCR Forward Primer (10 µM)	0.8 µl
PCR Reverse Primer (10 µM)	0.8 µl
ROX Reference Dye or Dye II (50X)	0.4 µl
RNase Free dH2O	5.2 µl
Total	18 µl

Up to18 µl of the above prepared Reaction mix was Transferred in 0.2 ml PCR tubes and tubes were closed. Then 2 µl of the earlier extracted RNA was added to each sample tube and mix well by pipetting up and down.

Calculation of ΔCq values with reference to ACTIN gene:

ΔCq (Gene – Reference)

Calculation of Fold Change: fold change was calculated using the formula

[\text {Fold Change} = 2^ {-\Delta\Delta Cq}]

Experimental design

The experiment was conducted to evaluate the effect of salinity stress on seed germination, callus formation and regeneration. The entire experiment was structured into three distinct phases to systematically evaluate seed viability, induce callus formation, and achieve plant regeneration. Initially, a seed viability test was conducted by inoculating seeds using plain Murashige and Skoog (MS) medium devoid of any hormonal supplementation. Germination was used as the primary indicator of viability. In the second phase, viable seeds were transferred to a callus induction medium composed of MS supplemented with 0.50 mg/L BAP (6-Benzylaminopurine) and 0.49 mg/L 2,4-D (2,4-Dichlorophenoxyacetic acid), which served as the control for callus formation. The third phase focused on plant regeneration, where calli were cultured on MS medium enriched with 1.0 mg/L IAA (Indole-3-acetic acid) and 0.5 mg/L Kinetin to promote shoot and root development. Each phase was carefully monitored to ensure consistency and reliability in assessing the biological responses under controlled conditions. Morphological, biochemical and molecular analysis were done to analyse the effect of Natrum muriaticum (6CH and12CH). Root to shoot ratio and callus weight were recorded as morphological parameters, while proline content, chlorophyll a, chlorophyll b, total chlorophyll content, DPPH antioxidant activity, and total phenolic

content were measured as biochemical parameters to assess the efficacy of the homeopathic remedies. To evaluate their effect at the molecular level, the expression of *NAC21* and *APX* genes was analyzed through RNA isolation and RT-qPCR, using *ACTIN* as the reference gene. Statistical analysis was performed using one-way ANOVA followed by paired t-tests to determine significant differences among treatments ($p < 0.05$).

Results and Discussion

Seed Germination and Viability Assessment

Seeds inoculated on Murashige and Skoog (MS) medium exhibited rapid germination within 3–4 days. Among the 60 seeds cultured on basal medium, a germination rate of 88.30% was recorded, indicating high seed viability Figure 1(A).

Effect of different concentration of salinity stress on seed germination-

Seed germination was adversely affected by NaCl concentrations of 100 mM and above, as evident from the results presented in Figure 1(B). This threshold was identified as the critical point beyond which salinity stress significantly impairs germination. Consequently, a concentration of 100 mM NaCl was selected as the toxic level for subsequent experiments.

Active concentration of remedy: After working at different concentrations of homeopathic remedies (NM 6CH and 12CH) from 0.1% to 1.2%, it was observed that seeds germinated well with 0.1%, 0.2% and 0.3% of remedy. At higher concentrations than this, no germination was observed. Best results were obtained at 0.1% of remedy and so considered as standard for conducting all the experiments.

Effect of Natrum muriaticum on seed germination under salinity stress*:* Under control conditions, seed germination took place in 3-4 daysFigure 1(A) while under stress (100mM NaCl), the seeds could not germinate Figure 1(B). To overcome this stress, the seeds were inoculated on MS media supplemented with 100mM NaCl and Natrum muriaticum 6 CH and Natrum muriaticum 12 CH individually with 0.1% concentration. It was observed that germination was achieved even at the toxic level of salinity i.e. 100mM NaCl. Germination of seeds can be observed in the Figure 1(D) and (E).

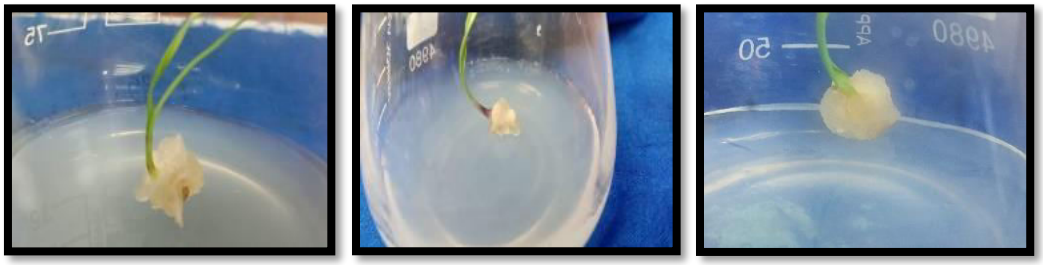
A B C

D E

Figure 1 (A) Viability testing of seeds, (B) No germination at 100mM NaCl, (C) Seed germination under control in the absence of 100mM NaCl. (D) Seed germination after 20 days with NM 6 CH at 100mM NaCl, (E) Seed germination after 20 days with NM 12 CH at 100mM NaCl

Effect of Natrum muriaticum on callus formation and regeneration under salinity stress

Under the influence of NM 6 CH and 12 CH, callus formation was initiated 10 days after seed inoculation in the presence of 100 mM NaCl, as well as in the control group, as depicted in Figure 3 (A, B, C). A substantial amount of callus was obtained, as detailed in Table 1. Approximately 100 mg of callus tissue was subsequently transferred to regeneration media, where regeneration began to appear within three days of inoculation, as illustrated in Figure 4 (A, B, C).



A B C

Figure 2(A): Callus formation under control conditions, (B) Callus formation after overcoming stress by the application of NM 6 CH(C) Callus formation after overcoming stress by the application of NM 12 CH

A B C

Figure.3: Regeneration observed with (A) control (B) Natrum muriaticum 6 CH + 100Mm NaCl (C) Natrum muriaticum 12 CH + 100Mm NaCl

Biochemical Analysis

Table 1 Summarizes key morphological parameters, root to shoot ratio and callus weight used to evaluate the impact of homeopathic treatments NM 6 CH and NM 12 CH under saline stress conditions (100 mM NaCl). As illustrated in Figure 4 (A), (B), seedlings were isolated from various flasks and analysed for root to shoot development. Seedlings treated with NM 6 CH exhibited the highest root to shoot ratio (3.45 ± 1.63), significantly outperforming both the control group (1.69 ± 0.26) and NM 12 CH treatment (2.05 ± 0.39). These results are consistent with findings byRodriguez et al.,2022, who reported enhanced root architecture in *Salicornia bigelovii* under hydroponic conditions. In contrast, callus formation was most pronounced in the control group (405.66 ± 24.00 mg), while both NM 6 CH (311.14 ± 11.42 mg) and NM 12 CH (299.05 ± 13.59 mg) treatments resulted in comparable values of callus weight under saline stress (100 mM NaCl) which are in accordance with the studies conducted by Klay et al.,2024.

(A) NM 12 CH (B) NM 6CH

Figure 4: Isolated seedlings subjected to 100mM NaCl and homeopathic remedies

Table 1: Morphological parameters to assess efficacy of NM 6 CH and 12 CH against saline stress

S. No.	Morphological Parameters	Plant Samples		
		Control	Natrum muriaticum 6 CH	Natrum muriaticum 12 CH
1.	Root-shoot ratio	1.69±0.26	3.45 ± 1.63**	2.05 ± 0.39***
2.	Callus weight	405.66 ± 24.00	311.14 ± 11.42***	299.05 ± 13.59**

Table 2 Presents the proline content across different plant tissuesat 50 µg/mL standard under saline stress conditions, highlighting the effects of homeopathic treatments NM 6 CH and NM 12 CH. In the absence of stress, proline levels in the control group were notably low. However, under treatment, distinct variations were observed across stem,

leaf, and root tissues. In stems, proline accumulation was comparable between NM 6 CH (0.167 ± 0.10) and NM 12 CH (0.169 ± 0.019). Leaf tissues exhibited the highest proline concentrations, with NM 6 CH (0.36 ± 0.03) and NM 12 CH (0.32 ± 0.07) showing similar levels. This elevated proline content in leaves aligns with previous studies[8,19] which have consistently reported leaf tissues as primary sites for Osmo protectant accumulation under stress. Root tissues displayed a different trend, with NM 12 CH (0.217 ± 0.019) inducing higher proline levels than NM 6 CH (0.148 ± 0.012) and the control (0.137 ± 0.005). These findings suggest that NM 6 CH may be more effective in enhancing root-level stress tolerance, while both treatments contribute significantly to proline accumulation in aerial parts, particularly leaves.

Table 2: Proline content (µ moles /g FW, Mean ± S.D.)

	Control	NM 6 CH	NM 12 CH
Root	0.137 ± 0.005	0.148 ± 0.012*	0.217 ± 0.019**
Stem	0.0018 ± 0.00	0.167 ± 0.10***	*** 0.169 ± 0.019
Leaf	0.020 ± 0.004	0.36 ± 0.03***	0.32 ± 0.07**

NM: Natrum muriaticum

Table 3. Summarizes the chlorophyll content- chlorophyll a, chlorophyll b, and total chlorophyll expressed in mg/g fresh weight across regenerated plant samples treated with NM 6CH and NM 12CH. Chlorophyll levels were significantly higher in NM 6CH (0.558 ± 0.013) compared to NM 12CH (0.445 ± 0.005), indicating a stronger retention of primary photosynthetic pigment under this treatment. Conversely, chlorophyll b was more abundant in NM 12CH (0.146 ± 0.005) than in NM 6CH (0.068 ± 0.0046).The total chlorophyll content was comparable for both the remedies i.e. NM 12 CH (22.18 ± 0.01) and NM 6 CH (21.13± 0.01). These results reflect a decline in chlorophyll concentration under stress conditions relative to the control, consistent with findings reported by Rashid et al., 2024.

Table 3: Chlorophyll content (mg/g FW, mean ± S.D.) under saline stress after treatment with NM 6CH and 12 CH compared to control.

	Control	**NM 6 CH**	**NM 12 CH**
Chl a	0.713 ± 0.0076	0.558 ± 0.013**	0.445 ± 0.005***
Chl b	0.634 ± 0.004	0.068 ± 0.0046***	0.146 ± 0.005***
Total chl	45.96 ± 0.01	21.13 ± 0.036*	22.18 ± 0.01**

Chl: Chlorophyll

Table 4 indicates % Radical scavenging activity of standard and samples treated with NM 6 CH and NM 12 CH under saline stress at selected dilutions against standard curve of ascorbic acid. Standard here refers to the values obtained by preparing dilutions of

Ascorbic acid. On most of the dilutions of root, stem and leaf extract the values were recorded higher for NM 12 CH. The highest value for % RSA was observed at 100 µL (88.83±0.18for root, 87.27±0.75 for stem, 84.87±0.48 for leaf) than NM 6 CH (88.83±0.18 for root, 83.29±0.68 for stem and 80.60±0.12 for leaf). These findings indicate that both NM 6 CH and NM 12 CH enhance radical scavenging activity under saline stress, with NM 12 CH demonstrating a more pronounced effect. This increase in antioxidant potential aligns with previous studies[6], which suggest that plants upregulate radical scavenging mechanisms under high stress conditions to mitigate the damaging effects of reactive oxygen species (ROS).

Table 4: % Radical scavenging activity of different test samples

Name of plant part		(Dilutions in µL) % RSA with different dilutions of metabolite extract				
		20	40	60	80	100
Root	Standard	65.20±1.01	72.75±1.10	80.87±0.61	88.41±0.98	92.88±0.41
	NM 6 CH	63.18±0.60*	74.56±0.64*	77.75±0.54*	78.95±0.42**	86.48±0.60*
	NM 12 CH	51.21±1.00**	66.72±0.49**	79.17±0.68ns	83.40±0.39**	88.83±0.18***
	NM 6 CH	46.33±0.55***	52.38±0.63***	68.63±0.56***	79.42±0.51***	83.29±0.68**
	NM 12 CH	43.79±0.57***	53.90±0.51***	73.56±0.34***	81.06±0.05***	87.27±0.75***
	NM 6 CH	54.63±0.24***	55.32±0.49***	65.16±0.62***	68.00±0.39ns	80.60±0.12***
	NM 12 CH	57.92±0.36***	60.44±0.55***	62.84±0.55***	74.99±0.60***	84.87±0.48***

Table 5 Indicate the values of total phenolic content as recorded with extract of root, stem and leaves of regenerated plants. The values of root and leaves were found to be higher forNM 6 CH (42.33±0.97and56.82±0.01) than NM 12 CH (28.17±5.39and30.32±0.02)respectively. Thevalues for stem were found to be similar for both the remedies24.64±0.01. The results of TPC are in accordance with the studies done byBitsgani et al.,2019.

Table 5: Total Phenolic Content in mg GAE/g of plant extract.

	Control	NM 6 CH	NM 12 CH
Root	7.20±0.53	42.33±0.97***	28.17±5.39**
Stem	51.9±0.55	24.64±0.01***	24.15±0.65***
Leaf	8.63±1.24	56.82±0.01***	30.32±0.02***

All experiments were conducted in triplicates. Values are represented as Mean ± SD. Paired T test was conducted in respect to control for each parameter. In each parameter, comparison in all 3 groups was done by one way ANOVA. Significance levels are represented as *** ($p<0.001$); ** ($p<0.01$); * ($p<0.05$); ns ($p>0.05$).

Gene expression analysis

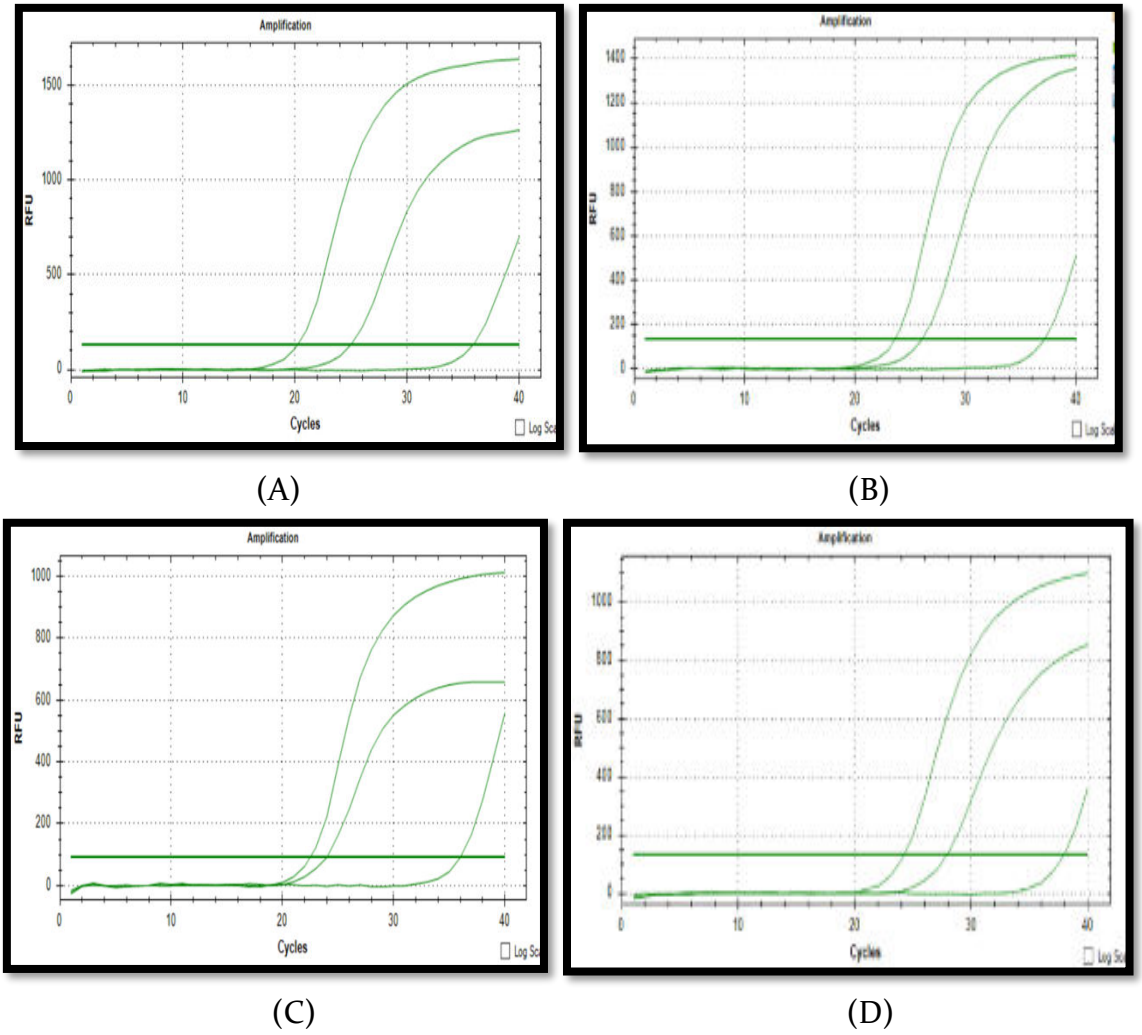


Figure 5: Amplification curves of stress-responsive genes in *Pennisetum glaucum* under saline stress (100 mM NaCl). The X-axis represents PCR cycles, while the Y-axis indicates relative fluorescence units (RFU). All curves demonstrate successful amplification, confirming detectable transcript levels of the target genes. (A) NM 12CH-treated shoot, (B) NM 12CH-treated root, (C) NM 6CH-treated shoot, and (D) NM 6CH-treated root.

X-axis in the plot represents PCR cycles (0 to 40) and Y-axis represent RFU (Relative Fluorescence Units, 0 to 1500). The steepness and early rise of a curve indicate high target abundance. The later the curve crosses the threshold, the lower the initial template concentration.

Table 6: Cq values from RTPCRof different target genes

S.No.	Sample	Target Gene	Cq Value	Cqwith reference to ACTIN- ΔCq = Cq(Target) – Cq(Reference)	Fold change	Interpretation
1.	NM 12CH shoot	ACTIN	25.01			Moderate expression
		NAC 21	20.24	–4.77	27	High expression
		APX	35.92	+10.91	0.0002	Very low expression
2.	NM 12CH root	ACTIN	26.07			Moderate expression
		NAC 21	23.58	–2.49	5.6	High expression
		APX	37.13	11.6	0.00047	Very low expression
3.	NM 6CH shoot	ACTIN	24.01			Moderate expression
		NAC 21	22.49	-1.52	2.87	Higher expression than ACTIN
		APX	36.00	11.99	0.00024	Very low expression
4.	NM 6CH root	ACTIN	27.94			Moderate expression
		NAC 21	24.23	-3.71	13.2	Higher expression than ACTIN
		APX	37.99	10.05	0.00093	Very low expression

Table 6 summarizes the Cq values obtained from RT-qPCR analysis of stress-responsive genes in *Pennisetum glaucum*. Quantitative RT-qPCR was performed to evaluate the relative expression of *NAC 21* and *APX* genes, with *ACTIN* serving as the internal reference.In the NM 12CH shoot, *NAC 21* exhibited strong transcriptional activation, with a Cq of 20.24 corresponding to ~27-fold upregulation relative to *ACTIN*. In contrast, *APX* showed late amplification (Cq 35.92), indicating substantially reduced transcript accumulation. Similarly, in the NM 12CH root under saline stress, *NAC 21* maintained strong expression (Cq 26.07), whereas *APX* was weakly expressed (Cq 37.13), consistent with minimal antioxidant response.

In the NM 6CH root, *NAC 21* was ~5.6-fold more expressed than *ACTIN*, confirming robust upregulation, while *APX* remained low, suggesting either minimal oxidative stress or delayed induction. In the NM 6CH shoot, *NAC 21* (Cq 22.49) showed ~2.9-fold higher expression than *ACTIN*, again supporting transcriptional activation. By contrast, *APX* (Cq 36) was weakly expressed. Notably, in the NM 6CH root, *NAC 21* (Cq 24.23) displayed ~13.2-fold higher expression than *ACTIN*, underscoring strong transcriptional activation, whereas *APX* (Cq 37.99) was nearly silent, suggesting transcriptional repression or negligible oxidative stress response. The stability of *ACTIN* across all samples validates its suitability as a normalization reference and supports the reliability of the data. Overall, these findings demonstrate that *NAC 21* is consistently upregulated under saline stress, whereas *APX* expression remains low, highlighting differential regulation of transcriptional and antioxidant pathways in *Pennisetum glaucum*. Previous studies in *Arabidopsis* have demonstrated that under saline stress, the expression of *pgNAC21* is significantly upregulated, thereby enhancing tolerance to salinity [24]. The NAC gene family is widely recognized for its strong adaptive responses to high salinity as well as a broad range of abiotic and biotic stresses [9]. Similarly, earlier reports have highlighted the critical role of *APX* (ascorbate peroxidase) in mitigating oxidative stress, with its expression typically elevated under conditions of high reactive oxygen species accumulation.

In contrast, the present study revealed consistently low *APX* transcript levels. This reduced expression may be attributed to the maintenance of cellular homeostasis facilitated by the applied homeopathic medicament, which likely minimized oxidative stress to a level where *APX* induction was not required. Such findings suggest that the medicament contributed to stabilizing redox balance, thereby reducing the necessity for a pronounced antioxidant response[22, 4].

Conclusion

The present study reinforces the potential of homeopathic remedies, particularly *Natrum muriaticum*, in mitigating saline stress and enhancing seed viability under adverse conditions. By incorporating ultra-dilutions directly into plant tissue culture media, a novel approach was demonstrated that yielded promising results in promoting vegetative growth and maintaining plant homeostasis. This method offers a viable alternative to conventional applications such as foliar spraying or seed priming, suggesting broader applicability in soil-based cultivation systems. Consistent with previous findings[7,26,14,20, 10], the study highlights that different potencies of homeopathic preparations exert varied effects across plant species, particularly under saline stress. The observed improvements in stem height, leaf development, flowering, and fruiting underscore the efficacy of these remedies in enhancing overall plant vigor.

Given the escalating challenges posed by climate change and soil degradation, the integration of environment friendly, cost-effective, and easily accessible solutions like homeopathic treatments presents a sustainable strategy for agricultural resilience.

Future studies should focus on acclimatization trials under natural field conditions to validate laboratory findings and establish practical protocols for large-scale application.

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Declaration of competing interest
The authors declare that there is no competing interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collected, analysis, decision to publish, or preparation of manuscript.

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