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Modulation of Salinity Stress Responses in *Pennisetum glaucum* by Homeopathic Phosphorus

Kalpana Agarwal and Pragya Dhakar*
Department of Botany, IIS (Deemed to be University), Gurukul Marg, SFS, Jaipur- 302020, India
*Correspondence for materials should be addressed to PD (email: pragyashu23@gmail.com)

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Abstract
Plant under saline stress show redundant growth or no germination. The present study was carried out to overcome this effect of salinity using homeopathic Phosphorus 6CH and 12CH on *Pennisetum glaucum* (L.) R. Br variety Raj 171. This study evaluates the potential of homeopathic Phosphorus 6CH and 12CH to mitigate salinity-induced growth inhibition. The toxicity threshold was determined by culturing seeds on Murashige and Skoog (MS) medium supplemented with 50–200 mM NaCl; 100 mM NaCl was identified as the critical level inhibiting germination. To assess stress alleviation, Phosphorus 6CH and 12CH were individually incorporated into MS medium under aseptic conditions. Seeds were surface sterilized and inoculated in seven replicates per treatment. Germination occurred only with Phosphorus 12CH, initiating on day 3, followed by callus formation (day 10) and plant regeneration (day 15). No germination was observed in control or Phosphorus 6CH treatments under saline stress. Regenerated plantlets were subjected to morphological and biochemical analyses, including chlorophyll a, b, total chlorophyll, total phenolic content, DPPH radical scavenging activity, seedling vigour index, root length, and plant height. From various experiments, it was concluded that Phosphorus 12CH enhances in vitro regeneration under saline stress.

Keywords: Seed; Germination; Phosphorus; Homeopathic; Salinity; Stress

Introduction

Seed germination is a fundamental biological process that marks the beginning of a plant's life cycle. It originates from the double fertilization of the ovule, resulting in a seed that encapsulates the potential to develop into a complete plant. Germination is considered successful when the seed gives rise to a viable embryo capable of sustained growth. This process is regulated by a complex interplay of physiological, biochemical, and environmental factors (Ranal and Santana, 2006; Ghadirnezhad et al., 2024; Zhang et al., 2025). Among the key regulators of germination are plant hormones such as abscisic acid (ABA), gibberellins (GA), auxins, and ethylene. Following imbibition, seeds must overcome the inhibitory effects of ABA, primarily through the synthesis of GA, which promotes germination by mobilizing stored reserves and suppressing inhibitory proteins. Additionally, seed coat structure plays a critical role; lignification of the pericarp can restrict oxygen and water availability, thereby impeding embryo development. These structural variations are often species-specific (Vishal and Kumar, 2018a; Shu et al., 2018b; Liu and Hou, 2018c).

Abiotic stresses such as salinity, drought, and low temperature pose significant challenges to seed germination, often reducing seed viability by up to 50% in major crops. Under such conditions, oxidative stress leads to the accumulation of reactive oxygen species (ROS), which damage cellular components including DNA, proteins, and lipids, thereby delaying or inhibiting germination (Ali and Elozeiri, 2017). Nevertheless, seeds possess adaptive mechanisms to maintain osmotic balance and water content, enabling survival under stress. Prolonged exposure to adverse conditions may induce dormancy due to elevated abscisic acid (ABA) levels, adversely affecting crop establishment

and yield. Conversely, favourable conditions stimulate gibberellic acid (GA) synthesis, counteracting ABA and restoring germination potential (Ravindran et al., 2020).

In light of these challenges, alternative approaches to mitigating abiotic stress effects are being explored. The present study investigates the efficacy of homeopathic remedies, specifically Phosphorus 6CH and 12CH, in alleviating salinity-induced stress in *Pennisetum glaucum* (L.) R. Br. var. Raj 171. By integrating homeopathic treatments into *in vitro* culture systems, this research aims to enhance seed germination and regeneration under saline conditions, offering a novel strategy for stress management in crop production.

Materials and methodology

The present study was conducted during 2022–2025 in the Department of Botany, IIS (Deemed to be University), Jaipur, Rajasthan, India. Seeds of *Pennisetum glaucum* (L.) R. Br. variety Raj 171 was procured from the Rajasthan Agriculture Research Institute, Jaipur. Homeopathic remedies (Phosphorus 6CH and 12CH), manufactured by Dr. Willmar Schwabe India Pvt. Ltd., were supplied through Vipassana Homeopathy.

All experiments were performed under *in vitro* conditions using standard plant tissue culture protocols. Given the sensitivity of tissue culture systems to microbial contamination, rigorous sterilization procedures were employed prior to seed inoculation.Seeds were initially rinsed with distilled water to remove surface dust. To eliminate microbial contaminants, seeds were treated with Tween-20, followed by three washes with distilled water to remove detergent residues. All subsequent steps were carried out under aseptic conditions in a laminar airflow cabinet. Seeds were then immersed in 70% ethanol for one minute, followed by three rinses with autoclaved distilled water. Final sterilization was achieved using 0.1% mercuric chloride ($HgCl_2$) for three minutes, followed by multiple washes with autoclaved distilled water to eliminate residual sterilant.

Seed Viability Assessment

To assess seed viability, seeds were inoculated into sterile culture flasks, with four seeds per flask. The viability test was conducted on hormone-free Murashige and Skoog (MS) medium. Germination was monitored, and radicle emergence was considered a reliable indicator of metabolic activity and seed viability.

Determination of Salinity Stress Threshold

To identify the toxic level of salinity stress, seeds were cultured on MS medium supplemented with varying concentrations of NaCl: 50 mM, 100 mM, 150 mM, and 200 mM. Each treatment was replicated three times to ensure statistical robustness. Germination rates were recorded to evaluate the impact of increasing salinity levels. A concentration of 100 mM NaCl was identified as the threshold at which germination ceased and was thus considered toxic.

Application of Homeopathic Remedies

To mitigate the adverse effects of salinity stress (100 mM NaCl), Phosphorus was applied as a biotherapeutic agent at homeopathic potencies of 6CH and 12CH. The objective was to evaluate its efficacy in enhancing stress tolerance during seed germination. Seeds were inoculated on MS medium supplemented with 100 mM NaCl and graded concentrations of Phosphorus (ranging from 0.25 µL to 3 mL per 250 mL of medium) for both potencies. Germination performance was systematically monitored to determine the optimal concentration and potency for stress alleviation.

Callus Induction and Subculture

Seeds were germinated on Murashige and Skoog (MS) basal medium without any hormonal supplementation. For callus induction under control conditions, MS medium was supplemented with 0.50 mg/L 6-Benzylaminopurine (BAP) and 0.49 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D). Induced calli were subsequently subcultured on MS medium containing 0.2 mg/L 2,4-D.

Stress Treatment: For seeds inoculated under saline stress, callus subculture was performed on MS medium supplemented with 0.2 mg/L 2,4-D and 100 mM NaCl.

Regeneration from Callus

Following the first subculture, 100 mg (fresh weight) of callus was transferred to regeneration medium containing 1 mg/L indole-3-acetic acid (IAA) and 0.5 mg/L kinetin. For regeneration under saline stress (100 mM NaCl), the medium was further supplemented with homeopathic phosphorus preparations at two potencies: Phosphorus 6CH and Phosphorus 12CH. Regeneration outcomes were recorded separately for each treatment.

The Seedling Vigor Index (SVI) was then calculated using the following formula under varied sampling conditions:
SVI = Average plumule length + Average radicle length x germination percentage (Sharma 2018).

Chlorophyll Estimation
Chlorophyll content was estimated following the Arnon method as modified by Manolopoulou et al. (2016). Fresh leaf tissue (2 g) was homogenized in 10 mL of 80% acetone for 2 minutes. The homogenate was centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected and volume-adjusted to 25 mL using 80% acetone.

Spectrophotometric Analysis: Absorbance was recorded at 645 nm and 663 nm using a UV-Vis spectrophotometer.

Calculations:

$$\textbf{Chlorophyll a- (mg/g FW)} = (12.7A_{663} - 2.69A_{645}) * x/1000 * n$$

$$\textbf{Chlorophyll b- (mg/g FW)} = (22.9A_{645} - 4.68A_{663}) * x/1000 * n$$

$$\textbf{Total Chlorophyll content (mg/g FW)} = (20.2A_{645} + 8.02A_{663}) * x/1000 * n$$

Where:
- (A {645}) and (A {663}) are absorbance values at respective wavelengths,
- (x) is the final volume of extract (mL),
- (n) is the dilution factor or sample weight (g).

DPPH Radical Scavenging Assay
The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as described by Marinova and Batchvarov (2011), with slight modifications. A total of 975 µL of DPPH solution (0.002 g DPPH dissolved in 100 mL methanol) was mixed with 25 µL of the sample extract. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 515 nm using a UV-Vis spectrophotometer. A blank was prepared by replacing the sample extract with 25 µL of methanol. A standard calibration curve was constructed using ascorbic acid (1 mg/mL in methanol) at varying volumes (20–100 µL). The percentage of radical scavenging activity (%RSA) was calculated using the following formula

$$\%RSA = \text{Control OD - Sample OD/ Control OD* 100}$$

***Estimation of total phenolic content* (Singleton et al., 1999)**
Total phenolic content (TPC) was determined following the Folin–Ciocalteu method as described by (Singleton et al. 1999). A 5 mL reaction mixture was prepared by combining 0.05 mL of the sample extract with 3.95 mL of distilled water and 0.25 mL of Folin–Ciocalteu reagent. Subsequently, 0.75 mL of 20% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated at room temperature, and absorbance was recorded at 760 nm using a spectrophotometer. A standard curve was generated using gallic acid (1 mg/mL in methanol) at concentrations ranging from 15 to 50 µg/mL (R^2 = 0.9919). Results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

Results and discussion
Seed Germination and Viability Assessment
Initiation of germination response of seeds inoculated on Murashige and Skoog (MS) medium was observed within 5–6 days. Among the 60 seeds cultured on basal medium, a germination rate of 88% was recorded, indicating high seed viability.

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 testing different concentrations of homeopathic remedies (NM 6CH and 12CH) ranging from 0.1% to 1.2%, it was observed that seeds germinated well at 0.1%, 0.2%, and 0.3%. At higher concentrations, no germination was observed. The best results were

obtained at 0.1%, which was therefore considered the standard concentration for conducting all experiments.

Effect of Natrum muriaticum on seed germination under salinity stress: Under control conditions, seed germination occurred within 5–6 days (Figure 1(A)), while under stress (100 mM NaCl), seeds failed to germinate (Figure 1(B)). To overcome this stress, seeds were inoculated on MS medium supplemented with 100 mM NaCl and Phosphorus 6CH or 12CH individually at 0.1% concentration. Germination was achieved even at the toxic level of salinity (100 mM NaCl), as shown in Figure 1(D) and Figure 1(E).

Table 1. Morphological parameters

S.No.	Sample	Seedling vigour index (Mean ± S.D.)	Root length (Mean ± S.D.)	Plant height (Mean ± S.D.)
1	Control	2.37 ± 0.23	1.69 ± 0.26	1 ± 0.0
2	P 6CH	NIL	NIL	NIL
3	P12CH	0.54 ± 0.2	5.04 ± 2.40	1 ± 0.0

As presented in Table 1, the seedling vigour index under control conditions (2.37 ± 0.23) was markedly higher compared to that observed with P12CH treatment (0.54 ± 0.20). The application of P 6CH did not demonstrate efficacy in mitigating stress-induced effects. Notably, in the absence of P12CH, seeds failed to germinate under stress conditions, underscoring the severity of the abiotic challenge. Stress exposure typically results in significant reductions in root and shoot length; however, treatment with P12CH elicited a positive response, counteracting the expected decline. This outcome contrasts with previous findings, which reported a consistent reduction in growth parameters under similar stress conditions (Sen et al., 2018; Shiade and Boelt, 2020).

Table 2. Chlorophyll content (mg/g FW, mean ± S.D.) under drought stress after treatment with Phosphorus 6CH and 12CH compared to control.

Parameter	Control	Phosphorus 6 CH	Phosphorus 12 CH
Chlorophyll a	0.71 ± 0.007	NIL	0.545 ± 0.01
Chlorophyll b	0.64 ± 0.013	NIL	0.0687 ± 0.005
Total chlorophyll	46.18 ± 0.20	NIL	21.02 ± 0.02

As presented in Table 1, the seedling vigour index under control conditions (2.37 ± 0.23) was markedly higher compared to that observed with P12CH treatment (0.54 ± 0.20). The application of P 6CH did not demonstrate efficacy in mitigating stress-induced effects. Notably, in the absence of P12CH, seeds failed to germinate under stress conditions, underscoring the severity of the abiotic challenge. Stress exposure typically results in significant reductions in root and shoot length; however, treatment with P12CH elicited a positive response, counteracting the expected decline. This outcome contrasts with previous findings, which reported a consistent reduction in growth parameters under similar stress conditions (Sen et al., 2018; Shiade and Boelt, 2020).

Table 3 presents the percent radical scavenging activity (%RSA) of plant samples treated with P 6CH and P12CH in comparison to the standard. According to Chaudhry et al. (2024) and Nadarajah (2020), radical scavenging activity tends to increase under elevated stress conditions as a physiological response to counteract excessive reactive oxygen species (ROS). Consistent with this, the current results demonstrate a notable enhancement in %RSA for stressed plants treated with P12CH (Root – 89.74 ± 0.5, Stem – 89.97 ± 0.15, Leaves – 90.56 ± 0.18), with the highest activity observed at 100 µL DPPH concentration. This suggests that P12CH may play a role in augmenting antioxidant defence mechanisms under abiotic stress.

Table 4 reveals a substantial increase in total phenolic content in plants treated with P12CH compared to the control. In root tissues, P 12CH-treated samples recorded 68.51 ± 0.015, exceeding the control value of 56.28 ± 0.005. This trend was even more pronounced in leaf tissues, where P 12CH treatment resulted in 73.54 ± 0.006, markedly higher than the control (25.52 ± 0.005). These findings suggest that P12CH enhances phenolic accumulation under stress conditions, potentially contributing to improved antioxidant defence. This observation aligns with previous studies indicating that phenolic content tends to increase in response to abiotic stress as part of the plant's adaptive mechanism (Bistgani et al., 2019).

Table 3. Percentage radical scavenging activity of different test samples

Name of plant part	Homeopathic Treatment	(Dilutions in µL) % RSA with different dilutions of metabolite extract				
		20	40	60	80	100
Root	Standard	65.34 ± 0.33	73.44 ± 0.42	83.28 ± 4.30	88.53 ± 0.37	93.62 ± 0.3
	Phosphorus 6 CH	NIL	NIL	NIL	NIL	NIL
	Phosphorus 12 CH	71.3 ± 0.21	78.49± 0.43	81.72 ± 0.46	85.45± 0.42	89.74 ± 0.5
Stem	Standard	65.34 ± 0.33	73.44 ± 0.42	83.28 ± 4.30	88.53 ± 0.27	93.62 ± 0.32
	Phosphorus 6 CH	NIL	NIL	NIL	NIL	NIL
	Phosphorus 12 CH	67.99 ± 0.32	71.09 ± 0.24	78.27 ± 0.18	83.29 ± 0.12	89.97 ± 0.15
Leaves	Standard	65.34 ± 0.33	73.44 ± 0.42	83.28 ± 4.30	88.53 ± 0.37	93.62 ± 0.32
	Phosphorus 6 CH	NIL	NIL	NIL	NIL	NIL
	Phosphorus 12 CH	74.97 ± 0.32	84.1 ± 0.24	84.97 ± 0.15	85.12 ± 0.12	90.56 ± 0.18

Table 4. TPC (mg GAE/g of extract) in regenerated plants.

Parameter	Control	Phosphorus 6 CH	Phosphorus 12 CH
Root	56.28 ± 0.005	NIL	68.51 ± 0.015
Stem	43.48 ± 0.005	NIL	34.25 ± 0.005
Leaf	25.52 ± 0.005	NIL	73.54 ± 0.006

TPC- Total phenolic content

Conclusion

The present study highlights agrohomeopathy as a promising eco-friendly alternative for mitigating the adverse effects of salinity stress in *Pennisetum glaucum*. In contrast to conventional approaches that often compromise soil health and require intensive inputs, homeopathic treatments such as Phosphorus 12CH demonstrated potential in enhancing seed germination, plant regeneration, and biochemical resilience under stress conditions. The observed improvements in antioxidant activity, chlorophyll retention, and phenolic accumulation underscore the therapeutic efficacy of these remedies. Given their minimal ecological footprint and ease of application, agrohomeopathic interventions warrant further validation through extensive field trials to establish standardized protocols and broaden their applicability across diverse agro-climatic zones.

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Author Contributions
KA and PD conceived the concept, wrote and approved the manuscript.

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