



# Optimization of pre-sowing treatments to break seed dormancy in wild finger millet (*Eleusine africana* L) for enhanced conservation and utilization of genetic resources

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Received: 3 September 2025 / Accepted: 16 October 2025 / Published online: 11 December 2025  
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**Abstract** Crop wild relatives of finger millet, a part of its genetic resources, are key to exploring the genetic potential of the crop for several climate-resilient and nutritional traits. Seed dormancy in wild *species* accessions poses a significant challenge to the conservation, evaluation, distribution, and their utilization in pre-breeding activities. In the present study, we tested 12 treatments to break dormancy and enhance germination in two wild accessions (IE 8414 and IE 8416). Germination of these two accessions under control conditions was 0%. Among the treatments tested, Thiourea at 0.5% for 16 h was the most effective treatment, achieving germination rates of 91.00% in IE 8414 and 95.25% in IE 8416. Potassium nitrate ( $\text{KNO}_3$ ) at 0.2% for 16 h also showed significant efficacy, with 50.75% germination in IE 8414 and 70.75% in IE 8416. Gibberellic acid ( $\text{GA}_3$ ) at 500 ppm for 2 h induced 51% germination in IE 8416, while <1% in IE 8414. Chemical treatments have been more effective, and while other treatments such as ethrel, cold stratification, and water immersion, were largely ineffective, with germination rates

remaining below 5%. These findings highlight the potential of Thiourea and  $\text{KNO}_3$  as effective tools for breaking dormancy in wild *Eleusine* accessions.

**Keywords** Wild relatives · Genetic conservation · Climate resilience · Dormancy · And germination

## Introduction

Finger millet is one of the most important millets among dry land crops with an allotetraploid nature ( $2n=4x=36$ ) and C4 photosynthetic machinery. Its cultivation has been spread across several countries; i.e., India, Ethiopia, Nepal, Uganda, Tanzania, Kenya, Zimbabwe, Zambia, Malawi, Eritrea, Mozambique, Rwanda, which accounts for around 20% of global millet area and 26% of global millet production (Gebreyohannes et al. 2024). India is one of the largest global producers of finger millet, with its cultivation spread across 1.162 million ha and with production of 1.692 million tons and productivity of 1456 kg/ha (<https://www.indiastat.com/>). In addition, it possesses a better nutritional profile with superiority in several nutrients (Backiyalakshmi et al. 2023). In the current scenario of the intensified impact of climate change, genetic resources, specifically the crop wild species, hold the key to addressing the spiraling challenges. Crop wild relatives are the reservoir of several desirable alleles for various traits (Dida et al. 2021) and play a crucial role in breeding for

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traits such as drought tolerance, pest resistance, and enhanced nutritional profiles (Hilu and Dewet 1976). However, seed dormancy has been identified as one of the major hurdles among the wild species and needs to be addressed.

Seed dormancy is the inability of the seed to germinate, though the embryo is intact and viable. While dormancy enhances survival in natural ecosystems, it poses a significant challenge in ex-situ conservation, where rapid and uniform germination is essential for conservation, regeneration, and breeding programs. It has been a major hurdle for the freshly harvested seeds for their immediate use, which has enhanced the need for standardization of several pre-sowing seed treatments for overcoming it. Seed dormancy is a complex trait, affected by multiple genes and environmental factors (Graeber et al. 2014; Lu et al. 2018) and an important component of plant adaptation (Donohue et al. 2005; Huang et al. 2015). It is regulated by the antagonistic hormones, abscisic acid (ABA) and gibberellic acid ( $GA_3$ ) (Finkelstein et al. 2008; Shu et al. 2016; Née et al. 2017). Moreover, seed dormancy is also considered an undesirable trait by farmers, affecting crop establishment. So, the dormancy-breaking pre-treatments have been found most useful in most of the crops; i.e., rice, wheat, maize, sorghum, pearl millet, chickpea, pigeon pea and many other crops (Burton 1969; Sahu et al. 1993; Matus-Cádiz and Hucl 2003; Shanmugavalli et al. 2007; Khadraji and Qaddoury 2023; Lamichaney et al. 2023). Compared to cultivated, the strong dormancy mechanisms present in wild genotypes often result in poor germination under standard conditions, complicating their conservation and utilization (Long et al., 2015). The study conducted by (Kannababu et al. 2025) reported several genes regulating seed vigour related traits in finger millet. The impact of seed dormancy breaking treatments on the expression of genes regulating seed vigour and related traits can elucidate further mechanisms involved.

Currently, ICRISAT genebank holds a global finger millet collection of 7536 accessions collected from 25 countries including 206 wild germplasm accessions (<https://genebank.icrisat.org/>). Seed viability testing, typically conducted through germination tests, is a critical component of routine genebank operations. To ensure that germination results accurately reflect true seed viability, it is essential to apply an effective dormancy-breaking treatment, but limited

information regarding the effective pre-sowing seed treatments and standardized protocol to overcome seed dormancy in wild species of finger millet is limiting their efficient conservation as well as exploration of their potential in crop improvement programs. Hence, the current study was initiated to unveil the impact of various pre-seed treatments to develop a more efficient treatment for routine use in viability testing for conservation and also to enhance the utility of crop wild relatives of finger millet.

## Materials and methods

### Plant material

The current study was carried out during the year 2023–24 at ICRISAT, Genebank, Patancheru, Hyderabad. The seeds belonging to the two wild finger millet accessions (IE 8414 and IE 8416) were sown in the glass house at ICRISAT in the month of October 2023. The seeds were grown in 12-inch pots in a mixture of red soil, sand and vermicompost in the ratio of 3:2:1. At the time of sowing, 2 g DAP was added to the mixture, and 2 g of urea was applied after 25 days of sowing. After thinning, only five seedlings were maintained in each pot, and the seeds from both accessions were harvested at maturity. The seeds were carefully harvested, threshed, and after harvesting, a sample of seeds from the accessions was taken in grip seal bags to determine the initial seed moisture content, which was 16.2% (Oven dry method). Seeds were dried in the sun (max. 30 °C, min. 22 °C) to bring the moisture content down to 12.6% and then transferred to the genebank drying room maintained at 15°C and 15% RH for further drying. The seeds were dried to a uniform moisture content (7.1%) before sampling for germination testing. The seeds were taken out from the drying room and kept at room temperature ( $25 \pm 2$  °C) for saturation before being immersed in different chemical solutions.

### Experimental design and treatments

The current experiment was carried out in a factorial completely randomized design with genotypes and treatments as two different factors and four

replications. The combinations of genotype  $\times$  treatments were applied randomly across replications.

### Seed Viability and dormancy evaluation

Before applying dormancy-breaking treatments, the seed viability of both the wild accessions was assessed using the tetrazolium (TZ) test following ISTA guidelines (ISTA 2021). For each accession, 100 seeds were preconditioned by soaking in distilled water at room temperature ( $23 \pm 2$  °C) for 16 h. The seeds were then longitudinally and carefully dissected under a magnifying lens to expose the embryo. Dissected seeds were immersed in a 1% tetrazolium chloride solution and incubated in the dark at 25 °C for two hours. Following staining, seeds were rinsed twice with distilled water. Seeds were carefully examined under a Tagarno magnifying microscope for evaluation. Seeds were considered viable if the embryo was uniformly stained red. The viability percentage for each accession was calculated as the proportion of seeds with completely stained embryos relative to the total number of seeds tested.

For dormancy evaluation, uniform and intact seeds were selected from the available seed, and a total of 400 seeds were randomly selected from them and divided into four replications of 100 seeds each. The seeds were then subjected to different kinds of pre-sowing seed treatments, including cold stratification and hydropriming with exposure for different number of days and variable periods, respectively. Moreover, the treatments include exposure to chemicals

like potassium nitrate ( $\text{KNO}_3$ ), thiourea, and also hormones like ethrel and gibberellic acid at different concentrations and time periods (Table 1). Following the treatments, after the soaking period was over, the seeds were taken out and washed thoroughly with distilled water before placing them on petri dishes for germination. Germination testing was performed at the seed laboratory of ICRISAT genebank, following the International Seed Testing Association rules (ISTA 2021) using the “Top of Paper” method, where seeds were evenly spaced on moistened germination sheets in petri dishes. The dishes were covered and incubated in a growth chamber under controlled conditions ( $25 \pm 1$  °C temperature and 85–90% relative humidity). Germination was recorded on the tenth day by counting normal seedlings, and the germination percentage was calculated as.

Germination percentage (%) = (Number of germinated seeds / Total seeds) \* 100.

### Statistical analysis

Germination percentage data were arcsine square root transformed before statistical analysis to stabilize the variance. The data were analyzed based on the linear model in which genotype, treatment, genotype  $\times$  treatment, and replication are considered fixed factors using R software (version 4.2.2) and the packages emmeans and multcompView. The interaction (Genotype  $\times$  Treatment) and main effect mean (treatment and genotype) were calculated. Further, post-hoc

**Table 1** Different kinds of pre-sowing seed treatments used for breaking seed dormancy

S. No	Treatment	Concentration/Condition
1	Control	Normal Incubation (25 °C for 10 days)
2	Cold stratification	4 °C for 7 days
3	Cold stratification	4 °C for 15 days
4	Hydropriming	Soaking in distilled water for 17 h
5	Hydropriming	Soaking in distilled water for 24 h
6	Chemical treatment	Potassium nitrate ( $\text{KNO}_3$ ) at 0.2% (16 h)
7	Chemical treatment	Potassium nitrate ( $\text{KNO}_3$ ) at 0.5% (16 h)
8	Chemical treatment	Ethrel 25 ppm (16 h)
9	Chemical treatment	Ethrel 50 ppm (16 h)
10	Chemical treatment	Thiourea 0.5% (16 h)
11	Chemical treatment	Thiourea 1% (16 h)
12	Chemical treatment	Gibberellic acid ( $\text{GA}_3$ ) at 500 ppm (2 h)
13	Chemical treatment	Gibberellic acid ( $\text{GA}_3$ ) at 1000 ppm (2 h)

analysis was performed using the Duncan multiple range test (DMRT) ( $\alpha = 0.05$ ).

## Results and discussion

Embryos of seeds from both wild finger millet accessions exhibited a uniform red coloration upon staining with 1% tetrazolium chloride (TZ) solution, indicating that all tested seeds were viable. The viability percentage recorded was 100% for the accession IE 8416, while it was 99% for the accession IE 8414. The complete and consistent staining of the embryo tissues suggests that cellular respiration was active, confirming the physiological integrity and viability of the seeds across both accessions. The differential impact of various dormancy-breaking treatments imposed on wild accessions of finger millet was evident (Table 2). Highly significant variation in germination rates among different treatments ( $p < 0.001$ ) and between accessions ( $p < 0.001$ ), with a significant genotype  $\times$  treatment interaction effect ( $p < 0.001$ ), was observed. The control groups of both *E. africana* accessions (IE 8414 and IE 8416) demonstrated absolute germination failure ( $0\% \pm 0.00$ ), confirming the persistence of primary dormancy under standard conditions ( $25\text{ }^\circ\text{C}$  for 10 days) (Table 3). In addition, a significant difference in germination (%) among the two wild genotypes has also been observed.

### Genotype\*treatment means

Among the various pre-sowing treatments (Table 3), similar to the control, no germination was observed in the case of ethrel under both concentrations (50 and 25 ppm) for a period of 16 h in both accessions. Though cold stratification of  $4\text{ }^\circ\text{C}$  for 7 days and 15 days has induced germination in both accessions, the germination percentage was lower than in other treatments, and differential response was observed

**Table 3** Grouping of genotypes\*treatment means of germination% based on Duncan multiple range test

Duncan's multiple range test _ treatments			
Accession	Treatment	Mean $\pm$ SEM	groups
IE 8416	Cold stratification $4\text{ }^\circ\text{C}$ 15 days	$10.25 \pm 1.49$	gh
IE 8414	Cold stratification $4\text{ }^\circ\text{C}$ 15 days	$0.25 \pm 0.25$	i
IE 8416	Cold stratification $4\text{ }^\circ\text{C}$ (7 days)	$13.50 \pm 0.65$	g
IE 8414	Cold stratification $4\text{ }^\circ\text{C}$ (7 days)	$0.50 \pm 0.29$	i
IE 8414	Control ( $25^\circ$ for 10 days)	$0.00 \pm 0.00$	i
IE 8416	Control ( $25^\circ$ for 10 days)	$0.00 \pm 0.00$	i
IE 8414	Ethrel-50 ppm(16 h)	$0.00 \pm 0.00$	i
IE 8416	Ethrel-50 ppm (16 h)	$0.00 \pm 0.00$	i
IE 8414	Ethrel-25 ppm (16 h)	$0.00 \pm 0.00$	i
IE 8416	Ethrel-25 ppm (16 h)	$0.00 \pm 0.00$	i
IE 8416	GA <sub>3</sub> -500 ppm (2 h)	$51.00 \pm 4.74$	cd
IE 8414	GA <sub>3</sub> -500 ppm (2 h)	$0.75 \pm 0.48$	i
IE 8416	GA <sub>3</sub> - 1000 ppm (2 h)	$46.25 \pm 3.90$	d
IE 8414	GA <sub>3</sub> - 1000 ppm (2 h)	$3.00 \pm 1.08$	i
IE 8416	KNO <sub>3</sub> -0.2% (16 h)	$70.75 \pm 2.14$	b
IE 8414	KNO <sub>3</sub> -0.2% (16 h)	$50.75 \pm 0.48$	cd
IE 8416	KNO <sub>3</sub> -0.5% (16 h)	$53.75 \pm 4.07$	c
IE 8414	KNO <sub>3</sub> -0.5% (16 h)	$0.25 \pm 0.25$	i
IE 8416	Thiourea-0.5%	$95.25 \pm 0.25$	a
IE 8414	Thiourea-0.5%	$91.00 \pm 0.58$	a
IE 8416	Thiourea-1%	$36.25 \pm 2.39$	e
IE 8414	Thiourea-1%	$27.50 \pm 2.84$	f
IE 8416	Water immersion (17 h)	$4.50 \pm 1.19$	hi
IE 8414	Water immersion (17 h)	$0.25 \pm 0.25$	i
IE 8416	Water immersion (24 h)	$3.75 \pm 0.75$	hi
IE 8414	Water immersion (24 h)	$2.00 \pm 1.68$	i

in the two accessions. Moreover, water immersion treatments (17 and 24 h) have not been very effective and resulted in poor germination (ranged between 2.38 to 2.88%) in the case of both accessions. GA<sub>3</sub>

**Table 2** Analysis of variance (ANOVA) for the germination percentage of *Eleusine africana* genotypes under various treatments

Source of variation	DF	Sum Sq	Mean Sq	F-Value	Pr(> F)
Accession	1	5835	5835	558.27	< 2e-16***
Treatment	12	47,798	3983	381.1	< 2e-16***
Accession* treatment	12	6138	512	48.94	< 2e-16***
Residuals	78	815	10	–	–
Total	103	60,586	–	–	–

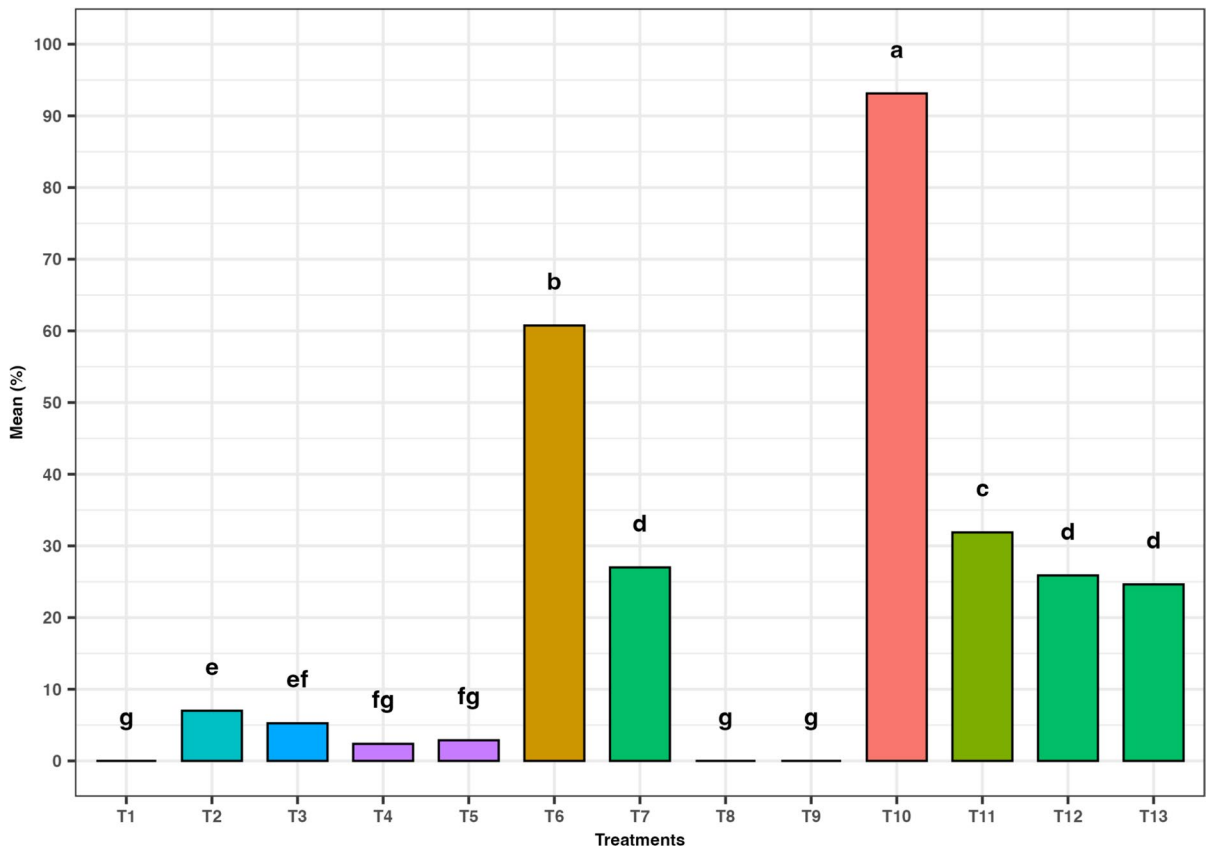
Significance codes: \*\*\* indicates  $P < 0.001$

has a significant positive impact on germination, but it was limited only to one of the two accessions: i.e., effective in IE 8416 with germination around 50% under both the concentrations; i.e., 500 ppm (2 h) and 1000 ppm (2 h). No significant difference in germination was observed in different concentrations of gibberellic acid; i.e., 500 ppm for 2 h and 1000 ppm for 2 h. Similar to  $GA_3$ ,  $KNO_3$  treatment for a period of 16 h also had a differential impact on germination in two accessions, with a greater positive impact on IE 8416. In addition, it also varied based on concentration (0.2 and 0.5%), which was not observed in the case of  $GA_3$ . The increase in the concentration of  $KNO_3$  resulted in a significant decrease in germination and it was much more drastic in the case of IE 8414 (50.25–0.25%). Apart from all the treatments, thiourea is the chemical treatment that has shown the

highest germination percentage (>90%) in both the accessions under 0.5% for 16 h while further increase in concentration (1% for 16 h) resulted in reduction in germination (~30%) (Fig. 1).

#### Treatment means

The means of germination % for various treatments (Table 4) and grouping based on significant differences (Fig. 1) can provide an overview of the impact of various treatments on finger millet wilds. No significant difference was observed among the treatments, control, ethrel (25 ppm for 16 h and 50 ppm for 16 h) and water immersion (17 h and 24 h) and these are not effective in breaking seed dormancy. The cold stratification treatment of 4 °C for 7 days and 15 days treatments are also similar



**Fig. 1** Bar graph representing treatment means of germination of finger millet wild genotypes % under various pre-sowing seed treatment. T1-Control, T2- Cold stratification 4 °C 7 days, T3-Cold stratification 4 °C 15 days, T4-Water immersion for 17 h, T5-Water immersion for 24 h, T6- $KNO_3$ -0.2% (16 h),

T7- $KNO_3$ -0.5% (16 h), T8-Ethrel-25 ppm (16 h), T9-Ethrel-50 ppm (16 h), T10-Thiourea-0.5% (16 h), T11-Thiourea—1% (16 h), T12- $GA_3$ -500 ppm (2 h), T13- $GA_3$ -1000 ppm (2 h)

**Table 4** Treatment grouping based on germination percentage using Duncan multiple range test (DMRT)

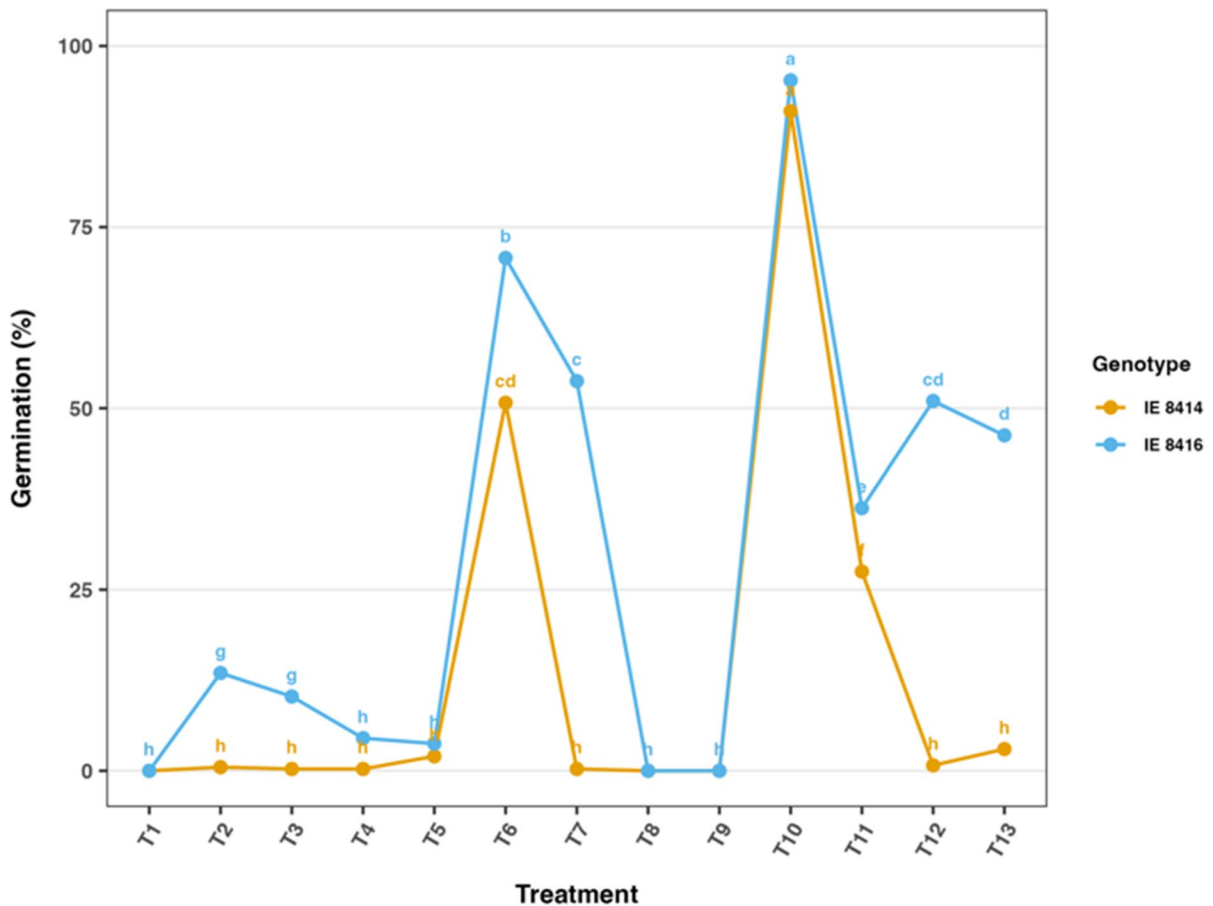
Treatment	Mean	Group
Thiourea-0.5% (16 h)	93.13	a
KNO <sub>3</sub> -0.2% (16 h)	60.75	b
Thiourea-1% (16 h)	31.88	c
GA <sub>3</sub> -1000 ppm (2 h)	24.63	d
GA <sub>3</sub> -500 ppm (2 h)	25.88	d
KNO <sub>3</sub> -0.5% (16 h)	27.00	d
Cold stratification 4 °C 7 days	7.00	e
Cold stratification 4 °C 15 days	5.25	ef
Water immersion for 17 h	2.38	fg
Water immersion for 24 h	2.88	fg
control	0	g
Ethrel-50 ppm (16 h)	0	g
Ethrel-25 ppm (16 h)	0	g

and had minimal impact on enhancing germination. No significant increase in germination percentage when the concentration of GA<sub>3</sub> was increased from 500 to 1000 ppm (24.63 and 25.88%). In the case of KNO<sub>3</sub> for a period of 16 h, a lower concentration of 0.2% shown a greater positive impact than a higher concentration (0.5%) with germination of 60.75 and 27% respectively. Finally, thiourea (0.5%) for

a period of 16 h is the chemical treatment that has been most effective and enhanced germination percentage levels of >90%, but a drastic reduction in was witnessed when its concentration was increased to 1%.(Fig. 3).

It was observed that the pre-sowing treatments effectively alleviated seed dormancy in wild finger millet accessions, IE 8414 and IE 8416, with efficacy varying significantly across treatments and between genotypes. Among the treatments evaluated, chemical applications demonstrated the highest overall effectiveness. Thiourea at 0.5% concentration for 16 h consistently emerged as the most effective treatment for breaking dormancy and enhancing percentage (Fig 2). Following thiourea (0.5% for 16 h), the next most effective treatments were potassium nitrate (KNO<sub>3</sub>) at 0.2%, thiourea at 1% for 16 h, and KNO<sub>3</sub> at 0.5%. GA<sub>3</sub> treatments showed moderate effectiveness, with 500 ppm for 2 h performing slightly better than 1000 ppm for 2 h. Physical treatments were considerably less effective; cold stratification at 4 °C for 7 days showed some benefit over cold stratification for 15 days, and water immersion for 24 h was marginally better than immersion for 17 h. Treatments involving ethrel at either 25 ppm for 16 h or 50 ppm for 16 h were entirely ineffective, showing results equivalent to the untreated control.

**Fig. 2** Germination test of finger millet wild germplasm (IE 8416) using the "Top of Paper" method under Thiourea treatment (0.5% for 16 h) (A) and control (B)



**Fig. 3** Genotype  $\times$  Treatment means of germination percentage under various pre-sowing seed treatments. T1-control, T2-Cold stratification 4 °C 7 days, T3-Cold stratification 4 °C 15 days, T4-Water immersion for 17 h, T5-Water immer-

sion for 24 h, T6-KNO<sub>3</sub>-0.2% (16 h), T7-KNO<sub>3</sub>-0.5% (16 h), T8-Ethrel-25 ppm (16 h), T9-Ethrel-50 ppm (16 h), T10-Thiourea-0.5% (16 h), T11-Thiourea-1% (16 h), T12-GA<sub>3</sub>-500 ppm (2 h), T13-GA<sub>3</sub>-1000 ppm (2 h)

Genotype-specific responses were notable. For genotype, IE 8414, the order of treatment effectiveness began with thiourea (0.5% for 16 h) as the best, followed sequentially by KNO<sub>3</sub> (0.2%), thiourea (1% for 16 h), GA<sub>3</sub> (1000 ppm for 2 h), water immersion (24 h), GA<sub>3</sub> (500 ppm for 2 h), and cold stratification (7 days). Treatments including water immersion (17 h), KNO<sub>3</sub> (0.5%), and cold stratification (15 days) showed similarly low effectiveness, while ethrel treatments and the control were the least effective. A significant genotype  $\times$  treatment interaction altered this pattern for IE 8416. Here, thiourea (0.5% for 16 h) was again the most effective, followed by KNO<sub>3</sub> (0.2%) for 16 h, then KNO<sub>3</sub> (0.5%) for 16 h, thiourea (1% for 16 h), GA<sub>3</sub> (500 ppm for 2 h), and GA<sub>3</sub> (1000 ppm for 2 h). Cold stratification for 7 days

was more beneficial than for 15 days. Interestingly, water immersion for 17 h was slightly more effective than the control but less effective than immersion for 24 h in this genotype. Ethrel treatments remained completely ineffective, matching the control. Crucially, most treatments induced a substantial positive response in IE 8416, enhancing germination by approximately 50% compared to the control.

The persistent dormancy observed despite extended cold stratification or water immersion (hydropriming), coupled with observed impermeable seed coats and a lack of imbibition and radicle emergence, strongly indicates the presence of physical dormancy in these genotypes (Baskin and Baskin 2004). Alarmingly, treatments utilizing ethylene-releasing compounds (ethrel at 25 for 16 h and

50 ppm for 16 h) and gibberellic acid ( $GA_3$  at 500 and 1000 ppm) resulted in severe phytotoxicity. Ethrel caused near-total (100%) seed mortality, while  $GA_3$  treatments resulted in mortality approaching 100%. Affected seeds frequently exhibited microbial rot or fungal proliferation. Microscopic examination revealed structural damage to both the seed coat and endosperm, confirming the phytotoxic effects of these compounds at the applied concentrations. Generally, the responsiveness of seed to pre-sowing treatments is genotype and species-specific (Corbineau 2024), but dormancy-breaking treatments are standardised considering benefits at a larger scale. Ethrel (25 ppm for 16 h) has been shown to enhance seed quality, germination, and seedling vigour in foxtail millet (*Setaria italica* L.) by effectively breaking dormancy, with treated seeds exhibiting 86.4% germination, increased seedling length and reduced electrolyte leakage (Sebastian et al., 2015). It has been reported that ethrel (25 ppm 16 h) enhanced seed quality, germination, and seedling vigour in crops such as soybean and rice (Ishibashi et al. 2013; Zhang et al. 2024). However, higher concentration of ethylene has negative impacts on germination in peanut (Cui et al. 2025). In the case of  $GA_3$ , it has a positive impact on the alpha-amylase activity, which had a key role in the regulation of seed germination (Brasileira et al. 2002). The alpha-amylase activity estimation in both genotypes may provide an understanding of their differential response towards  $GA_3$  treatment. Though there was a huge difference in germination % between the genotypes due to  $GA_3$  treatment, no significant increase in germination % was observed when the concentration of  $GA_3$  increased from 500 ppm for 2 h to 1000 ppm for 2 h.

In the case of thiourea, it is the establishment of the proper redox equilibrium within a cellular environment that plays a key role in stimulatory effects on germination and growth, and the development of various crops (Vikas Yadav Patade et al. 2020). The study carried out by (Gupta et al. 2011) has also reported the negative impact of the increase in  $KNO_3$  concentration on germination. The mechanism involved in it needs to be elucidated at the biochemical and molecular levels. Further, treatments like cold stratification and hydro priming treatments have been ineffective; the combination of these treatments with various chemical treatments can be studied in the future and their species-specific impact needs further elucidation in terms of finger millet wilds.

## Conclusion

From the above study, it was concluded that *Thiourea@0.5%* for 16 h was most effective in alleviating dormancy of freshly harvested wild finger millet germplasm. This treatment offers an effective method for breaking seed dormancy in wild finger millet species and can be adopted by genebanks, researchers, and breeders to facilitate improved germination and promote broader utilization of these genetic resources. Identification of treatments with similar potential and a combination of chemical treatments and hydropriming or water soaking can be more cost-effective and environmentally friendly. The availability of standardized pre-sowing seed treatments for finger millet wild species for alleviating the impact of dormancy can improve the efficiency of conservation and distribution of finger millet wild genetic resources. Further, the distribution of these genetic resources can initiate several pre-breeding programs for trait exploration.

**Acknowledgement** We would like to acknowledge the valuable support provided by the research technicians of the ICRI-SAT genebank seed laboratory, especially Ms Rajasri Bandaru and Ms Nagajyothi Kollati, in conducting this experiment.

**Authors contribution** O.P. Wrote the manuscript K.J. Prepared the figures and tables. V.M. and K.S. Reviewed the manuscript.

**Funding** This study was undertaken as part of the CGIAR Genebank Initiative and is part of the Research Program on Accelerated Crop Improvement, ICRISAT.

**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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