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# Seed priming with Heme compound for improvement of seed germination and seedling establishment of rice (*Oryza sativa* L.) under salinity stress

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**Abstract**
Three rice (*Oryza sativa* L.) varieties, saline tolerant (TNAU Rice TRY 3) and saline sensitive (ADT 56 and ADT 57), were used in the study to assess the effects of seed priming with hematin, an inducer of heme oxygenase-1 (HO-1), on seed germination and seedling establishment under salt stress condition. The experiment's findings demonstrated that the administration of hematin at various doses (1,3,5,7, and 9 µM) reduced the salt stress-induced impairment of rice seed germination and subsequent seedling growth in a variety of ways. Seed primed with 1 µM hematin showed maximum germination in all three varieties as compared to hydroprimed and unprimed control seeds. It also enhanced speed of germination, seedling length, vigour index, and dry matter production regardless of salt concentrations. It is obvious that seed primed with 1 µM hematin was efficient in reducing the effects of salt stress on rice during germination and seedling establishment, even at elevated salt concentrations.

**Keywords:** Rice, Salt stress, Seed priming, Hematin, Seed germination, Seedling growth

## Introduction
More than half of the world's population consumes rice (*Oryza sativa* L.), one of the major food crops. More than a hundred nations cultivate it, with Asia producing 90% of the world's supply. Numerous abiotic impediments, including salt stress, which is one of the biggest ones, have made rice production extremely difficult. Saline stress has a substantial impact on the growth and development of rice seedlings and causes a number of morphological, physiological, biochemical, and molecular abnormalities.

Salt stress typically has a detrimental effect on seed germination, seedling growth, and even plant productivity. By reducing the amount of water accessible, affecting the mobilisation of stored reserves, and changing the structural organisation of proteins, salinity slows or stops seed germination. In order to improve seed germination and stand establishment in saline conditions, many techniques are applied.

A well-known pre-sowing seed treatment for enhancing seed quality is seed priming. Primed seeds provide good crop yields and have high levels of biotic and abiotic stress resistance, as well as enhanced germination rates and seedling vigour (Paparella *et al*., 2015) [9]. Before the radicle emerges, seed priming weakens the endosperm and controls the activity of many enzymes involved in mobilising reserves (such as α and β amylases) (Anese *et al*., 2011) [3]. In general, seedpriming with organic compounds improves seed germination and seedling growth in many crops.

Heme compounds are distinctive chemical substances that can be used to improve the quality of seeds, plant growth, and yield as well as to alleviate the negative impacts of various abiotic stresses on plant growth and crop performance. Hematin, an inducer of heme oxygenase-1 (HO-1) production, degrades heme into ferrous iron (Fe2+), biliverdin-IXa (BV-IXa), and carbon monoxide(CO) (Wu *et al*., 2011) [11]. The germination of rice seeds is greatly accelerated by hematin (Liu *et al*., 2007). Wheat seeds that had been primed with hematin prior to sowing significantly boosted the shoot and root dry weight of seedlings under salt stress (Huang *et al*., 2006) [5].

The current study set out to determine how rice seeds and seedlings responded to saline stress and whether seeds primed with hematin might potentially decrease the effects of saline stress.

## Materials and Methods
Experiments were conducted at Department of Seed Science and Technology, Tamil Nadu

Agricultural University, Coimbatore during 2022. The seeds of three different rice varieties *viz*., salt tolerant (TNAU TRY 3) and salt sensitive (ADT 56, ADT 57) were chosen for the study. The seeds were primed with hematin at various concentrations of 1,3,5,7 and 9 µM in the respective solution for the duration of 12 hours @ 1:1 seed to solution ratio. The seeds were then removed from the solutions and shade dried at room temperature in order to restore the seeds' original moisture content before being used to measure various aspects of seed quality.

The germination test was conducted in line with the guidelines in employing between paper method in order to ascertain the effect of salt stress on seed germination. The germination test was conducted using germination papers that had been pre-soaked in saline solution at three different concentrations: 0, 100, and 150 mM. Four replicates of 100 seeds for each treatment were used in the germination test, which was carried out in a room that was kept at a constant temperature of 25+2 °C and a relative humidity of 95+3%. After 14 days, the seedlings were assessed, and the normal seedlings were counted and expressed as a percentage (ISTA, 2011) [6].

At the time of the germination count using the roll towel method, ten healthy seedlings were randomly selected from each replication and used to measure the length of the seedlings. The values were calculated and expressed in centimetre.

The seedlings used to measure growth were wrapped with paper and dried in the shade for 24 hours before passing another 24 hours in an oven that was maintained at 85+2 °C. The dried seedlings were removed from the hot air oven and allowed to cool in the desiccators over silica gel. The dry weight was recorded, and the average values were shown in g per 10 seedlings. The speed of germination was calculated, and the outcome was given as a number. The vigour index was computed, and the mean values were shown as whole numbers (Abdul-Baki and Anderson, 1973) The critical differences (CD) were assessed at a 5% and 1% probability level, according to Gomez & Gomez (1984). Data from each experiment were subjected to an analysis of variance, and treatment differences were assessed for significance (P = 0.05). The percentage data were transformed to arc-sine values before to analysis, wherever necessary.

## Results and Discussion

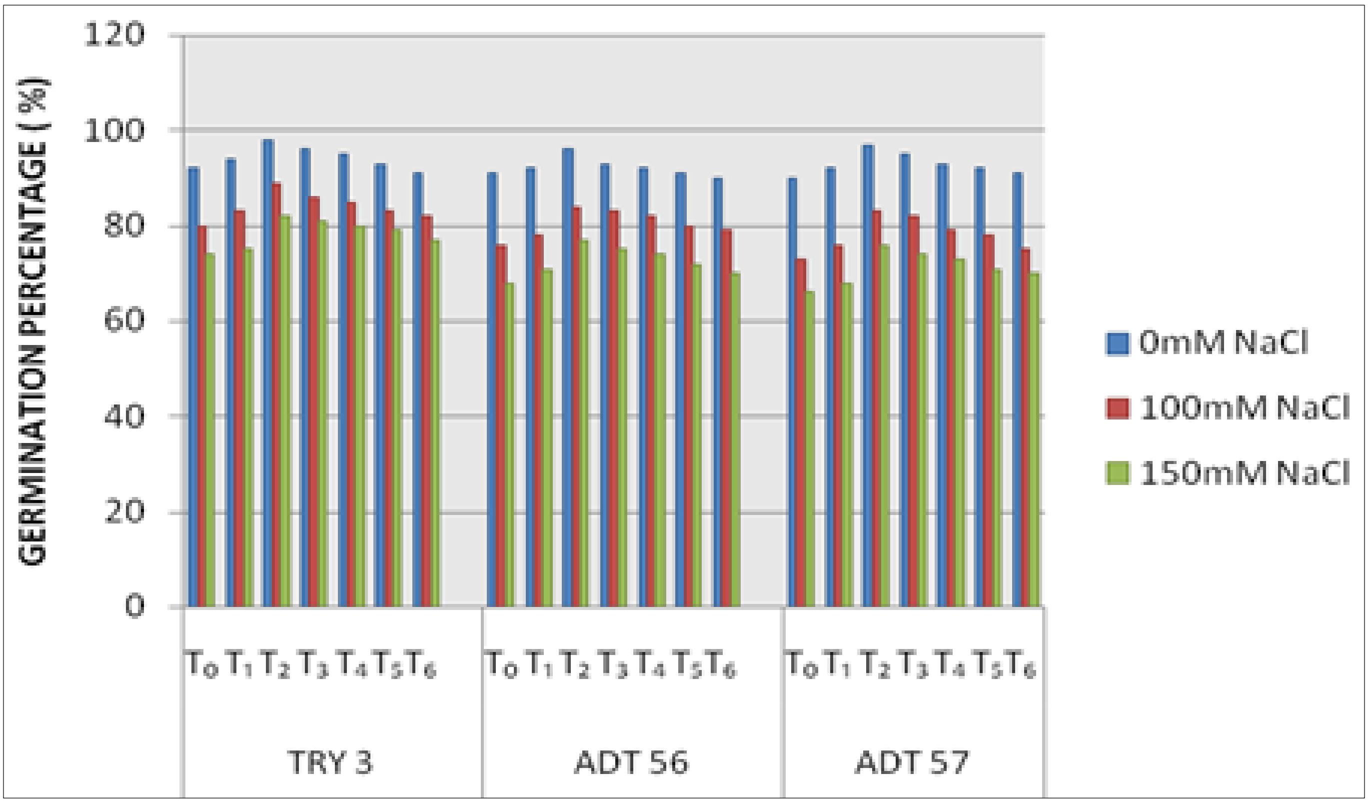
Salinity significantly hampered the growth and development of seedlings. Physiological parameters such as germination, seedling growth and vigour index were adversely affected by high level of salinity.

When different salinity stress were applied using sodium chloride at 100 and 150 mM levels, a greater reduction in germination was seen in unprimed seeds in all of the varieties at the salinity level of 150 mM. Additionally, all of the cultivars' seedling length and vigour index decreased as a result of salinity stress. One of the most likely causes of reducing seedling length is that seedlings are taking in less water more slowly, which should lead to less cell growth and a reduction in turgor pressure at relatively high salinities (Migahid *et al*., 2019) [8]. A delay in seed germination is also observed under salt stress condition. All three types underwent priming treatment, which greatly increased germination percentage at various salinity levels. The greatest seedling length and vigour index were recorded by TNAU TRY 3 regardless of salt concentration and priming treatment. In comparison to hydropriming and unprimed seeds, seed priming with hematin dramatically increases germination, root and shoot length, speed of germination, and dry matter production regardless of variety or salt level.

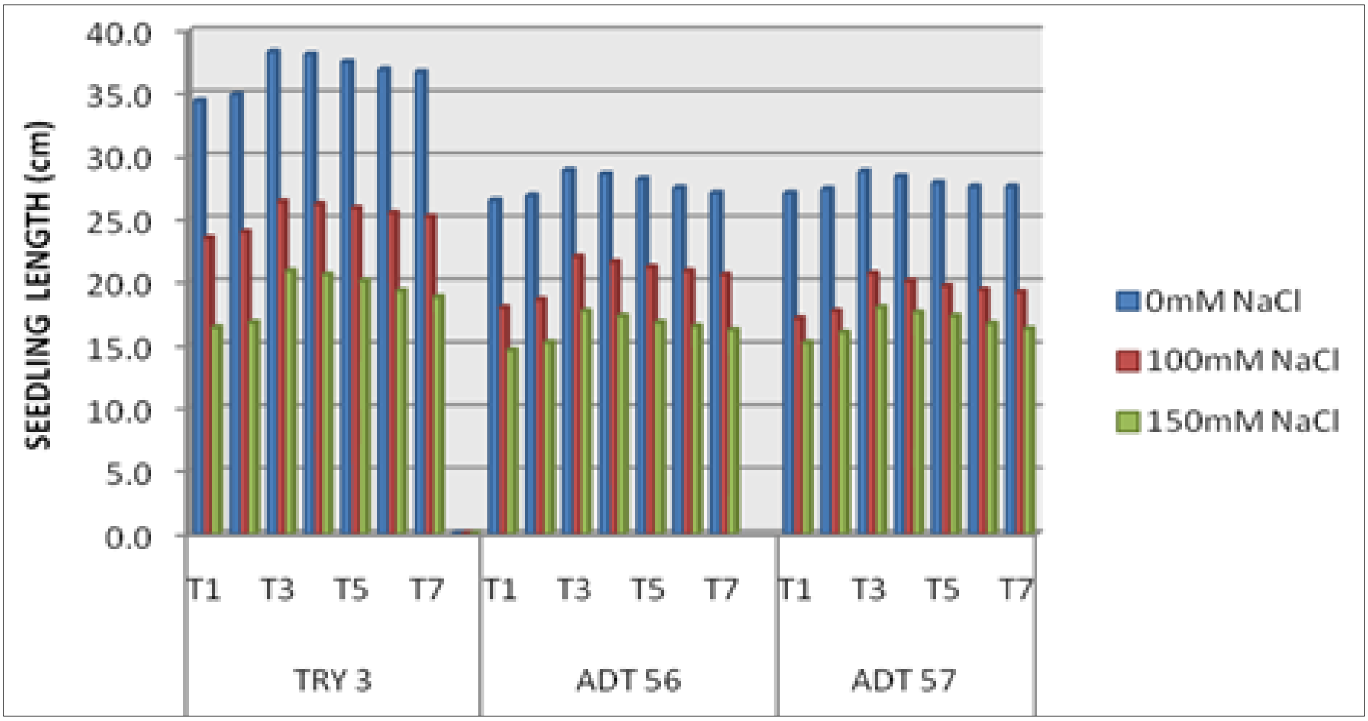
Hematin might differentially affect seed germination and seedling growth at the lowest concentration tested, 1 µM. At 150 mM salinity, primed seed exhibits a germination percentage of 82, 77, and 76, root length of 13, 12, and 12.5 cm, shoot length of 7, 5.3, and 5.5 cm, speed of germination of 6.4, 5.9, and 5.9, drymatter production of 0.104, 0.089, and 0.086 g of 10 seedlings$^{-1}$, and vigour index of 1715, 1361, and 1324 in TNAU TRY 3, ADT 56 and ADT 57 respectively.Whereas control seed of TNAU TRY 3, ADT 56 and ADT 57 recorded comparatively reduced germination percentage of 74, 68 & 66, root length of 10.4, 10.1& 10.2cm, shoot length of 6, 4.4 & 4.9 cm, speed of germination of 5.6, 4.8 &4.6, dry matter production of 0.092, 0.078& 0.076 g 10 seedlings$^{-1}$ and vigour index 1211, 986 & 1000 respectively. At 100 mM salinity, unprimed seeds of TNAU TRY 3 recorded 80% germination while primed seeds exhibited 9% improvement in germination. Similarly, primed seeds of saline sensitive varieties such as ADT 56 and ADT 57 also showed 8 and 10% improvement in germination respectively over control (Fig 1A).

The vigour index showed a declining trend from 0 to 150 mM of salt concentration. At greater salt concentration (150 mM), there was 55% fall in vigour index compared to unstressed condition. All priming treatments demonstrated better vigour index when compared to unprimed seeds due to increase in seedling length. Seed priming with hematin @ 1 µM recorded the highest vigour index among the priming treatments. Compared to unprimed control and hydropriming, hematin @ 1 µM treatment resulted in improvement of 20 and 16 percentage respectively. At 150 mM salinity, salt tolerant variety, TRY 3 showed an improvement of 29 percentage over unprimed seeds as a result of seed priming with 1 µM hematin, while salt sensitive varieties such as ADT 56 and adt 57 showed improvement of 27.5 and 24.5 percentage respectively. Similarly when the salt concentration ranged from 0 to 150 mM, the dry matter production also displayed a diminishing trend. Due to an increase in seedling length, all priming treatments produced more dry matter than unprimed seeds did. At higher salinity level(150Mm) salt tolerant variety, TRY 3 exhibited 11 percentage improvement in dry matter production after priming treatment with hematin @1 µM. Where as, salt sensitive varieties such as ADT 56 and ADT 57 exhibited 12 and 11% improvemt respectively.

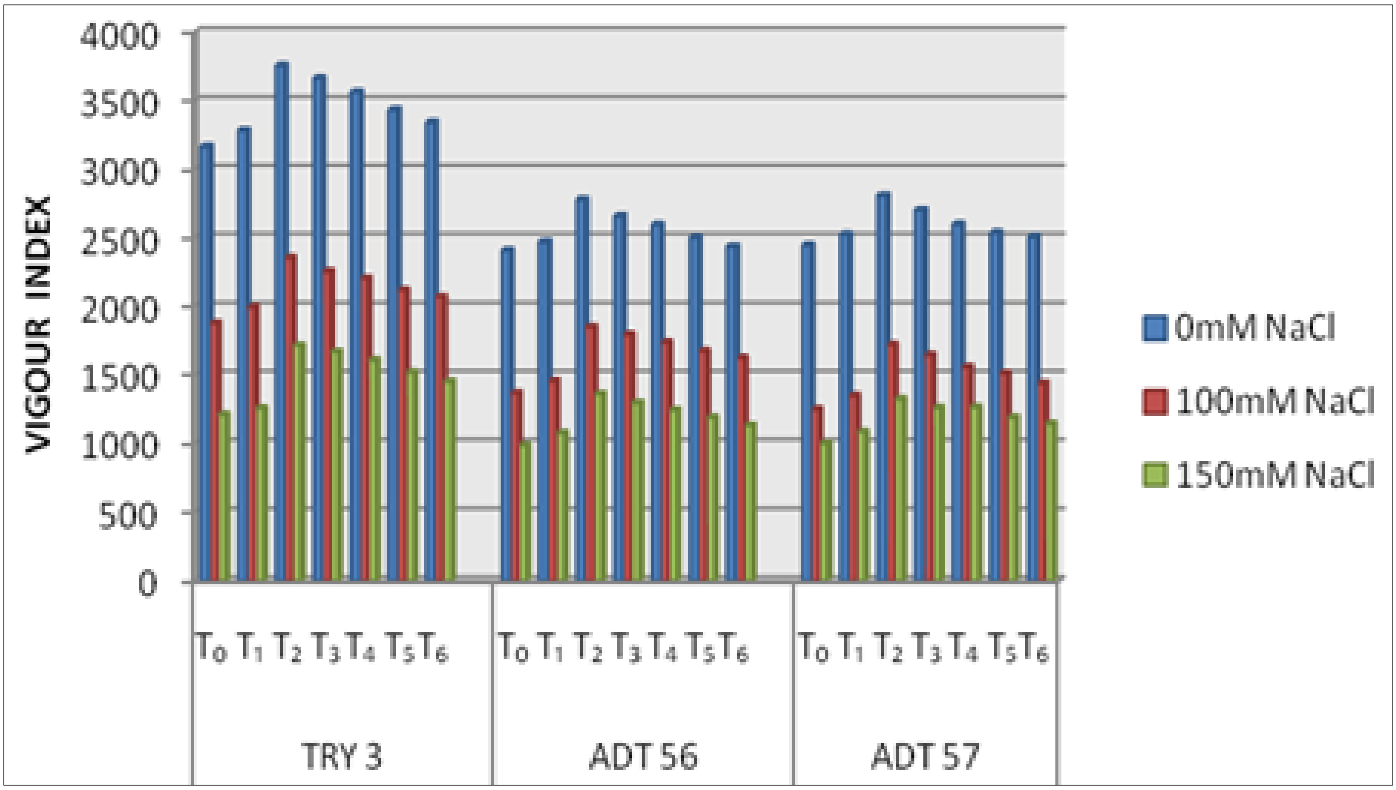
From this, it is clear that seed priming with heme compound can enhance the performance of salt sensitive varieties under moderate saline condition. Similar result was previously reported in alfalfa seeds (Amooaghaie *et al*., 2017) [2]. Role played by the hematin in salt alleviation has been reported in wheat (Xu *et al.*, 2006) [12] and rice (Liu *et al*., 2007) [7]. In a time-dependent way, pretreatment of wheat seeds with heme compound was able to reduce salinity-driven seed germination inhibition, which was compatible with the responses of other germination parameters like germination energy (GE) and germination index (GI). Additionally, it boosted the activity of α- and β-amylase, which aided in the process of turning starch into soluble sugar and reducing sugar (Xu *et al.*, 2011) [13]. The induction of catalase and superoxide dismutase activities by hematin and CO aqueous solution in *Oryza sativa* was demonstrated to alleviate salt-induced oxidative damage and lower the amount of TBARS (Liu *et al*., 2007) [7]. Higher sodium ion concentrations are harmful to cellular metabolism and can hinder the activity of numerous vital enzymes, cell division and growth, membrane disruption and osmotic imbalance, all of which can ultimately inhibit the growth of the seedlings (Singh and Sengar, 2014) [10].




A)




B)




C)

$T_0$ – Control $T_1$ – Hydropriming
$T_2$ –Seed priming with hematin @ 1 µM
$T_3$ – Seed priming with hematin @ 3 µM
$T_4$ – Seed priming with hematin @ 5 µM
$T_5$ – Seed priming with hematin @ 7 µM
$T_6$ – Seed priming with hematin @ 9 µM

**Fig 1:** The effect seed priming with hematin on seedling growth of rice under salt stress condition A) Germination percentage (%). B) Seedling length (cm) C) Vigour index

The mitigation of salinity stress by hematin is due to the up-regulation of HO1 expression which can protect the plants against oxidative damage caused by abiotic stress. Among the byproducts of heme, BV-IXa is an efficient ROS scavenger and CO act as a potent cyto-protectant against oxidative stress. Therefore, heme compounds can be used to improve the seed physiological parameters, plant growth and also to alleviate the adverse effect of different abiotic stresses on plant growth and crop yield.

## Conclusion

The study's findings supported the advantages of heme compound-assisted seed priming on seed germination and establishment in saline environments. It is well known that using hematin at a lower quantity enhances rice's physiological characteristics even when the soil is saline. Therefore, seed priming with 1 mM hematin could be recommended for enhancing seedling growth under salt stress conditions; however, more research on the responsiveness of plants to salinity and priming is warranted.

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