Influence of spermidine priming on rice (*Oryza sativa*) seed germinability and vigour under heat stress

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ABSTRACT

Polyamines (PAs) play a vital role in plants’ response to various abiotic stresses, including high temperature (HT) stress. The present study was carried out during 2020–23 at the Indian Agricultural Research Institute, New Delhi to explore how spermidine treatment affects rice (*Oryza sativa* L.) seeds’ ability to withstand heat and the availability of sugars for seedling growth during the radicle stage in heat-susceptible (IR64) and tolerant (N22) varieties. The seeds were primed with 1 mM spermidine using PEG (-1 MPa) as the priming medium, followed by exposure to heat stress. Results showed that in unprimed seeds, heat stress significantly reduces the seed germination, vigour and sugar availability in both the varieties and to a greater extent in IR64 compared to N22. This difference resulted in a substantially lower percentage of normal seedlings and seed vigour index in IR64 than in N22. However, spermidine seed priming enhanced thermotolerance in both varieties, more in IR64, equating the normal seedling percentage and seedling growth with control, i.e. without heat stress. The priming treatment also notably augmented α-amylase activity and reducing sugar availability, particularly in N22, enabling better seedling growth under heat stress conditions. This study underscores the importance of seed priming with spermidine to allow the seedlings to tolerate elevated temperatures and maintain better seedling growth due to the enhanced availability of reducing sugars during germination and early seedling growth.

Keywords: α-Amylase activity, High-temperature stress, PEG, Seed Priming, Thermotolerance

Rice (*Oryza sativa* L.), an important staple food crop, occupies the second position among cereals on a global scale (Singh 2022). The ideal temperature range for successful rice cultivation typically spans 25 to 35°C. Temperatures surpassing this range can have adverse consequences for rice, particularly in germination and early seedling stage, causing inhibition in seedling establishment and development, leading to poor crop stand or complete death of seedlings (Hussain *et al.* 2019). Seed priming is one of the effective strategies to mitigate the effect of heat stress. Priming involves controlled hydration of seeds to trigger pre-germinative metabolic events followed by drying back to their initial moisture content. Polyamines (PAs) are small organic molecules that play essential roles in various physiological processes in plants, including stress responses. Different polyamines were used in multiple studies; among them, spermidine is more closely associated with plant stress tolerance (Paul *et al.* 2017).

Various studies have reported the practical application of exogenous spermidine during the flowering stage for mitigating abiotic stresses such as chilling (Fu *et al.* 2020), salinity (Paul *et al.* 2017) and heat stress (Tang *et al.* 2018). However, limited reports are available on the impact of spermidine seed priming in alleviating heat stress during germination and early seedling stages. Further, most polyamine priming studies involve soaking seeds directly in spermidine solution. Seed priming is based on the interplay between water imbibition and the water potential of the medium. Adjusting the water potential of the medium through osmotic agents slows down the imbibition rate, affording seeds more time for repair processes (Bewley *et al.* 2012). Only a few studies have explored seed priming involving the combination of spermidine and PEG (Basra *et al.* 1994).

Seed priming with spermidine led to early radicle emergence and vigorous seedling growth due to enhanced starch metabolism in wheat (Farooq *et al.* 2011). Moreover, earlier studies have highlighted how exogenous spermidine spraying can safeguard against stress-induced damage (Li *et al.* 2014) and a notable increase in reducing sugars (Paul *et al.* 2017) and amylase activity (Sheteiwy *et al.* 2017).
Therefore, the present study aimed to elucidate the effect of spermidine priming through PEG to induce thermotolerance in heat-susceptible (IR64) and tolerant (N22) rice varieties at the radicle stage.

MATERIALS AND METHODS

The study was carried out at ICAR-Indian Agricultural Research Institute, New Delhi during 2020–23 using seeds of heat-susceptible IR64, a popular IRRI-developed variety, and N22 (Nagina 22), a famous drought and heat-tolerant Indian rice variety. The seeds were multiplied at ICAR-IARI during rainy (kharif) season of 2020 and stored in sealed aluminium packets at lab bench conditions for 2 years after drying to 12–13% moisture. The germination and vigour of the fresh and 2-year-old seeds were measured. The two-year-aged seeds were primed using spermidine (1 mM) dissolved in PEG solution (-1.0 MPa water potential) at 25°C. The seeds primed with PEG (-1 MPa) solution alone were used as a positive control, and the unprimed seeds were used as a negative control. Based on Michel and Kauffman's (1973) equation, a -1.0 MPa water potential was achieved by dissolving 29.57 g of PEG-6000 in water to prepare a 100 ml solution at 25°C. For priming, 50 seeds in two replications were imbibed for 56 h in PEG or Spd+PEG solutions, then dried at 26°C and 60% RH to their original moisture content and placed for germination along with unprimed seeds. The primed seeds were germinated as per ISTA Rules (2022) by placing them between two layers of moistened paper (with water/PEG/Spd+PEG) and incubated at 25°C with continuous dark for 72 h. The seeds with 1–1.5 cm radicles were exposed to the previously identified (Archana et al. Unpublished data) high-temperature stress (47°C for 30 min) using a water bath. After heat stress, the seeds were acclimatized to room temperature for 2 h before placing them at 25°C and 98% RH for further seedling growth. The following parameters were measured in all three treatments in both varieties (Fig 1).

Germination (%): Following heat treatment, germination was measured after 7 days (i.e. 13 days from imbibition) at 25°C and 98% RH. The germination (%) was calculated based on the number of normal seedlings obtained against the total number of seeds kept for germination.

Seedling length: Ten normal seedlings were randomly selected, and their size was measured using a ruler and the mean was expressed in cm.

Seed vigour index: The seed vigour index (SVI) was calculated as:

\[
\text{Seed vigour index} = \text{Germination (\%)} \times \text{Seedling length (cm)}
\]

Thermotolerance index: It was calculated as the ratio

![Fig 1 Methodology used in the experimentation.](image-url)
of number of seedlings that survived to the total number of seedlings that emerged from the heat-stressed seeds at the radicle stage after 7 days of heat treatment.

α-amylase activity: Seeds at radicle stage (0.5 g) were ground in 5 ml of ice-cold 10 mM calcium chloride solution and incubated for 3 h at room temperature. The supernatant was extracted, and the α-amylase activity was measured following standard methodology (Salleh et al. 2020).

Reducing sugars: The heat-treated seeds (at radicle stage) were homogenized in 5 ml of 80% preheated ethanol, centrifuged at 20000 g for 15 min, and the supernatant was used for estimating reducing sugars using DNSA reagent (Miller 1972).

Statistical analysis: All the data were subjected to Two-way ANOVA to identify the significant variations between varieties, treatments and their interaction. The means were compared using Tukey’s HSD.

RESULTS AND DISCUSSION
The radicle stage during seed germination is highly susceptible to heat stress, and spermidine is a stress-alleviating polyamine commonly present in plants. The usefulness of spermidine priming for inducing thermostolerance during early seedling growth was tested by subjecting the primed seeds to severe heat stress conditions, and the seedling growth and survivability were measured using germination and vigour parameters.

Germination and vigour: The 2-year lab bench storage of seeds under sealed conditions led to an asymptomatic deterioration, with a marginal 10% decline in germination in both IR64 (89%) and N22 (91%) from initial germination (99 and 100% in IR64 and N22, respectively). However, the seed vigour index reduced drastically, with a 70% decline in IR64 (640.6) and a 67% decline in N22 (669.6) compared to the initial vigour indices (2167.8 and 2025 in IR64 and N22, respectively). Loss of seed vigour precedes loss of viability (Bewley et al. 2012, Sharma et al. 2022). The repair of cellular damage during priming affects the seed vigour per se rather than total physiological germination.

Therefore, these seeds with asymptomatic deterioration were used to test the efficacy of priming. Among the different priming treatments, spermidine-primed (Spd+PEG) seeds had significantly higher germination than control and PEG priming (Table 1). Germination was considerably higher in N22 (75.7%) than in IR64 (60.8%) across the treatments. A significant interaction effect was observed between varieties and treatments, where the heat-tolerant variety (N22) showed substantially higher normal seedlings (NS) than the susceptible variety (IR64) under control conditions (unprimed seed). However, due to priming (both PEG and Spd+PEG), the NS% increased more in IR64, even after heat stress, resulting in no significant difference between N22 and IR64 (Table 1). The seedling length did not differ significantly between the varieties, and no interaction was observed between varieties and treatments (Table 1). However, spermidine priming helped substantially recover seedling size after heat stress compared to the control (Fig 2, Table 1). Priming helps extend the lag phase of water uptake where activation of repair mechanisms initiates, and the seed can rectify the cellular damages due to various stresses, resulting in increased vigour (Bewley et al. 2012). In the present study, the seed vigour index (SVI) improved with priming and, more specifically, with spermidine priming. A significant interaction effect was observed, where N22 recorded a significantly higher vigour index than IR64 in unprimed seeds. However, with priming, the difference between varieties was non-significant (Table 2).

Seeds are sensitive to high temperatures during germination and early seedling stages, resulting in a poor germination rate and reduced seedling vigour (Xu et al. 2021). In the present study, heat stress reduced seedling length significantly (Fig 2). The growth retardation due to heat stress can be attributed to the disruption of various metabolic reactions and reserve mobilization (Akter and Islam 2017). A similar finding was made by Hussain et al. (2019) in rice, where exposure to high-temperature stress drastically decreased the coleoptile and radicle length. However, when the seeds were primed with spermidine,

### Table 1: Effect of spermidine priming on germination and seedling length of two rice varieties under heat stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Seeding length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IR64</td>
<td>N22</td>
</tr>
<tr>
<td>CK</td>
<td>22.5(28.3a)</td>
<td>57.5 (49.3b)</td>
</tr>
<tr>
<td>PEG</td>
<td>67.5 (55.2b)</td>
<td>72.5(58.4b)</td>
</tr>
<tr>
<td>Spd+PEG</td>
<td>92.5(74.1b)</td>
<td>95(77.1a)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>60.83 (51.25b)</strong></td>
<td><strong>75(60.00a)</strong></td>
</tr>
<tr>
<td>Variety (V)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

The values in parentheses are arcsine converted values.

Values superscripted with the same alphabet are not significantly different from each other at P≤0.05; Variety (V) means, and treatment (T) means are superscripted with capital letters; V×T interaction means are superscripted with lowercase letters; The mean comparisons of germination and seedling length are independent of each other.

Control (CK), No priming (negative control); PEG, PEG priming (positive control); Spd+PEG, Spermidine with PEG priming.
they showed reduced injury and produced more normal seedlings than unprimed seeds (Fig 2, Table 1). Polyamines, including spermidine, are good biostimulants that boost plant stress tolerance (Tyagi et al. 2023), mainly by modulating the homeostasis of reactive oxygen species (Liu et al. 2015). In the present study, PEG priming also enhanced...
germination and vigour than control. This aligns with the reports of Tamindzic et al. (2023), who found better seedling establishment in PEG-primed seeds during heat stress.

Several authors (Jagadish et al. 2010, Poli et al. 2013) reported that N22 exhibits higher thermostolerance than IR64, mainly during the reproductive stage of plant growth. Kilasi et al. (2018) showed a more significant impact of high temperature on IR64 than N22, resulting in significantly lower seedling growth in IR64. The present results, where N22 fared well for germination and seed vigour under heat stress, are consistent with previous findings. However, no significant difference was observed after seed priming between IR64 and N22, indicating that priming effectively alleviated heat stress in both varieties, with a better response in the susceptible variety.

**Thermotolerance index (TI):** It is a helpful parameter for assessing the plant’s ability to grow under heat stress and can be used to screen the genotypes (Azhurudeen et al. 2013). The heat-tolerant N22 showed a significantly higher (0.89) TI than heat-susceptible IR64 (0.74). TI negatively correlates with lipid peroxidation and oxidative stress (Azhurudeen et al. 2013). In the present study, the Spd+PEG and PEG-primed seeds had a considerably higher TI than unprimed seeds (Table 2), indicating reduced oxidative stress due to priming.

**α-amylase activity:** α-amylase enzyme hydrolyzes stored starch into sugars that provide energy for rice seedling growth (Kaneko et al. 2002). The spermidine priming had a strong positive effect in enhancing the α-amylase activity after heat stress. It was substantially better than PEG priming and control (Table 3). The α-amylase activity across treatments was significantly higher in N22 (8.050 mg maltose/g FW) than in IR64 (7.318 mg maltose/g FW). The interaction was significant, with N22 showing higher amylase activity than IR64. Seed priming per se accelerates amylase activity and starch breakdown (Ella et al. 2011). Under water stress conditions, spermidine priming improved seed germination and vigour in white clover by improving the starch metabolism through increased activity of α-amylase and increased content of reducing sugars like glucose and fructose (Li et al. 2014). In the present study also, the α-amylase activity increased significantly after heat stress due to spermidine priming (Table 3). Sheteiwy et al. (2017) found that chilling stress in rice reduced germination and seedling growth, while spermidine priming increased these physiological parameters, including α-amylase activity, thus mitigating the adverse effects of chilling stress. Therefore, the higher germination percentage and seed vigour due to spermidine priming under heat stress observed in the present study could be attributed to the increased α-amylase activity. Thermo-tolerant rice genotypes can withstand heat stress injury by increasing the activity of protective and metabolizing enzymes, including α-amylase (Sari 2021). In the present study, the heat-tolerant N22 produced more normal seedlings with better growth under heat stress and had higher α-amylase activity than IR64. This further substantiates the role of α-amylase in influencing seedling growth and its increased activity with spermidine priming in mitigating heat stress.

**Reducing sugar content:** Partial starch hydrolysis leads to increased reducing sugar content. The increased activity of α-amylase increased reducing sugars due to spermidine priming (0.32 µg glucose/g FW) under heat stress conditions compared to control (0.26 µg glucose/g FW). However, the mean reducing sugar content in heat-tolerant N22 was significantly higher (0.316 µg glucose/g FW) than in susceptible IR64 (0.258 µg glucose/g FW). The interaction between varieties and treatments was significant, with a substantially higher reducing sugar content in N22 than in IR64 under control and primed conditions (Table 3). Higher levels of reducing sugars in spermidine-primed seeds compared to unprimed seeds observed in the present study were consistent with the work of Collado-Gonzalez et al. (2020), who reported increased sugar contents after spermidine treatment during heat stress in cauliflower.

Seed germination and early seedling growth are energy-demanding processes, and reducing sugars play an essential role as an energy source to support these processes (Chen et al. 2013). The heat-tolerant N22 showed a significantly higher (0.89) TI than heat-susceptible IR64 (0.74). TI negatively correlates with lipid peroxidation and oxidative stress (Azhurudeen et al. 2013). In the present study, the Spd+PEG and PEG-primed seeds had a considerably higher TI than unprimed seeds (Table 2), indicating reduced oxidative stress due to priming.

<table>
<thead>
<tr>
<th>Variety (V)</th>
<th>Treatment (T)</th>
<th>V×T</th>
<th>Variety (V)</th>
<th>Treatment (T)</th>
<th>V×T</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR64</td>
<td>N22</td>
<td>Mean</td>
<td>IR64</td>
<td>N22</td>
<td>Mean</td>
</tr>
<tr>
<td>CK</td>
<td>6.279&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.900&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.589&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.220&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.292&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>PEG</td>
<td>7.385&lt;sup&gt;A&lt;/sup&gt;</td>
<td>7.985&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.685&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.261&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.309&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spd+PEG</td>
<td>8.291&lt;sup&gt;B&lt;/sup&gt;</td>
<td>9.266&lt;sup&gt;A&lt;/sup&gt;</td>
<td>8.778&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.293&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.347&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>7.318&lt;sup&gt;B&lt;/sup&gt;</td>
<td>8.050&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
<td>0.258&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.316&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values superscripted with the same alphabet are not significantly different from each other at P≤0.05; Variety (V) means, and treatment (T) means are superscripted with capital letters; V×T interaction means are superscripted with lowercase letters; The mean comparisons of α-amylase activity and reducing sugar content are independent of each other.

Control (CK), No priming (negative control); PEG, PEG priming (positive control); Spd+PEG, Spermidine with PEG priming.
et al. 2019). The spermidine priming in rice enhanced seed germination and seedling growth under heat stress conditions, coinciding with increased availability of reducing sugars, the critical energy sources. Zhou et al. (2017) reported increased soluble sugar content in thermo-tolerant plants compared to susceptible plants during heat stress. This report aligns with the higher reducing sugar accumulation observed in the heat-tolerant N22 than in susceptible IR64.

In conclusion, rice seed priming with spermidine improves germination percentage, seedling growth and seed vigour index under heat stress, particularly during the radicle stage. The heat-tolerant variety N22 had better germination and vigour than the susceptible variety IR64 under heat stress due to increased starch metabolism and availability of reducing sugars. Spermidine priming enhanced heat tolerance in susceptible (IR64) and tolerant (N22) varieties, with a more significant effect in susceptible ones. The increased α-amylase activity due to spermidine priming enhanced the starch metabolism, raising the reduced sugar content. The increased availability of reducing sugars helped to realize better germination and seedling growth even after heat stress. Thus, the pre-sowing seed priming with 1 mM spermidine through -1 MPa PEG medium can be used to mitigate heat stress during germination and early seedling growth of rice varieties.

REFERENCES


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