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Short Communication

Low-Dose Hydrogen Peroxide Seed Priming Improves Early-Seedling Stress Tolerance in Rice (*Oryza sativa*)

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Rice (*Oryza sativa* L.) feeds about half the world's population, yet its establishment in direct-seeded systems is set back by drought, heat, salinity and, on contaminated soils, heavy metals, all of which act most severely at the seedling stage (Muthayya *et al.,* 2014; Prasad *et al.,* 2015). Direct-seeded rice lowers labour and water use but exposes young seedlings directly to these stresses, so cheap pre-sowing treatments that harden the seedling are of practical value. Seed priming conditions the antioxidant and osmotic machinery before germination, allowing primed seedlings to respond faster once stress arrives (Ashraf *et al.,* 2018). Hydrogen peroxide (H_2O_2) is attractive for this purpose because at low doses it acts as a signalling molecule that induces cytosolic ascorbate peroxidase and related scavenging enzymes rather than as a damaging oxidant (Morita *et al.,* 1999; Hossain *et al.,* 2015). This study tested whether priming rice seed with 10 µM H_2O_2 improves seedling performance under combined drought and heat stress and under salinity and chromium stress, and whether the response differs among genotypes. Despite this interest, three gaps remain. First, most hydrogen peroxide priming studies in rice have tested a single stress in isolation, whereas seedlings in direct-seeded fields commonly experience drought and heat together, and the response to a combined stress is not predictable from separate single-stress responses (da Costa *et al.,* 2021; Yadav *et al.,* 2022). Second, the recent priming trials in direct-seeded rice have addressed salinity (Zhang *et al.,* 2025), while heavy-metal contamination, a growing constraint on peri-urban and industrially affected rice soils, remains largely untested for seed priming (Srivastava *et al.,* 2021). Third, priming responses are strongly cultivar-dependent, so a treatment validated on one genotype cannot be assumed to transfer to others (Zulfiqar *et al.,* 2022). The present study, therefore, pairs two complementary experiments within one system. The first addresses the magnitude of the priming effect and which pigments and photo protective traits it acts on, using a single widely grown cultivar under combined drought and heat; it establishes the physiological signature of priming. The second experiment shows how far that effect extends across genetic backgrounds and two chemically distinct stresses, using a seedling screen of nine genotypes under salinity and chromium stress; it tests the generality of the response. Altogether, the two experiments address effect size and transferability, which a low-cost pre-sowing treatment must meet before it can be recommended.

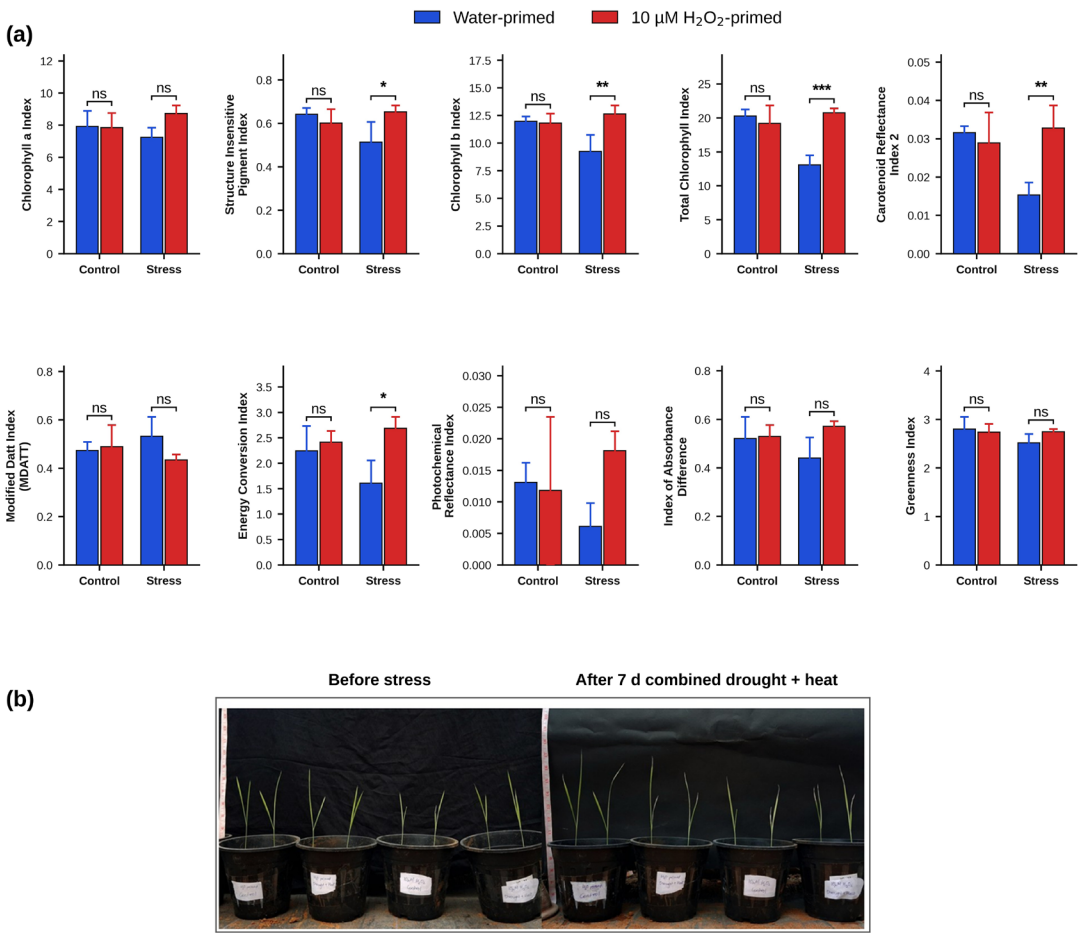
Seeds were surface-sterilised (0.1% NaOCl), rinsed and soaked for 16 h in 10 µM H_2O_2 or in distilled water as the control. For the combined drought and heat experiment, primed seed of cv. Dhaksha was raised in pots (3:1 soil:coco-peat) in a factorial completely randomised

design with two priming levels and two environments in three replicates. The 16 h soak was chosen since it allows rice seed to complete phase-I imbibition and early metabolic activation associated with priming without radicle emergence, allowing the treated seed to be subsequently handled as ordinary seed (Cheng et al., 2016). The 10 µM dose was selected as a low, sub-toxic concentration consistent with the role of hydrogen peroxide as a signalling molecule at low doses and as a damaging oxidant at higher concentrations (Hossain *et al.,* 2015; Zulfiqar *et al.,* 2022). The experiment was conducted at CHRIST (Deemed to be University), Bengaluru. Plants were grown under ambient greenhouse conditions at approximately 25–28 °C, with regular moisture monitoring to prevent waterlogging or excessive drying. Twenty-day-old plants were droughted by withholding water for seven days. Soil moisture during drought treatment was progressively reduced through a three-day restricted-watering pre-conditioning period followed by seven days of complete irrigation withdrawal. Heat stress was imposed concurrently at 40 °C for 2 h day^{-1} for seven consecutive days using a calibrated hot-air oven. Following stress imposition, plants were allowed a seven-day recovery period under optimal growth conditions, during which normal irrigation was restored and pots were maintained at field capacity; a second application of NPK 19:19:19 was supplied during recovery. Leaf pigments and reflectance indices were measured non-destructively with a CI-710s SpectraVue leaf spectrometer (CID Bio-Science, USA). For the seedling screen, primed seed of nine genotypes (KMP220, KMP225, RNR15048, MSN99, IR64, GV Sona, Jyothi, Thanu and Dhaksha) was germinated in paper rolls moistened with 120 mM NaCl or 10 ppm potassium dichromate ($K_2Cr_2O_7$); root length, shoot length and root-to-shoot ratio were recorded after seven days.

The combined drought and heat experiment was analysed using a two-way ANOVA with priming, environment and their interaction as fixed effects, with n = 3 independent biological replicates per priming × environment combination. The priming × environment interaction was considered the principal test of interest and was evaluated for each physiological and spectral index. The seedling screen was analysed using a two-way ANOVA with priming and genotype as fixed effects, followed by Šídák's multiple-comparison test for the planned water-primed versus H_2O_2-primed contrast within each genotype. The seedling screen included nine rice genotypes, with independent seedlings evaluated for each priming × genotype combination. All values are presented as mean ± standard error of the mean (SEM). Pearson correlation coefficients were calculated across treatment means (n = 4 observations for the 2 × 2 priming × environment comparison) and were interpreted as associations only. Statistical analyses and graphical representations were performed using GraphPad Prism version 10.5.0.

Under combined drought and heat stress, H_2O_2-primed plants stayed visibly greener and more turgid than water-primed plants (Fig. 1). Priming raised total chlorophyll ($p \leq 0.001$) and chlorophyll b ($p \leq 0.01$) in stressed plants, while chlorophyll a was higher but not significantly. Among reflectance indices, the Energy Conversion Index increased ($p \leq 0.05$) and Carotenoid Reflectance Index 2 increased more strongly ($p \leq 0.01$), and the Structure Insensitive Pigment Index, which tracks photoprotective pigments, was higher in primed stressed plants ($p \leq 0.05$); the Photochemical Reflectance Index and Index of Absorbance Difference were higher under priming but not significantly. Priming therefore stabilised the pigment pool and the photo protective indices as a whole rather than lifting any single pigment, consistent with H2O2 sustaining antioxidant capacity and protecting chloroplast pigments during and after stress (Hossain *et al.,* 2015; Nounjan *et al.,* 2020). Since, no antioxidant enzyme activity, reactive oxygen species, lipid peroxidation, or osmolyte measurements were made in this study, the reading is a hypothesis to be tested rather than as a demonstrated mechanism. Under control conditions no index differed between the two priming treatments (all $p > 0.05$; Fig. 1a).

In the seedling screen, the priming effect depended on genotype and on the stress applied (Fig. 2a, b). IR64 responded to priming in opposite directions under the two stresses (Fig. 2c). Under salinity, priming increased root length with no change in shoot length, so the root-to-shoot ratio increased. Under chromium, by contrast, priming reduced root length, while had no change in shoot length (5.96 ± 0.45 versus 5.90 ± 0.34 cm, $p > 0.05$), so the root-to-shoot ratio fell. Neither effect was observed in unstressed IR64 ($p > 0.05$), indicating that both were stress-inducible. Among the eight remaining genotypes, root length improved significantly with priming only



*Bars are mean ± SE, n = 3 biological replicates per treatment. ns, p > 0.05; *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001.*

Fig. 1. Effect of 10 µM H_2O_2 seed priming on rice cv. Dhaksha under combined drought and heat stress. (a) Leaf pigment contents and reflectance indices in water-primed (blue) and H2O2-primed (red) plants under control and combined-stress conditions: chlorophyll a index, Structure Insensitive Pigment Index, chlorophyll b index, total chlorophyll index, Carotenoid Reflectance Index 2, Modified Datt index, Energy Conversion Index, Photochemical Reflectance Index, Index of Absorbance Difference and Greenness Index. (b) Representative plants before (left) and after (right) seven days of combined drought and heat stress; images were edited to enhance contrast only. Bars are mean ± standard error of the mean, n = 3 independent replicates per treatment combination. ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

under chromium, in KMP225 (4.53 ± 0.67 to 8.08 ± 0.72 cm, $p \leq 0.001$) and RNR 15048 (3.51 ± 0.59 to 6.37 ± 0.86 cm, $p \leq 0.01$). The increases in Dhaksha (6.15 ± 0.76 to 8.11 ± 0.18 cm), KMP220, MSN99, GV Sona, Jyothi and Thanu were not significant. Under salinity no genotype showed a significant change in root length (all $p > 0.05$), although the direction of the effect was positive in seven of the eight genotypes. Shoot length responded mainly under chromium, increasing in six of the eight genotypes (Dhaksha, KMP220, RNR 15048, KMP225, Jyothi and Thanu; all $p \leq 0.0001$) but not in MSN99 or GV Sona. Under salinity the only significant shoot response was in Dhaksha (2.93 ± 0.31 to 3.81 ± 0.17 cm, $p \leq 0.05$). The direction of the change in root-to-shoot ratio therefore depended on the stress. Under salinity, where shoot length was largely unaffected, responsive genotypes shifted towards root growth, a shift that aids water and nutrient capture and is a recognised salt-tolerance trait (Zhang *et al.,* 2025). Under chromium the shoot response was the larger of the two, so the ratio moved in the opposite direction in most genotypes. Separating these two patterns is important, because a single summary statement about root-to-shoot ratio would obscure the fact that priming acts on different organs under the two stresses.

The two stresses differed less in the overall size of the priming benefit than in the organ on which it acted. Under chromium, shoot length increased in six of the eight genotypes and root length in only two, while under salinity the responses were smaller and largely non-significant, although root length moved in the positive

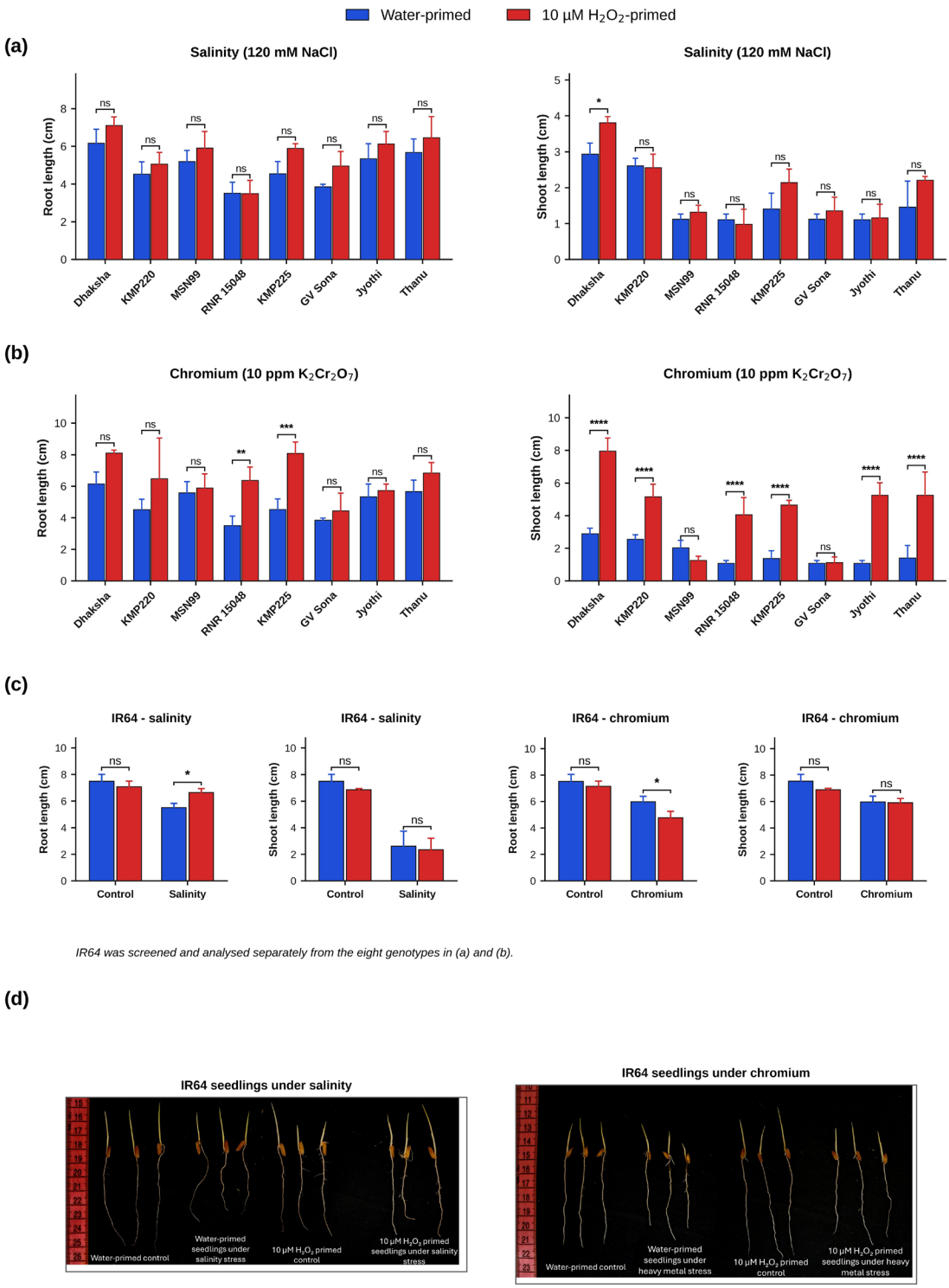


Fig. 2. Genotype-dependent effect of 10 µM H_2O_2 seed priming on rice seedlings after seven days in paper rolls. (a) Root and shoot length of eight genotypes under salinity (120 mM NaCl). (b) Root and shoot length of the same genotypes under chromium (10 ppm $K_2Cr_2O_7$, supplying Cr(VI)). (c) IR64, which was screened and analysed separately, under salinity and under chromium. (d) Representative IR64 seedlings under each stress; images were edited to enhance contrast only. Blue, water-primed; red, H_2O_2-primed. Bars are mean ± standard error of the mean, n = 10 seedlings per genotype x priming combination. ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

ARI1, Anthocyanin Reflectance Index 1; CHLT, Total Chlorophyll Index; CHLA/CHLB, Chlorophyll a / b Index; SIPI, Structure Insensitive Pigment Index; PRI, Photochemical Reflectance Index; MDATT, Modified Datt Index; CRI2, Carotenoid Reflectance Index 2; ECI, Energy Conversion Index; FRI, Flavonoid Reflectance Index; IAD, Index of Absorbance Difference; G, Greenness Index.

Fig. 3. Pearson correlation matrix relating leaf spectral indices to seedling root and shoot length. Cell colour and the printed coefficient give the strength and direction of each linear association (green, positive; red, negative). Coefficients were computed across treatment means (n = 3 observations) and describe association only; they do not establish causation. ARI1, Anthocyanin Reflectance Index 1; CHLT, total chlorophyll index; CHLA and CHLB, chlorophyll a and b indices; SIPI, Structure Insensitive Pigment Index; PRI, Photochemical Reflectance Index; MDATT, Modified Datt index; CRI2, Carotenoid Reflectance Index 2; ECI, Energy Conversion Index; FRI, Flavonoid Reflectance Index; IAD, Index of Absorbance Difference; G, Greenness Index.

direction in seven of the eight genotypes. Root extension under chromium was thus the least consistent response recorded, improving in two genotypes and declining in IR64.. One possible explanation, which the present data can suggest but not test, is that H_2O_2 priming works mainly through osmotic and ionic adjustment and antioxidant pre-conditioning, while metal toxicity additionally requires chelation and vacuolar sequestration that priming does not directly provide (Sharma *et al.*, 2012). Since these measurements were not made, this is a hypothesis for future work. It is relevant that the chromium source used here, potassium dichromate, supplies Cr(VI), the more toxic and more mobile oxidation state, and that Cr exposure is itself known to raise phenolic, flavonoid, and anthocyanin levels in rice tissue (Kang *et al.*, 2024) - a response that may contribute directly to the pigment-index associations described below. Across the dataset the pigment indices tracked growth: Anthocyanin Reflectance Index 1 correlated with root and shoot length (r = 0.93 and 0.80) and total chlorophyll with chlorophyll b (r = 0.99), while the Flavonoid Reflectance Index and Modified Datt index correlated negatively with growth (Fig. 3), linking priming-associated pigment retention to seedling extension, although these associations do not by themselves prove causation.

In summary, seed priming with 10 µM H_2O_2 improved early-stage stress tolerance in rice. Primed plants of cv. Dhaksha retained more chlorophyll and higher photoprotective reflectance indices under combined drought and heat stress, and priming increased root length in KMP225 and RNR 15048 under chromium and in IR64 under salinity, and increased shoot length in most genotypes under chromium, so that the direction

of the root-to-shoot response depended on the stress applied. Because these results come only from controlled pot and laboratory experiments, they should be read as a promising indication rather than a recommendation for practice. H_2O_2 priming is a low-cost, easily applied candidate treatment for direct-seeded rice, but its benefits are both cultivar- and stress-dependent, and it reduced root length in IR64 under chromium. Multi-season field validation across representative soils, sowing dates and stress environments is needed before any recommendation for direct-seeded systems can be made, and responsive genotypes would have to be identified first. Because the present evidence rests on non-destructive spectral indices and short-term seedling growth, pairing these with extracted-pigment, oxidative-marker and ion-content data and with multi-season field testing is the logical next step.

Several limitations should be stated explicitly. The physiological assessment rests on non-destructive leaf spectral indices; chlorophyll fluorescence (Fv/Fm), gas exchange, membrane stability, relative water content, and biomass accumulation were not measured, so the effect of priming on photosynthetic function and on growth itself is inferred rather than demonstrated. No biochemical markers of oxidative status were determined, so every mechanistic statement above is a hypothesis rather than a conclusion. Chromium partitioning between the root and shoot was not measured, so no uptake or detoxification mechanism is proposed. The correlation analysis is computed across treatment means and describes only the association; it cannot establish that pigment retention causes seedling extension, and the small number of observations means individual coefficients should be read as indicative. Finally, the drought-heat experiment used a single cultivar, so its physiological findings should not be generalised across genotypes without further testing.

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Author Contributions

GKR Conceptualized the study, GKR & TT executed the study, GKR & TT written and interpreted the study; all authors contributed to manuscript writing and approved the final version.

Declaration of Competing Interest

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

Use of AI Tools

AI tools were used for review of literature and language improvement.

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