

SEED INVIGORATION TECHNIQUES IMPROVE CROP ESTABLISHMENT AND PRODUCTIVITY IN DRY DIRECT SEEDED RICE

G. Senthil Kumar^{*1}, S. Manonmani¹, R. Suresh¹, N. Sritharan¹, A. Thanvarshni², and R. Krishnan²

^{*1}Department of Rice, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu

²Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu

^{*}Corresponding Author: G. Senthil Kumar, *Email: senthilkumar.g@tnau.ac.in

ABSTRACT

Dry direct-seeded rice (DSR) is an alternative to conventional puddled transplanted rice because of its lower labour and water requirements. However, poor and non-uniform crop establishment under aerobic soil conditions remains a major constraint. A field experiment was conducted during the Kharif seasons of 2023 and 2024 at the Department of Rice, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, to evaluate different seed invigoration techniques for improving seedling vigour and productivity of dry DSR. The experiment was laid out in a randomized block design with seven treatments and three replications. The treatments comprised hydropriming, seed hardening with 1% potassium chloride (KCl), priming with 1% sodium chloride (NaCl) solution, seed treatment with CPB-1 (chitinolytic bacterium), *Trichoderma asperellum*, *Bacillus subtilis* and an untreated control. Seedling vigour index at 14 and 21 days after sowing (DAS), yield attributes, grain yield and straw yield were recorded. Priming with 1% NaCl recorded the highest seedling vigour index at 14 DAS (1094.32) and 21 DAS (2682.11), whereas CPB-1 recorded the highest productive tillers (332 m⁻²), panicle weight (2.75 g), grain yield (5406 kg ha⁻¹) and straw yield (7658 kg ha⁻¹). Although NaCl priming produced the highest early seedling vigour, CPB-1 resulted in superior yield performance, indicating that early vigour alone did not directly determine final productivity. The higher grain yield under CPB-1 was associated with improved productive tiller production and panicle weight. Overall, the results suggest that CPB-1 and seed hardening with 1% KCl have potential as practical seed invigoration techniques for improving crop establishment and productivity of dry DSR.

KEYWORDS: chitinolytic bacterium; grain yield; seed hardening; seed priming; seedling vigour.

INTRODUCTION

Dry direct-seeded rice (Dry DSR) is emerging as a promising alternative to conventional puddled transplanted rice owing to its lower labour requirement, reduced water consumption, earlier crop maturity and lower production costs. This technology has gained increasing attention in regions facing labor shortages and declining water availability. However, successful adoption of dry DSR largely depends on rapid and uniform crop establishment, which is frequently constrained by erratic rainfall, inadequate soil moisture, high soil temperatures and intense weed competition during the early stages of crop growth. Under aerobic soil conditions, poor and uneven germination often results in sparse plant stands, delayed seedling emergence and reduced crop competitiveness against weeds, ultimately leading to substantial yield losses (1,2,3).

Seedling vigour is one of the most critical determinants of successful crop establishment in dry direct-seeded rice. Vigorous seedlings emerge rapidly, establish a stronger root system, utilize available soil moisture and nutrients more efficiently and exhibit greater tolerance to early-season abiotic stresses. Consequently, enhancing seedling vigour through simple and economical agronomic interventions has become an important strategy for improving the productivity and sustainability of dry DSR (4,5).

Among the various seed enhancement technologies, seed priming has emerged as one of the most effective, economical and farmer-friendly pre-sowing techniques. Seed priming involves controlled hydration of seeds using water or suitable osmotic or biological solutions for a predetermined period, followed by drying the seeds back to their original moisture content before sowing. This controlled hydration initiates the early metabolic processes associated with germination without permitting radicle protrusion. Consequently, primed seeds complete the remaining phases of germination more rapidly after sowing, resulting in faster and more uniform emergence compared with untreated seeds (6,7).

The benefits of seed priming are particularly pronounced under moisture-limited and stress-prone environments such as dry DSR (6,7). Primed seeds exhibit enhanced germination, improved seedling emergence and better stand establishment under aerobic soil conditions. The accelerated emergence enables rice seedlings to establish earlier than weeds, thereby improving crop competitiveness and reducing the adverse effects of weed infestation during the critical establishment phase. In addition, seed priming promotes the development of a vigorous root system, allowing seedlings to access water and nutrients more effectively, particularly under intermittent soil moisture stress (6-8).

The physiological advantages of seed priming are attributed to the activation of several biochemical and metabolic processes before sowing. Priming stimulates hydrolytic enzymes responsible for reserve mobilization, enhances antioxidant enzyme activity, improves membrane stability, accelerates DNA and protein synthesis and facilitates efficient energy metabolism during germination. These physiological and biochemical changes improve stress tolerance, reduce oxidative damage and support rapid seedling growth under sub-optimal environmental conditions. Consequently, primed plants generally produce more productive tillers, exhibit improved panicle development, achieve better grain filling and ultimately realize higher grain yield (8-10).

In recent years, biological seed treatments using beneficial microorganisms such as *Trichoderma*, *Bacillus* and chitinolytic bacteria have also gained importance as eco-friendly seed invigoration technologies. These beneficial microbes enhance seed germination and seedling vigour through the production of plant growth-promoting substances, improved nutrient availability, suppression of soil-borne pathogens and induction of systemic tolerance against abiotic stresses (11-13). Likewise, seed hardening and priming with sodium chloride (NaCl) have been reported to improve drought tolerance by enhancing osmotic adjustment, maintaining cellular water balance and improving physiological efficiency during early crop growth (14,15).

Chitinolytic bacteria have attracted attention as potential biological seed treatments because of their ability to produce chitin-degrading enzymes and other plant-beneficial metabolites, which may contribute to improved seedling growth and suppression of soil-borne pathogens. CPB-1 was selected for the present study based on its chitinolytic potential and its previously reported beneficial effects on rice growth and/or seedling establishment. However, its effectiveness as a seed treatment under dry direct-seeded rice (DSR) conditions, particularly in comparison with physical and chemical seed invigoration practices, has not been adequately evaluated. Therefore, CPB-1 provides a biologically based treatment for assessing whether microbial seed treatment can improve early seedling vigour and subsequent productivity under the challenging aerobic conditions of dry DSR.

Although several seed invigoration techniques have been reported to improve germination, seedling vigour and crop establishment in rice, previous studies have largely evaluated individual physical, chemical or biological approaches. Comparative evaluation of these seed invigoration strategies under dry direct-seeded rice (DSR) conditions remains limited. In particular, the potential of chitinolytic bacterial seed treatment, including CPB-1, in improving early seedling vigour and subsequent productivity under dry DSR conditions has received limited attention. Therefore, the present study was undertaken to compare physical, chemical and biological seed invigoration treatments and to identify an effective and economical approach for improving seedling vigour and productivity of dry direct-seeded rice.

Therefore, the present investigation was undertaken to evaluate the effectiveness of different seed priming and seed treatment techniques on crop establishment, growth and grain yield of dry direct-seeded rice and to identify a suitable and promising seed invigoration technology for enhancing the productivity and sustainability of dry DSR cultivation.

MATERIALS AND METHODS

Experimental Site

A field experiment was conducted during the Kharif seasons of 2023 and 2024 at the Department of Rice, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, to evaluate the influence of different seed priming and seed treatment techniques on crop establishment and productivity of dry direct-seeded rice (DDSR). The experimental site is situated in the western agro-climatic zone of Tamil Nadu and experiences a tropical semi-arid climate with distinct wet and dry seasons. The weather conditions during the cropping seasons of 2023 and 2024 have been provided. During Kharif 2023, the average temperature was 28.7°C, with maximum and minimum temperatures of 34.1°C and 23.2°C, respectively. During Kharif 2024, the average temperature was 29.2°C, with maximum and minimum temperatures of 33.4°C and 22.4°C, respectively. Morning relative humidity ranged from 74–80% in 2023 and 76–82% in 2024, while evening relative humidity ranged from 21–45% and 26–48%, respectively. The total rainfall received during the cropping period was 154 mm in 2023 and 178 mm in 2024. During the experimental period, the mean air temperature was 28.9°C, with a maximum temperature of 34.5°C and a minimum temperature of 23.2°C, which provided favourable conditions for the growth and development of the rice crop. Geographically, the experimental site is located at an elevation of 426.72 m above mean sea level, at 11° N latitude and 77° E longitude.

Before sowing, soil samples were collected from the experimental field at a depth of 0–15 cm using a soil auger. Soil cores were collected from different locations following a zig-zag sampling pattern across the experimental area, avoiding bunds and other non-representative spots. The collected cores were thoroughly mixed to obtain a representative composite soil sample for each experimental area. The samples were air-dried, gently crushed and passed through a 2-mm sieve before analysis. Soil texture was determined by the hydrometer method. Soil pH was measured potentiometrically in a 1:2.5 soil-to-water suspension using a calibrated pH meter, while electrical conductivity (EC) was measured in the same soil-water extract using a conductivity meter. Soil organic carbon was determined by the Walkley–Black wet oxidation method. The analytical procedures followed standard methods commonly used for soil characterization. The soil was clay loam in texture with a slightly alkaline reaction (pH 8.07), electrical conductivity (EC) of 0.51 dSm⁻¹, indicating non-saline conditions and an organic carbon content of 0.52%. These soil characteristics were considered suitable for evaluating the response of seed priming treatments under dry direct-seeded rice cultivation.

Experimental Design and Crop Establishment

The experiment was laid out in a Randomized Block Design (RBD) with seven treatments and three replications with gross plot of 5.0 x 4.0 m and net plot of 4.0 × 3.0 m. The treatments consisted of different seed priming and biological seed treatment techniques, viz., hydropriming (T₁), seed hardening (T₂), seed priming with 1% sodium chloride (NaCl) solution (T₃), seed treatment with CPB-1 (Chitinolytic bacterium) (T₄), seed treatment with *Trichoderma asperellum* (T₅), seed treatment with *Bacillus subtilis* (T₆) and an untreated control (T₇). The selected treatments represented physical, chemical and biological seed enhancement approaches aimed at improving seed germination, seedling vigour, crop establishment and grain yield under dry direct-seeded conditions.

For hydropriming (T₁), seeds were soaked in clean water at a seed-to-water ratio of 1:2 (w/v) for 12 h at 25–30°C, ensuring complete immersion of the seeds throughout the soaking period. After soaking, the water was drained and the seeds were shade-dried at room temperature until they regained their original moisture content. The hydroprimed seeds were then used for sowing (16). For seed hardening, seeds were subjected to seed hardening by soaking in a 1% potassium chloride (KCl) solution for 16 hrs. After soaking, the seeds were drained and shade-dried at ambient room temperature until they regained their original moisture content and were subsequently used for sowing. The hardened seeds were then used for subsequent sowing. For seed priming with 1% sodium chloride (NaCl) solution (T₃), seeds were primed by soaking in a 1% sodium chloride (NaCl) solution, prepared using distilled water, for 24 h at 25 ± 2 °C using a 1:5 (w/v) seed-to-solution ratio. After priming, the seeds were removed from the solution, washed thoroughly with distilled water to remove residual salt and shade-dried at room temperature until they regained their initial pre-priming weight. The dried seeds were then used for sowing.

The chitinolytic bacterial isolate CPB-1, previously identified by 16S rRNA gene sequencing, was used for seed treatment. The isolate was maintained as CPB-1 and sub-cultured on nutrient agar, followed by incubation at 30 ± 2 °C for 48 h. The bacterial culture was suspended in sterile distilled water and adjusted to approximately 1 × 10⁸ CFU mL⁻¹. Rice seeds were soaked in the bacterial suspension for 30 min, drained, shade-dried under ambient conditions until surface dry, and sown immediately. A spore suspension of *Trichoderma asperellum* was prepared by culturing the fungus on potato dextrose agar (PDA) and incubating at 28 ± 2 °C until adequate sporulation. The spores were harvested in sterile distilled water, filtered through sterile muslin cloth, and adjusted to a concentration of approximately 2 × 10⁴ spores mL⁻¹. Rice seeds were soaked in the spore suspension for 30 min, drained, shade-dried to a surface-dry condition under ambient temperature, and sown immediately. For the *Bacillus subtilis* treatment, rice seeds were treated with a talc-based formulation of *B. subtilis* at 10 g kg⁻¹ seed and thoroughly mixed to obtain uniform coating. The treated seeds were subsequently soaked in 1 L of water per kg of seed overnight and maintained under suitable conditions for 24 h to facilitate sprouting before sowing. The excess water was decanted before the sprouting period. The treated seeds were sown immediately after sprouting. Thus, this treatment involved *B. subtilis* seed treatment followed by hydration and pre-germination. Seeds in the untreated control (T₇) received no seed treatment prior to sowing.

It should be noted that the seed treatment protocols differed in soaking duration, treatment medium, microbial concentration and post-treatment handling. In particular, the *Bacillus subtilis* treatment included an additional overnight hydration and 24 h pre-germination step, unlike the other treatments. These protocol differences may have contributed to differences in emergence, seedling vigour and yield in addition to the effects of the respective seed treatments.

The suitable direct-seeding rice variety CO 53 was used for the experiment. Prior to sowing, seeds were subjected to the respective priming or seed treatment protocols according to the treatment schedule described above. After completion of the respective seed treatment protocols, the seeds were prepared for sowing according to the treatment-specific procedures. Sowing was carried out by the dibbling method in well-prepared dry soil at a spacing of 30 cm between rows and 10 cm between hills using a seed rate of 40 kg ha⁻¹. Uniform crop management practices were followed throughout the cropping period to ensure optimum crop growth.

Nutrient and Water Management

The crop received the recommended fertilizer dose of 75:50:37.5 kg N, P₂O₅, K₂O/ha through urea, single superphosphate (SSP) and muriate of potash (MOP), respectively. The entire quantity of phosphorus was applied as a basal dose before sowing to ensure adequate phosphorus availability during the early stages of crop growth. Nitrogen and potassium were each applied in three equal splits at 20-25, 40-45 and 60-65 days after sowing to synchronize nutrient availability with crop demand and improve nutrient use efficiency.

The experiment was conducted under unpuddled dry direct-seeded rice (DSR) conditions. Irrigation was provided during the initial crop establishment period to ensure uniform germination and seedling establishment. Subsequently, irrigation was managed following the alternate wetting and drying (AWD) principle, based on the field conditions (attain hairline crack), without maintaining continuous standing water. Since the experimental field was laid out in clay loam soil, irrigation was generally provided at about weekly intervals, depending on the prevailing soil moisture conditions. Thus, the crop was maintained under intermittent soil moisture conditions rather than continuous flooding, representing the moisture regime of dry DSR. Other agronomic practices, including weed management, plant protection measures and intercultural operations, were carried out uniformly across all experimental plots following the recommendations to avoid any confounding effects on treatment performance.

Observations recorded

Seedling vigour was assessed at 14 and 21 days after sowing (DAS). Ten randomly selected seedlings from each plot were carefully uprooted and washed to remove adhering soil. Seedling length was measured from the root tip to the tip of the longest leaf. The seedling vigour index (SVI) was calculated using the following formula (1).

$SVI = \text{Germination percentage} \times \text{Mean seedling length (cm)} \quad (1)$

Field emergence was assessed at 14 days after sowing (DAS) by counting the number of visibly emerged seedlings in each experimental plot. A seedling was considered emerged when the plumule had emerged above the soil surface. The percentage of field emergence was calculated based on the total number of seeds sown in each plot using the following formula (2). The resulting field emergence percentage was used in the calculation of the seedling vigour index along with seedling length.

$\text{Field emergence (\%)} = (\text{Number of emerged seedlings at 14 DAS} / \text{Total number of seeds sown}) \times 100 \quad (2)$

Growth and yield parameters were recorded at appropriate crop growth stages following standard procedures. At maturity, productive tillers were counted by placing a quadrat at representative locations within the net plot area. The number of tillers bearing panicles was recorded and expressed as the number of productive tillers per square metre (No. m^{-2}). For determination of panicle weight, representative panicles were harvested, air-dried and weighed using an electronic balance and the mean panicle weight was expressed in grams (g). Filled grain percentage was determined by separating filled and unfilled grains from randomly selected panicles and was calculated using the following equation (3).

$\text{Filled grain percentage (\%)} = (\text{Number of filled grains} / \text{Total number of grains}) \times 100 \quad (3)$

At physiological maturity, the crop was harvested from the undisturbed net plot area to avoid border effects. The harvested produce was threshed, cleaned, dried to a standard moisture content and the grain yield was recorded. The grain yield was subsequently converted and expressed as kilograms per hectare (kg ha^{-1}). After threshing, the remaining above-ground biomass was sun-dried to constant weight and expressed as straw yield (kg ha^{-1}). All observations were recorded separately for each treatment and replication during both years of experimentation.

Statistical analysis

For the combined analysis of the two Kharif seasons (2023 and 2024), year was considered as an environmental factor, and the treatment $\times$ year interaction was included in the analysis of variance to assess the consistency of treatment responses across years. The statistical model included the effects of year, treatment, and treatment $\times$ year interaction. Since the treatment $\times$ year interaction was non-significant ($P > 0.05$), the treatment effects were considered consistent across the two years and the data were pooled for combined analysis. Homogeneity of variance across years was also verified before pooling.

The data were subjected to analysis of variance (ANOVA), and treatment means were compared using the appropriate critical difference (CD) at the 5% probability level. The standard error of the mean (SE) was calculated for each parameter.

The experimental data collected during both years were subjected to analysis of variance (ANOVA) appropriate for the Randomized Block Design to evaluate the significance of treatment effects. Homogeneity of variance across years was examined before pooling the data and pooled analysis was performed wherever the treatment $\times$ year interaction was found to be non-significant. The significance of treatment differences was tested using Fisher's F-test at the 5% probability level ($P \leq 0.05$). Wherever significant differences were observed, treatment means were compared using the Critical Difference (CD) at the 5% level of significance. Statistical analyses were performed following the standard procedures described and the results were interpreted accordingly (17).

RESULTS AND DISCUSSION

Effect of seed invigoration treatments on seedling vigour of dry direct seeded rice

Seed enhancement treatments significantly influenced the seedling vigour index at both 14 and 21 days after sowing (Table 1, Figure 1). The improvement in seedling vigour under different priming and biological seed treatment techniques clearly indicates the effectiveness of these treatments in enhancing early crop establishment, which subsequently contributed to improved growth and productivity under dry direct-seeded rice.

Seedling vigour index showed marked improvement due to seed enhancement treatments. At 14 DAS, priming with NaCl (1%) recorded the highest seedling vigour index (1094.32), which was statistically comparable with seed treatment with CPB-1 (1075.07) and seed hardening (944.02). However, it exhibited significantly higher seedling vigour than hydropriming (741.24), seed treatment with *Bacillus subtilis* (632.24), seed treatment with *Trichoderma asperellum* (586.33) and the untreated control (577.32). The untreated control consistently recorded the lowest seedling vigour, indicating poor germination and slower seedling growth under dry direct-seeded conditions.

A similar trend was observed at 21 DAS. Seed priming with NaCl (1%) produced the highest seedling vigour index (2682.11) and was statistically comparable with seed hardening (2205.25) and seed treatment with CPB-1 (2218.33). Hydropriming recorded an intermediate value (1655.90), whereas *Bacillus subtilis* (1435.61), untreated control (1346.67) and the *Trichoderma asperellum* (1317.23) remained significantly inferior. The superiority of NaCl priming, seed hardening and CPB-1 treatment was evident even after three weeks of crop establishment, indicating that these treatments imparted prolonged physiological advantages beyond germination.

CPB-1 showed better seedling establishment than *Trichoderma asperellum* and *Bacillus subtilis*. The relatively high response to CPB-1 might be associated with the functional traits of the chitinolytic bacterial isolate including its ability to rapidly colonize the rhizosphere and produce metabolites that promote early seedling growth. However, the efficacy of *Trichoderma* and *Bacillus* as plant-growth-promoting microorganisms may depend on strain-specific features and environmental conditions affecting their establishment and activity. Thus, the relatively lower response obtained with these treatments in the present study should not be taken as an intrinsic inferiority of these microorganisms, but rather a differential response under the experimental conditions.

NaCl priming may improve seed hydration dynamics and stimulate pre-germinative metabolic processes, thereby contributing to rapid and uniform seedling establishment. Controlled hydration initiates membrane repair, enhances respiratory activity, activates hydrolytic enzymes such as α -amylase and accelerates mobilization of stored food reserves, resulting in rapid radicle emergence and vigorous seedling growth. NaCl priming also improves osmotic adjustment, enabling seedlings to withstand transient moisture stress commonly encountered under dry direct-seeded rice ecosystems (18,19).

The superior performance of NaCl seed priming over hydropriming may be related to differences in the regulation of water uptake during seed hydration. Hydropriming rapidly initiates metabolic activation and repair processes, whereas NaCl treatment can impose a controlled hydration environment that may prevent excessive water uptake while allowing pre-germinative metabolic processes to proceed. Such controlled hydration may improve physiological readiness of the seed and contribute to more uniform germination and early seedling establishment. However, as physiological parameters such as osmotic adjustment, antioxidant activity and enzyme activity were not measured in the present study, these mechanisms remain plausible explanations rather than directly demonstrated responses.

Similarly, seed hardening improves membrane integrity, increases antioxidant enzyme activity and enhances cellular tolerance against dehydration stress. Hardened seeds are capable of maintaining better cellular hydration and exhibit improved root development immediately after germination. The stronger root system facilitates efficient water and nutrient uptake, resulting in vigorous seedlings capable of sustaining rapid vegetative growth during the critical establishment phase (19).

The superior performance of CPB-1 observed in the present study may be associated with functional traits previously reported for chitinolytic and plant growth-promoting bacteria. Such microorganisms have been reported to produce extracellular enzymes and growth-promoting metabolites, and may contribute to root development, nutrient acquisition and suppression of soil-borne pathogens. These potential mechanisms could have contributed to the improved seedling vigour and subsequent crop performance observed with CPB-1 under the present field conditions. However, rhizosphere colonization, phytohormone production, nutrient acquisition and pathogen suppression were not directly measured in the present study; therefore, these mechanisms should be considered possible explanations based on previous literature rather than responses demonstrated by the present experiment.

The relatively lower seedling vigour recorded under *Trichoderma asperellum* and *Bacillus subtilis* treatments compared with CPB-1 suggests that although these beneficial microorganisms improve crop growth, their growth-promoting effects during the very early stages of crop establishment were comparatively less pronounced under the prevailing experimental conditions.

The present findings indicate that improved early seedling vigour may contribute to subsequent crop performance. However, higher seedling vigour did not necessarily translate directly into the highest grain yield. NaCl priming recorded the highest seedling vigour index at both 14 and 21 DAS, whereas CPB-1 produced the highest grain yield. Nevertheless, treatments with comparatively higher seedling vigour generally performed better than the untreated control for subsequent crop growth and yield. Thus, improved seedling vigour may be considered one of the contributing factors associated with better crop performance under dry direct-seeded rice conditions, rather than a sole determinant of final productivity (19-22).

The superior performance of hydropriming, seed hardening, NaCl priming and CPB-1 treatments was associated with improved seedling vigour and higher subsequent crop productivity. Seedling vigour index at 14 DAS was highest and statistically comparable under NaCl priming and CPB-1, while at 21 DAS, NaCl priming recorded the highest value and was statistically comparable with seed hardening and CPB-1. These treatments also recorded higher grain yields than the untreated control. The results therefore suggest that improved early seedling vigour may be one of the factors associated with better crop performance under dry direct-seeded rice conditions, although the present study does not establish a direct causal relationship between seedling vigour and grain yield.

Effect of seed invigoration treatments on yield attributes of dry direct seeded rice

The pooled analysis of two years of experimentation revealed that seed priming and biological seed treatments significantly influenced the yield attributes and productivity of dry direct-seeded rice (DDSR). Among the treatments, seed treatment with CPB-1 (Chitinolytic bacterium) consistently recorded the highest values for yield attributes, grain yield and overall crop performance.

The number of panicles per square metre was significantly affected by different seed treatments (Table 2, Figure 2). Seed treatment with CPB-1 produced the highest productive tillers (332 m^{-2}), which was statistically on par with seed hardening (318 m^{-2}) and priming with NaCl 1% (312 m^{-2}) and hydropriming (302 m^{-2}). Hydropriming and seed treatment with *Bacillus subtilis* recorded intermediate values of 302 and 301 panicles m^{-2} , respectively,

while seed treatment with *Trichoderma asperellum* (288 m⁻²) and the untreated control (256 m⁻²) recorded comparatively lower panicle numbers.

A similar trend was observed for panicle weight. The highest panicle weight (2.75 g) was recorded with seed treatment using CPB-1, statistically comparable with seed hardening (2.59 g), NaCl priming (2.48 g) and hydropriming (2.36 g). The untreated control registered the lowest panicle weight (2.09 g). The improvement in these yield attributes indicates that seed priming and beneficial microbial treatments enhanced early crop establishment, tillering and assimilate partitioning, thereby contributing to superior reproductive growth.

The filled grain percentage ranged from 86.51 to 89.22 per cent among the treatments. There was no significant difference among the treatments. Numerically, seed treatment with *Bacillus subtilis* recorded the highest filled grain percentage (89.22 %), followed by seed hardening (89.04 %), priming with NaCl 1% (88.92%), seed treatment with CPB-1 (88.87 %), hydropriming (88.33 %), *Trichoderma asperellum* (87.12 %) and control (86.51 %). However, the differences among treatments were smaller than the critical difference, indicating that the treatments did not significantly influence grain filling. The consistently high filled grain percentage across treatments suggests that the seed enhancement techniques primarily affected crop establishment and yield formation through increased tillering and panicle production rather than altering the grain-filling process. Grain filling in rice is largely governed by the availability of assimilates during the reproductive phase and environmental conditions prevailing after flowering. Since all treatments experienced similar environmental conditions during grain filling, the observed variation remained statistically non-significant.

Interestingly, the filled grain percentage did not differ significantly among treatments, despite differences in grain yield. This indicates that the yield advantage associated with seed treatments was unlikely to have resulted from a substantial alteration in the proportion of grains that became filled. Instead, the higher grain yield may have been associated more strongly with improved crop establishment and vigour and their subsequent effects on productive tiller formation, panicle development and grain production per unit area. The non-significant response in filled grain percentage therefore suggests that seed treatments primarily influenced the establishment and growth phases of the crop rather than directly altering grain-filling efficiency.

Effect of seed invigoration treatments on grain yield and straw yield

The grain yield differed significantly among the treatments (Table 3). Seed treatment with CPB-1 produced higher grain yield (5406 kg ha⁻¹), which was comparable with seed hardening (5217 kg ha⁻¹). This was followed by NaCl priming (5136 kg ha⁻¹) and hydropriming (4967 kg ha⁻¹). Seed treatments with *Bacillus subtilis* (4762 kg ha⁻¹) and *Trichoderma asperellum* (4711 kg ha⁻¹) also improved grain yield over the untreated control (4217 kg ha⁻¹).

The trend in straw yield (Table 3) largely followed that of grain yield. Seed treatment with CPB-1 recorded the highest straw yield (7658 kg ha⁻¹), followed by seed hardening (7234 kg ha⁻¹), NaCl priming (7102 kg ha⁻¹), hydropriming (6626 kg ha⁻¹) and *Bacillus subtilis* (6603 kg ha⁻¹). The control recorded a straw yield of 6781 kg ha⁻¹, indicating that the higher straw production did not translate into higher economic yield. Overall, CPB-1 seed treatment resulted in the highest straw and grain yields, whereas the untreated control recorded the lowest grain yield despite producing a comparatively moderate straw yield.

Overall, the study demonstrates that while filled grain percentage remained unaffected by seed enhancement treatments, substantial improvement in seedling vigour during the initial stages of crop growth significantly enhanced crop establishment and ultimately contributed to higher yield realization. These findings emphasize that successful crop establishment through appropriate seed enhancement technologies is a key determinant of productivity in dry direct-seeded rice systems. The findings demonstrate that seed treatment with CPB-1 and seed hardening are effective and practical seed enhancement techniques for improving the productivity of dry direct-seeded rice under field conditions (1,2).

CONCLUSION

The present study demonstrated that seed invigoration techniques significantly improved crop establishment and productivity of dry direct-seeded rice by enhancing early seedling vigour and subsequent yield attributes. Among the treatments evaluated, seed treatment with CPB-1 (chitinolytic bacterium) recorded the highest productive tillers and panicle weight. Although filled grain percentage remained unaffected, the higher grain yields achieved, particularly under CPB-1 and seed hardening, were primarily associated with improved seedling vigour, better crop establishment and enhanced tiller production. The findings indicate that early seedling vigour is a critical determinant of productivity in dry direct-seeded rice. Seed treatment with CPB-1 and seed hardening, being simple, economical and farmer-friendly technologies, can be recommended as effective seed invigoration practices for improving crop establishment and grain yield under dry direct seeded rice cultivation.

The study indicates that seed invigoration, particularly CPB-1 and seed hardening, has potential to improve crop establishment, early seedling vigour and productivity of dry direct-seeded rice. However, further research is needed to elucidate the long-term effects of CPB-1 application on soil microbial communities and soil health and to evaluate its integration with other components of integrated weed management for sustainable dry direct-seeded rice production. For wider farmer adoption, future studies should focus on developing a stable, cost-effective and easy-to-use CPB-1 seed treatment formulation, including optimization of carrier materials, shelf life, dosage and application methods. In addition, multi-location and multi-season validation under diverse soil, climatic and crop

management conditions is required to confirm the consistency, adaptability and economic feasibility of CPB-1 and other promising seed invigoration practices before large-scale recommendation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language editing and improving the clarity and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Table 1. Effect of treatments on seedling vigour index of dry direct seeded rice (Pooled analysis of two years data)

Treatments	Germination percentage	Implied seedling length (cm)		Seedling vigour index	
		14 DAS	21 DAS	14 DAS	21 DAS
T ₁ - Hydropriming	86	8.62	19.25	741.24 ^b	1655.90 ^{bc}
T ₂ - Hardening	90	10.49	24.50	944.02 ^a	2205.25 ^a
T ₃ - Priming with NaCl 1%	94	11.64	28.53	1094.32 ^a	2682.11 ^a
T ₄ - Seed treatment with CPB-1 (Chitinolytic bacterium)	93	11.56	23.85	1075.07 ^a	2218.33 ^{ab}
T ₅ - Seed treatment with Trichoderma asperellum	80	7.33	16.47	586.33 ^c	1317.23 ^c
T ₆ - Seed treatment with Bacillus subtilis	84	7.53	17.09	632.24 ^{bc}	1435.61 ^c
T ₇ - Control	79	7.31	17.05	577.32 ^c	1346.67 ^c
Exp. mean				844.36	1814.87
CV (%)				12.83	15.81
SEd				88.21	255.15
SEm (±)				62.38	180.42
CD (0.05)				188.34	527.38

Table 2. Effect of treatments on panicle number /m², panicle weight (g) and filled grain (%) of dry direct seeded rice (Pooled analysis of two years data)

Treatments	Productive tillers m ⁻²	Panicle weight (g)	Filled grain (%)
T ₁ - Hydropriming	302 ^{ab}	2.36 ^{bc}	88.33
T ₂ - Hardening	318 ^{ab}	2.59 ^{ab}	89.04
T ₃ - Priming with NaCl 1%	312 ^{ab}	2.48 ^{abc}	88.92
T ₄ -Seed treatment with CPB-1 (Chitinolytic bacterium)	332 ^a	2.75 ^a	88.87
T ₅ -Seed treatment with Trichoderma asperellum	288 ^{bc}	2.20 ^{cd}	87.12
T ₆ - Seed treatment with Bacillus subtilis	301 ^{ab}	2.32 ^{bc}	89.22
T ₇ - Control	256 ^c	2.09 ^d	86.51
Exp. Mean	301.29	2.40	88.29
CV (%)	19	4.95	-
SEd	15	0.11	4.2
SEm (±)	10.61	0.08	2.97
CD (0.05)	36	0.23	NS

**Table 3. Effect of treatments on Grain yield and Straw yield of dry direct seeded rice
(Pooled analysis of two years data)**

Treatments	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)
T ₁ - Hydropriming	4967.34 ^{bc}	6626.35 ^{bc}
T ₂ - Hardening	5217.28 ^{ab}	7234.28 ^{ab}
T ₃ - Priming with NaCl 1%	5136.47 ^b	7102.64 ^{ab}
T ₄ - Seed treatment with CPB-1 (Chitinolytic bacterium)	5406.62 ^a	7658.47 ^a
T ₅ - Seed treatment with Trichoderma asperellum	4711.39 ^c	6427.32 ^c
T ₆ - Seed treatment with Bacillus subtilis	4762.18 ^c	6603.58 ^{bc}
T ₇ - Control	4217.56 ^d	6781.26 ^{bc}
Exp. mean	4916.69	6918.70
CV	11.23	12.60
SEd	112	214
SEm (±)	79.20	151.32
CD (0.05)	237	355

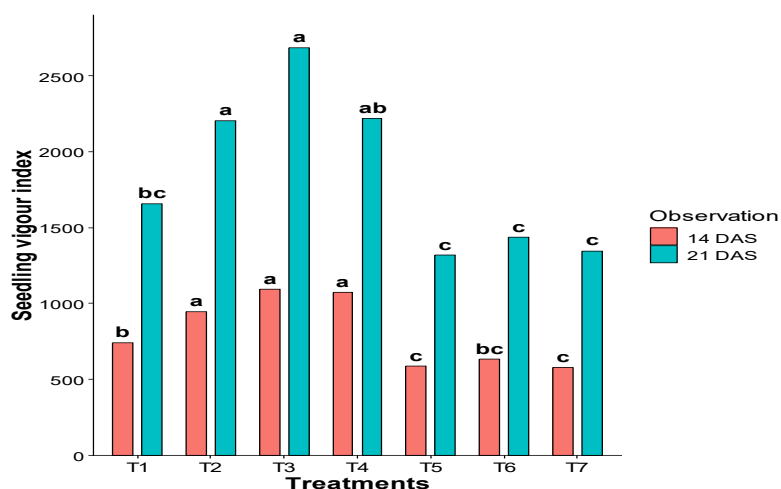


Fig. 1 Effect of treatments on seedling vigour index of dry direct seeded rice on 14 DAS and 21 DAS. Bars sharing the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.

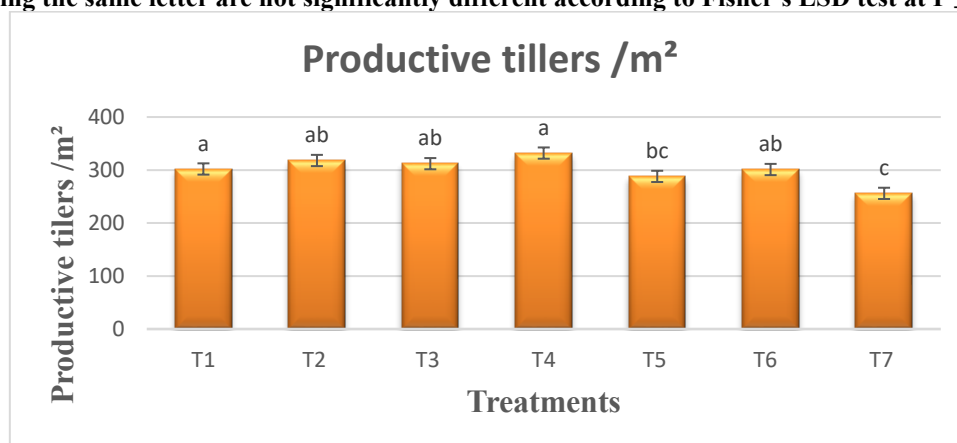


Fig. 2 Effect of seed invigoration treatments on productive tillers per square meter. Bars represent mean $\pm$ SEM (Standard error of mean). Bars sharing the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$ (P, Probability value).

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Authors' contributions

GS¹ conceptualized and wrote the original draft. GS¹ conceptualized, supervised and reviewed writing and editing. SM¹, RS², NS³, AT⁴, RK⁵ contributed to the review the manuscript.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest related to this study.

Ethical Issues: No ethical issues are associated with this research