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RESEARCH ARTICLE

Effect of seed treatments on drought tolerance, growth, yield and biochemical responses of rice under varying moisture regimes

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Abstract

A pot experiment was conducted at the Instructional Farm, College of Agriculture, Vellanikkara, during late rabi 2024 to study the effect of seed treatments on drought stress management and their impact on growth, yield and biochemical characteristics. The treatments consisted of seven seed treatments, viz., S_1-designer seed, S_2-*Piriformospora indica* at 10 mL kg^{-1} of seed, S_3-plant growth promoting rhizobacteria (PGPR) mix-1 at 50 g kg^{-1} of seed, S_4-proline at 0.1 %, S_5-calcium chloride ($CaCl_2$) at 2 %, S_6-hydro priming and S_7-control (without seed treatment), along with three moisture stress levels, viz., I_1-50 % field capacity (FC) at all growth stages, I_2-stress at maximum tillering (MT) and panicle initiation (PI) stages and I_3-no moisture stress. Seed treatments had a significant influence on growth, yield and biochemical parameters under different moisture levels. Plant growth promoting rhizobacteria seed treatment recorded a significantly higher grain yield (29.2 g $plant^{-1}$) under 50 % FC and under moisture stress at MT and PI stages (21.4 g $plant^{-1}$). Seed treatment with PGPR and $CaCl_2$ resulted in higher yield compared to the control treatment without seed treatment. Thus, seed treatments were found to be effective in mitigating drought stress and improving crop yield.

Keywords: calcium chloride; designer seed; drought stress; hydropriming; *Piriformospora indica*; plant growth promoting rhizobacteria; proline

Introduction

Rice is the second largest cereal crop cultivated worldwide and serves as a staple food for more than 3.5 billion people. However, rice cultivation is increasingly affected by climate change, including rising temperatures, heavy rainfall, frequent floods and a higher incidence of extreme weather events. Among this drought stress is one of the most severe constraints, causing reduced growth, impaired reproductive cycle, affecting panicle emergence and grain filling. It also increases weed competition and adversely affects physiological, morphological, biochemical functions of rice plants. These changes ultimately lead to a significant reduction in yield, posing a serious threat to food security.

Therefore, efforts must be aligned in mitigating the effects of drought stress on rice cultivation and productivity. One of the potential approaches is seed treatment, which enhances seed tolerance to stress and improves viability under adverse conditions. It is a low-cost and risk-free pre-planting method for managing water stress (1–3). Seed treatment improves plant resistance to stresses such as drought, salinity and biotic factors. Pre-soaking seeds in optimal concentrations of phytohormones enhances germination, growth and yield. Priming under stress conditions enhances nutrient reserve mobilisation, physiological activity and root proliferation, thereby improving plant establishment and stress tolerance (4, 5). There is a lack of knowledge in understanding how seed priming would make changes in crop growth and productivity under different moisture regimes. Hence, the present study was undertaken to evaluate the effect of seed treatments under different moisture stress conditions and their impact on rice productivity.

Materials and Methods

A pot culture experiment was conducted at Instructional Farm, Kerala Agricultural University, Vellanikkara, Kerala, India during late rabi 2024 with the objective to manage drought in rice. The mean average temperature was above 40 °C and experienced a rainless period for over 100 days. The crop days were exposed to high temperature up to maturity stage. Medium duration rice variety Uma was used for the study. The experimental design followed was factorial completely randomised design (FCRD) which was replicated thrice. Treatments consisted of 7 seed treatments viz., S_1-designer seed, S_2-*Piriformospora indica* at 10 mL kg^{-1} of seed, S_3-plant growth promoting rhizobacteria (PGPR) mix-1 at 50 g kg^{-1} of seed, S_4-proline 0.1 %, S_5-2 % calcium chloride ($CaCl_2$), S_6-hydro priming, S_7-control (without seed treatment) and 3 moisture stress levels viz., I_1-50 % field capacity (FC) (at all growth stages), I_2-stress at maximum tillering (MT) and panicle initiation (PI) stage, I_3-no moisture stress. In I_1-50 % FC was calculated using gravimetric method. At I_2 moisture stress was imposed at MT and PI stage by withdrawing irrigation for 5 days then providing irrigation

after 90 % of plants exhibited leaf rolling symptoms. After 5 days, the irrigation was given and maintained as unsaturated condition. The study was conducted in ultraviolet (UV) stabilised grow bag with size of 40 × 24 × 24 cm^3, with 600 G (150 M) thickness. Fertilisers, lime and farmyard manure (FYM) was applied as per KAU package of practice recommendation (6). Urea (46 % N), rock phosphate (20 % P_2O_5), muriate of potash (MOP-60 % KCl) was used as the source of nitrogen, phosphorus and potassium respectively. Lime was applied at 250 kg ha^{-1} and mixed thoroughly two weeks before sowing crop. Farmyard manure at 5 t ha^{-1} was applied 3 days before sowing of crop.

Seed treatment

In the case of designer seed treatment (Fig. 1), rice seeds were first soaked in KAU Sampoorna multimix at 100 ppm for 3 hr then dried back to original moisture content thereafter dried seeds were mixed with polymer at 3 mL kg^{-1} (for increasing stickiness of chemical added) then with Imidachloprid WS/FS at 5 mL kg^{-1} and PGPR mix -1 at 50 g kg^{-1} of seeds. In S_2 *P. indica* fungal strain was obtained from Department of Microbiology, Vellayani, Thriuvanathapuram. *Piriformospora indica* was cultured on agar containing potato dextrose agar (PDA) media. For S_3 PGPR mix-1 is a consortium of



Fig. 1. Designer seeds after treatment.

beneficial bacterial strain which was obtained from Department of Microbiology, Vellanikkara, Thrissur. Seed treatments such as proline, $CaCl_2$ and hydropriming was done a day before sowing by soaking with respective chemical around 4 hr.

Data collection

Growth parameters such as plant height, leaf area, root length, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, dry matter production was observed at 30, 60 and 90 days after sowing (DAS). Number of days for flowering and yield parameters such as number of panicles per plant, number of grains per panicle, grain yield, 1000-grain weight, straw yield were recorded. Biochemical parameters such as relative water content (RWC), specific leaf weight (SLW), total chlorophyll content (CHL), carotenoid content, proline content, cell membrane stability index (CMS), malondialdehyde (MDA) and enzyme activities such as catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) were also estimated after imposing stress to the rice.

Proline estimation

Proline content in control and water-stressed seedlings was estimated (7). Root and shoot samples (300 mg) were homogenised in 5 mL of 3 % sulfosalicylic acid and centrifuged at 22000 × g for 20 min. Subsequently, 2 mL of the supernatant was mixed with 2 mL of freshly prepared acid ninhydrin solution and 2 mL of glacial acetic acid. The mixture was heated in a boiling water bath at 100 °C for 1 hr. After cooling, the reaction mixture was extracted with 5 mL of toluene and vortexed for 15 sec. The tubes were then kept undisturbed for 10 min at room temperature to facilitate separation of the aqueous and toluene phases. The upper toluene layer was carefully collected and absorbance was recorded at 520 nm using a spectrophotometer. L-proline was used as the standard and proline content was expressed as µmol g^{-1} fresh weight.

Enzyme assay of catalase, ascorbate peroxidase, glutathione reductase activity

Catalase, APX and GR enzyme activities were determined using leaf samples (0.1 g) homogenised in 50 mM sodium phosphate buffer (pH 6.8). The homogenate was centrifuged and the resulting supernatant was used for enzyme assays. Catalase activity was estimated (8) where one unit of CAT activity was defined as the quantity of enzyme required to decompose 1 µmol of hydrogen peroxide (H_2O_2) per min. Ascorbate peroxidase activity was studied (9). One unit of APX activity corresponded to the amount of enzyme that oxidised 1 µmol of ascorbic acid per min and the reduction in ascorbic acid concentration was monitored at 290 nm. Glutathione reductase activity was measured (10), in which one unit of GR activity represented the amount of enzyme responsible for degrading 1 µmol of nicotinamide adenine dinucleotide phosphate (NADPH) per min. The decline in NADPH concentration was recorded at 340 nm.

Localisation of superoxide anion and hydrogen peroxide using nitroblue tetrazolium and 3,3-diaminobenzidine staining

Localisation of superoxide anion ($O_2^{\bullet-}$) in situ was detected in the leaves of the control and water deficit-stressed seedlings using nitroblue tetrazolium (NBT) as substrate (11). Leaf discs (3–5 mm) were immersed in 6 mM NBT (prepared in 10 mM sodium-citrate buffer, pH 6.0) at 25 °C for 8 hr under light. Nitroblue tetrazolium reacts with $O_2^{\bullet-}$, producing dark-blue insoluble formazan spots in the leaves, which indicate the accumulation of reactive oxygen species (ROS) in rice under water-deficit conditions. The leaves were then immersed in boiling ethanol (95 %) for 10 min to decolourise the leaves except for the dark-blue insoluble formazan deposits produced by the reaction of NBT with $O_2^{\bullet-}$. After cooling, leaf discs were photographed. Histochemical detection of H_2O_2 in situ was done (12). Leaf discs, 3–5 mm, were incubated in a solution containing 1 mg mL^{-1} of 3,3-diaminobenzidine (DAB) prepared in hydrochloric acid (HCl) acidified (pH 3.8) water. After 8 hr of incubation at 25 °C, leaf discs were kept in boiling 95 % ethanol for 10 min and were then cleared in saturated chloral hydrate. Leaf discs were then examined under a light microscope. Reddish-brown spots in leaves is a characteristic feature of the reaction between DAB and H_2O_2, indicating presence of H_2O_2 accumulation in the leaf tissues. Nitroblue tetrazolium staining for $O_2^{\bullet-}$ and DAB staining for H_2O_2 were performed to assess ROS accumulation.

Statistical analysis

Data collected was analysed statistically using analysis of variance (ANOVA) method using software GRAPES KAU version 1.1.0 to identify significant differences among rice under different moisture regimes and among treatments (13). Least significant difference (LSD) test was applied for comparisons among treatment means.

Results and Discussion

Growth parameters

Seed treatment had significant influence on growth parameters of rice under varying moisture stress. Plant height was significantly influenced by seed treatments and moisture stress at different growth stages, but the interaction of two factors influenced only at 60 DAS (Table 1). At 60 DAS seeds treated with *P. indica* at 10 mL kg^{-1} reported taller plants compared to other treatments when stress was imposed at MT, PI stage and no moisture stress. Possible reasons for this could be due to earlier seedling emergence and better utilisation of photoperiod at early growing vegetative phase. Similar results were observed in previous studies (14, 15), in which treatment with the endophyte *P. indica* enhanced early seedling growth and emergence in rice. The study describes the initial events occurring during seed germination in seeds that have lost viability due to long-term storage or artificial ageing, as well as in seeds that retain high moisture content at maturity, commonly referred to as recalcitrant seeds. However, under 50 % FC seed treated with $CaCl_2$ at 2 % observed taller plants. This might be due to calcium helping to regulate membrane permeability and water transport, ensuring better hydration of tissues. This leads to improved cell expansion and overall taller growth, especially under mild stress conditions (16). Likewise, fresh shoot weight was significantly influenced at initial stages of growth with $CaCl_2$ treatment. At 30 DAS, seeds treated with 2 % $CaCl_2$ exhibited the highest fresh shoot weight (Table 2) compared with all other seed treatments, irrespective of whether water stress was imposed at the MT and PI stages or under non-stress conditions. Like this, dry shoot weight was positively influenced at 30 DAS by seed treatments and moisture stress levels. Highest dry shoot weight and dry matter production were observed in seed treated with $CaCl_2$ at 2 % when moisture stress was imposed at MT and PI (2.5 g $plant^{-1}$).

At 50 % FC highest fresh shoot weight was observed in proline seed treatment. Proline stabilises cell membranes, enzymes and proteins, preventing denaturation under stress. This helps maintain metabolic activity and photosynthesis, supporting continued shoot growth and biomass accumulation (17). Similarly root length was also positively influenced by proline seed treatment. At 30 DAS the longest root length (30.15 cm) was observed in seeds treated with proline at 0.1 % under 50 % FC. When moisture stress was imposed at MT and PI stage, longer root length was noticed in proline treatment which was about 29 cm. Fresh root weight was significantly influenced only at 30 DAS by seed treatments as well as moisture stress levels. A similar trend was observed for fresh root weight, with the highest value recorded in seeds treated with 0.1 % proline under 50 % FC at 30 DAS. When moisture stress was imposed at MT and PI stage highest fresh root weight was observed in *P. indica* seed treatment (7.55 g). Under 50 % FC highest dry shoot weight was observed in seeds treated with proline at 0.1 % (2.4 g $plant^{-1}$). Like fresh shoot weight, dry matter production was also influenced only at 30 DAS. At 30 DAS the longest dry matter production was observed in seeds treated with proline at 0.1 % under 50 % FC (3.4 g $plant^{-1}$). Thus, seed treatment with proline has improved roots as well as shoot biomass at initial stages of growth.

Table 1. Effect of seed treatments and moisture stress on plant height at different growth stages

Treatments	Plant height											
Seed treatments	**30 DAS**				**60 DAS**				**90 DAS**			
Factor A-seed treatments	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
S_1-designer seed	31.9	34.2	31.9	32.7	61.7	50.5	47.5	53.2	67.7	84.8	85.2	79.2
S_2-*Piriformospora indica* at 10 mL kg^{-1} of seed	32.6	39.6	41.3	37.84	53.4	62.5	63.6	59.8	79.77	85.77	87.57	84.4
S_3-PGPR mix at 50 g kg^{-1} of seed	31.9	35.3	35.7	37.8	58.9	57.5	63.1	59.8	95.9	85.7	81.8	87.8
S_4-proline 0.1 %	38.5	34.3	33.4	34.3	59.5	60.0	52.0	57.2	66.2	72.2	43.4	60.6
S_5-$CaCl_2$ 2 %	32.5	38.7	35.8	35.4	62.3	55.2	56.5	58.0	86.7	88.4	97.1	90.7
S_6-hydro priming	31.9	33.3	35.9	35.6	51.5	53.5	41.1	48.7	91.7	94.9	91.8	92.8
S_7-control	31.9	34.2	31.9	33.8	43.2	50.3	43.7	45.7	100.9	99.0	86.4	95.5
Mean	32.9	35.7	35.7		55.7	55.6	52.5		84.1	87.3	81.9	
CD (0.05)-seed treatments	**3.01**				**6.07**				**10.57**			
CD (0.05)-moisture stress	**1.97**				**1.39**				**NS**			
Interaction CD (0.05)	**NS**				**10.52**				**NS**			

DAS: days after sowing; PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; NS: non significant.

Table 2. Effect of seed treatments and moisture stress on fresh shoot weight at different growth stages

Treatments	Fresh shoot weight											
Seed treatments	**30 DAS**				**60 DAS**				**90 DAS**			
Factor A-seed treatments	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
S_1-designer seed	4.8	4.1	6.2	5.0	31.7	33.9	37.5	34.3	59.6	49.1	51.2	53.3
S_2- *Piriformospora indica* at 10 mL kg^{-1} of seed	4.0	6.7	5.8	5.5	20.7	17.5	19.9	19.4	54.3	51.1	51.5	52.3
S_3-PGPR mix at 50 g kg^{-1} of seed	5.8	3.5	8.8	6.0	16.3	28.7	20.6	21.9	58.4	45.6	50.6	51.6
S_4-proline 0.1 %	11.1	5.2	5.4	7.2	22.2	18.2	20.6	20.3	55.0	59.6	49.2	54.6
S_5- $CaCl_2$ 2 %	6.1	9.5	9.9	8.5	26.1	20.5	37.3	27.9	61.1	48.6	55.5	55.0
S_6-hydro priming	7.8	4.6	4.7	5.7	13.1	16.9	15.2	15.1	53.8	50.9	53.9	52.9
S_7-control	5.6	3.8	7.7	5.7	21.1	17.9	16.0	18.3	54.2	54.3	51.1	53.2
Mean	6.4	5.31	6.90		21.6	21.93	23.86		56.6	51.32	51.87	
CD (0.05)-seed treatments	**1.4**				**4.5**				**NS**			
CD (0.05)-moisture stress	**0.9**				**NS**				**NS**			
Interaction CD (0.05)	**2.6**				**7.9**				**NS**			

DAS: days after sowing; PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; NS: non significant.

However, the tiller number and leaf area were not influenced by seed treatments as well as moisture stress levels at any stages of growth.

Yield parameters

The yield parameters such as number of panicles per plant, number of grains per panicle, 1000-grain weight, grain yield (g plant^{-1}), straw yield (g plant^{-1}) were observed and presented in Table 3. Interaction of seed treatments and moisture stress levels had significantly influenced number of panicles per plant, grain yield and straw yield positively. Higher number of panicles per plant was observed in PGPR seed treatment under 50 % FC as well as under no moisture stress. Stress imposed at MT and PI stage, highest number of panicles per plant was observed under PGPR seed treatment.

Under no moisture stress, highest grain yield was obtained from designer seed followed by *P. indica* seed treatment. At 50 % FC moisture stress maximum grain yield was obtained from PGPR seed treatment (Table 4). In PGPR the bacterial consortium such as *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* colonise the rhizosphere and stimulate root elongation. They produce phytohormones (auxins, gibberellins and cytokinins) that promote better root architecture, leading to increased absorption of nutrients and water. Thus, through seed treatment a comparable yield was obtained from 50 % FC moisture stress and no moisture situation. Much research aligned to similar findings PGPR consortium had improved biomass and grain yield (18). Further under 50 % FC yield increase with PGPR seed treatment was 35 % more compared to no seed treatment.

Like 50 % FC condition stress imposed at MT and PI stage found highest grain yield from PGPR seed treatment, it was closely followed by $CaCl_2$ seed treatment. The yield increases when stress imposed at MT and PI stage with PGPR and $CaCl_2$ seed treatment was 51.77 and 51.06 % respectively over control without seed treatment. Calcium is important secondary nutrient; it acts as secondary messenger and helps to regulate stress response. Stress tolerance in rice seeds is imparted by seed priming with $CaCl_2$. Calcium imparts stress tolerance by regulating antioxidant enzymes and reducing oxidative stress.

Following the similar trend as of grain yield and no. of panicles per plant, the no. of grains per panicle was highest under PGPR seed treatment. Second highest no. of grains per panicle was observed under $CaCl_2$ seed treatment. So, the maximum grain yield from seed treatment with PGPR and $CaCl_2$ could be contributed by higher no. of panicles per plant as well as higher no. of grains per panicle. As PGPR microbial consortium would have mobilised nutrients in soil that could have helped plant roots with higher nutrient uptake and utilisation. Better nutrient uptake could have

Table 3. Effect of seed treatments and moisture stress on yield parameters

	Yield parameters											
Treatments	**Number of panicles per plant**				**Number of grains per panicle (g plant^{-1})**				**Straw yield**			
Factor A-seed treatments	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
S_1-designer seed	9.0	9.0	11.7	9.9	79.0	84.3	123.0	95.4	48.7	73.0	64.4	62.0
S_2- *Piriformospora indica* at 10 mL kg^{-1} of seed	9.3	9.0	11.0	9.7	85.3	102.7	102.7	96.9	56.0	42.9	67.3	55.4
S_3-PGPR mix at 50 g kg^{-1} of seed	13.3	8.3	12.7	11.4	104.3	119.7	95.7	106.6	60.4	52.9	52.0	55.1
S_4-proline 0.1 %	8.7	7.7	10.0	8.7	94.0	67.7	55.0	72.2	50.5	50.3	47.0	49.3
S_5-$CaCl_2$ 2 %	9.2	9.7	9.8	9.4	106.3	99.0	95.7	100.3	63.3	68.8	50.8	60.9
S_6-hydro priming	10.2	10.7	10.1	10.5	63.7	78.3	64.7	68.9	64.7	68.5	47.5	60.2
S_7-control	6.3	6.5	7.3	7.1	82.3	90.7	88.3	87.1	48.8	39.9	45.5	44.7
Mean	10.3	8.7	9.8		87.9	91.8	89.3		56.1	56.6	53.5	
CD (0.05)-soil amendments	**1.74**				**25.2**				**9.51**			
CD (0.05)-moisture stress	**1.14**				**NS**				**NS**			
Interaction CD (0.05)	**3.03**				**NS**				**5.77**			

DAS: days after sowing; PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; NS: non significant.

Table 4. Effect of seed treatments and moisture stress on grain yield

Treatments	**Grain yield (g plant^{-1})**			
Seed treatments	**I_1-50 % FC (at all growth stages) (g plant^{-1})**	**I_2- Stress at MT and PI stage (g plant^{-1})**	**I_3- No moisture stress (g plant^{-1})**	**Mean**
S_1-designer seed	15.0	15.8	29.4	20.1
S_2- *Piriformospora indica* at 10 mL kg^{-1} of seed	17.5	20.8	24.7	18.0
S_3-PGPR mix at 50 g kg^{-1} of seed	29.2	21.4	23.4	24.6
S_4-proline 0.1 %	18.1	11.6	10.7	13.5
S_5-$CaCl_2$ 2 %	20.5	21.3	21.2	21.0
S_6-hydro priming	18.3	14.3	13.6	15.4
S_7-control	18.9	14.1	15.4	16.1
Mean	19.6	17.0	18.5	
CD (0.05)-seed treatments	**6.2**			
CD (0.05)-moisture stress	**NS**			
Interaction CD (0.05)	**NS**			

PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; NS: non significant; FC: field capacity; MT: maximum tillering; PI: panicle initiation.

enhanced source sink partitioning that would have contributed to the better grain formation grain filling finally resulting in good yields (19).

Straw yield was significantly influenced by seed treatment, but moisture stress and the interaction of the two factors didn't influence straw yield. Highest biomass was obtained under designer seed.

Biochemical parameters

Biochemical parameters such as RWC, SLW, CHL, carotenoid content, proline content, CMS and MDA were estimated after imposing stress on the rice.

Photosynthesis and all allied activities depend on light harvesting structures such as chlorophyll. In this study chlorophyll content was also significantly influenced by seed treatments and moisture stress levels. Seed treatment with proline at 0.1 % showed maximum chlorophyll content in both 50 % FC moisture stress and stress imposed at MT and PI stage (Fig. 2). Similarly, proline content was higher in stressed conditions such as 50 % FC (1.28 µmol g^{-1} fresh weight) in proline treatment. However, stress at MT and PI under PGPR seed treatment, maximum proline content was noticed (1.2 µmol g^{-1} fresh weight) (Fig. 3). Studies have shown that proline seed treatment had reduced osmotic stress and protected scavenging from ROS that maintained chlorophyll and other cellular contents (20–22). Further proline treatment can increase antioxidant enzyme activities, thereby protecting chlorophyll and other cellular components from oxidative damage (23). Proline treatment improved physiological traits such as chlorophyll retention and stress tolerance at the vegetative stage, but these improvements were not sufficient to enhance assimilate partitioning and reproductive development under drought stress. Higher chlorophyll content indicates better maintenance of the photosynthetic apparatus; however, grain yield is a complex trait influenced by several factors including panicle formation, spikelet fertility, grain filling and translocation of photosynthates. Under severe moisture stress, the beneficial effect of proline on chlorophyll stability may not have adequately supported reproductive processes, resulting in comparatively lower grain yield. Additionally, exogenous proline

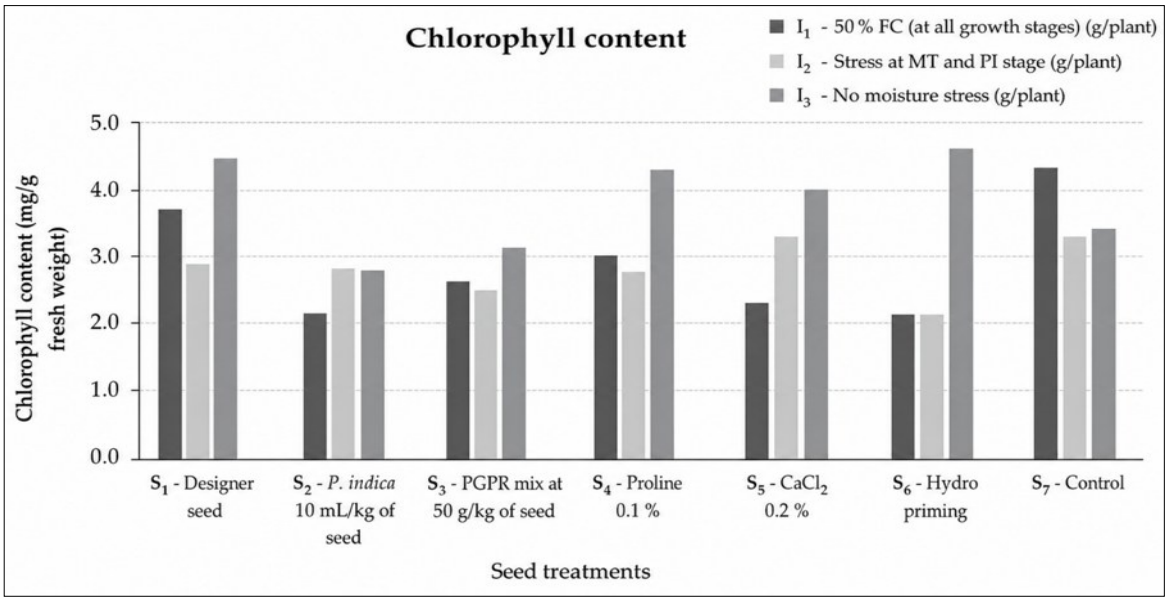


Fig. 2. Effect of seed treatments and moisture stress on chlorophyll content ($p \leq 0.05$). PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; FC: field capacity; MT: maximum tillering; PI: panicle initiation; *P. indica*: *Piriformospora indica.*

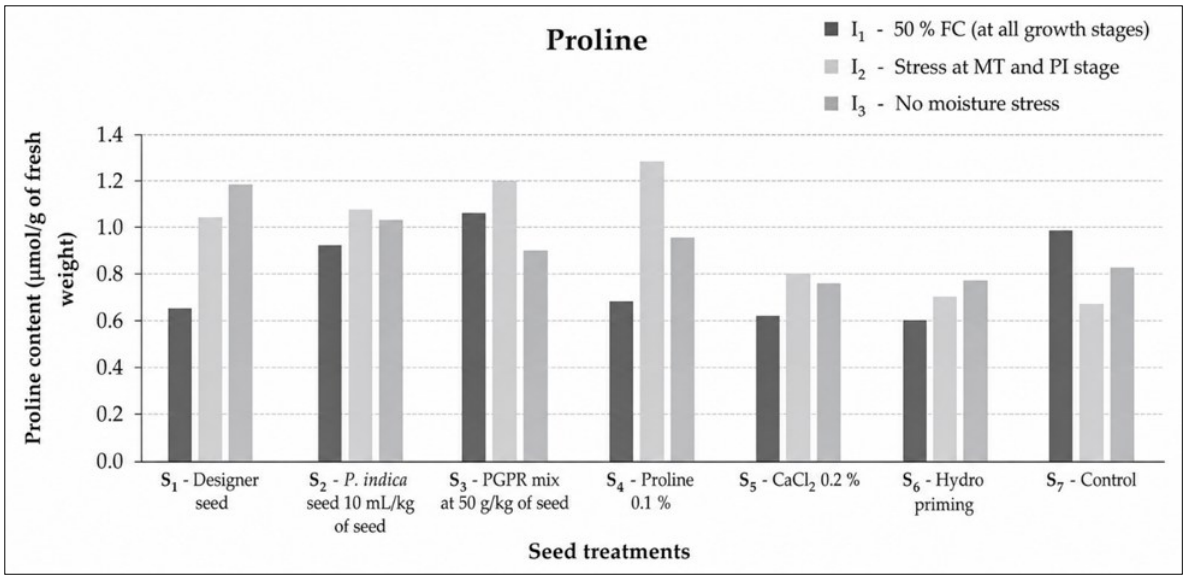


Fig. 3. Effect of seed treatments and moistures stress on proline content ($p \leq 0.05$).

PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; FC: field capacity; MT: maximum tillering; PI: panicle initiation; *P. indica*: *Piriformospora indica.*

application might have enhanced stress protection rather than directly stimulating yield-contributing parameters.

Membrane stability describes the strength of cell membrane, especially when plants are exposed to stress. It plays a vital role in plant survival by preserving the membrane's selective permeability, which controls the movement of substances into and out of the cell and shields it from injury. This parameter is commonly used to evaluate and select plants with greater tolerance to environmental stress conditions. In this work cell membrane study was positively influenced by seed treatments and moisture stress levels. In hydropriming, the cell membrane showed higher values (Table 5). Possible reason for this might be in hydropriming seeds before germination itself gives seed hardening and resistance that might have carried towards letter stages of development. Indirectly hydropriming could have triggered activation of antioxidant enzyme activities helping plants to cope with oxidative stress caused by lower water conditions. Similar findings reported in a study revealed that hydropriming had increased the oxidative and moisture stress tolerance of rice in Asian regions (24). However, RWC, SLW and MDA content was not influenced by any of seed treatments or moisture stress levels. Hydropriming may have improved crop growth rate and CMS during the early vegetative stage by enhancing seed hydration, enzyme activation and initial seedling vigour. However, these early physiological advantages may not have been sustained throughout the crop growth period under drought stress conditions. Grain yield depends largely on reproductive stage processes such as PI flowering, spikelet fertility and grain filling. Although hydropriming enhanced physiological performance initially, it may not have provided prolonged stress protection during critical reproductive stages, leading to reduced assimilate translocation and poor grain development, ultimately resulting in lower yield.

Enzyme activities

Enzyme activities such as CAT, APX and GR were estimated after imposing stress on the rice. The data on enzyme activities such as CAT, APX and GR is presented in Fig. 4A–C. Catalase is one of the important enzymes that has got potential role in dismutase of H_2O_2 into water and oxygen and this very important for ROS scavenging during any kind of stress in plants (25). Among different seed treatments highest CAT was observed in seed treatment *P. indica* and proline compared to other treatments (Fig. 4A). Since the ROS generation could be higher in this treatment, higher CAT activity was noticed for scavenging the ROS. Similar results were observed in a previous study (26) with the stress induced at different levels.

Ascorbate peroxidase belongs to the family of heme containing peroxidases that catalyse the H_2O_2-dependent oxidation of a wide range of organic molecules. It is present across a wide spectrum of plant kingdoms and plays a crucial role in growth regulation. The APX content was evaluated under different seed treatments and moisture stress levels (Fig. 4B). The highest APX content was observed in the control treatment, without any seed treatment, across all moisture stress levels. The elevated APX activity observed in the control treatment may be attributed to increased oxidative stress due to the absence of stress mitigation strategies. Enhanced APX activity reflects an induced antioxidant defence mechanism to counteract excessive ROS. In contrast, treated plants exhibited relatively lower APX activity, possibly due to reduced ROS generation and improved physiological stability under stress conditions.

Glutathione reductase is essential for plant defence against oxidative stress by ensuring the continued availability of reduced glutathione (GSH). As a crucial component of the ascorbate-glutathione cycle, GR actively scavenges ROS to protect cells. The expression and activity of this enzyme increase to counteract environmental stresses such as drought, high salinity, extreme temperatures, mechanical damage, heavy metal toxicity and herbicide exposure (27). Highest GR content was observed (Fig. 4C) in PGPR mix -1 followed by *P. indica*.

Nitroblue tetrazolium (Fig. 5A-G) and DAB (Fig. 6A-G) staining revealed darker blue colouration on control without any seed treatment indicating accumulation of more ROS species and H_2O_2 than seed treatments symbolising plants are more stressed in control without seed treatment.

Economics of cultivation

This study demonstrates the potential for enhancing net returns from rice cultivation under drought conditions through appropriate management practices. Economic parameters, including gross returns, net returns and benefit : cost (B : C) ratio, are presented in Table 6. The highest gross and net returns were recorded with PGPR and $CaCl_2$ seed treatments under 50 % FC. The cost of cultivation associated with PGPR and $CaCl_2$ treatments was comparatively lower than other treatments, while the returns were substantially

Table 5. Effect of seed treatments and moisture stress on cell membrane stability

Treatments	Moisture stress			
Seed treatments	**I_1-50 % FC (at all growth stages)**	**I_2-stress at MT and PI stage**	**I_3-No moisture stress**	**Mean**
S_1-designer seed	88.5	91.4	90.3	90.0
S_2-*Piriformospora indica* at 10 mL kg^{-1} of seed	85.14	79.1	81.2	81.8
S_3-PGPR mix at 50 g kg^{-1} of seed	87.47	83.9	90.7	87.4
S_4-proline 0.1 %	82.8	83.6	83.13	83.2
S_5-$CaCl_2$ 2 %	89.0	89.5	87.03	88.5
S_6-hydro priming	91.1	87.9	87.3	91.1
S_7-control	87.8	82.4	86.4	82.3
Mean	87.9	86.4	84.6	
CD (0.05)-seed treatments		**2.2**		
CD (0.05)-moisture stress		**1.5**		
Interaction CD (0.05)		**3.8**		

PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; NS: non significant; FC: field capacity; MT: maximum tillering; PI: panicle initiation.

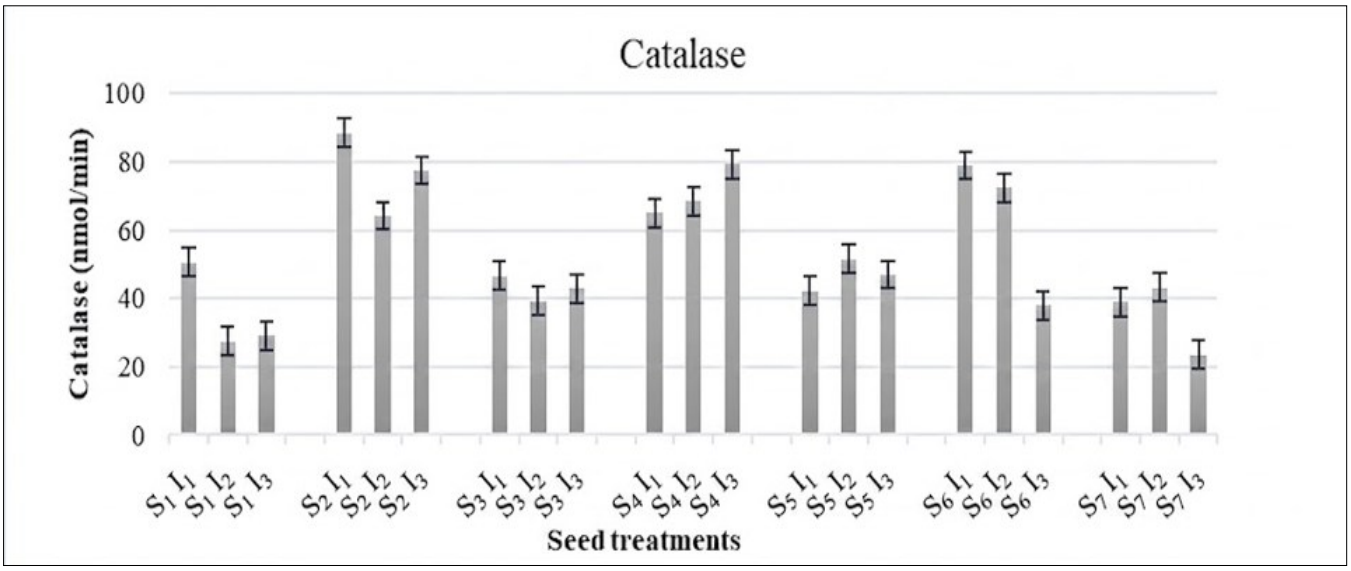


Fig. 4A. Effect of seed treatments and moisture stress on catalase ($p \leq 0.05$).

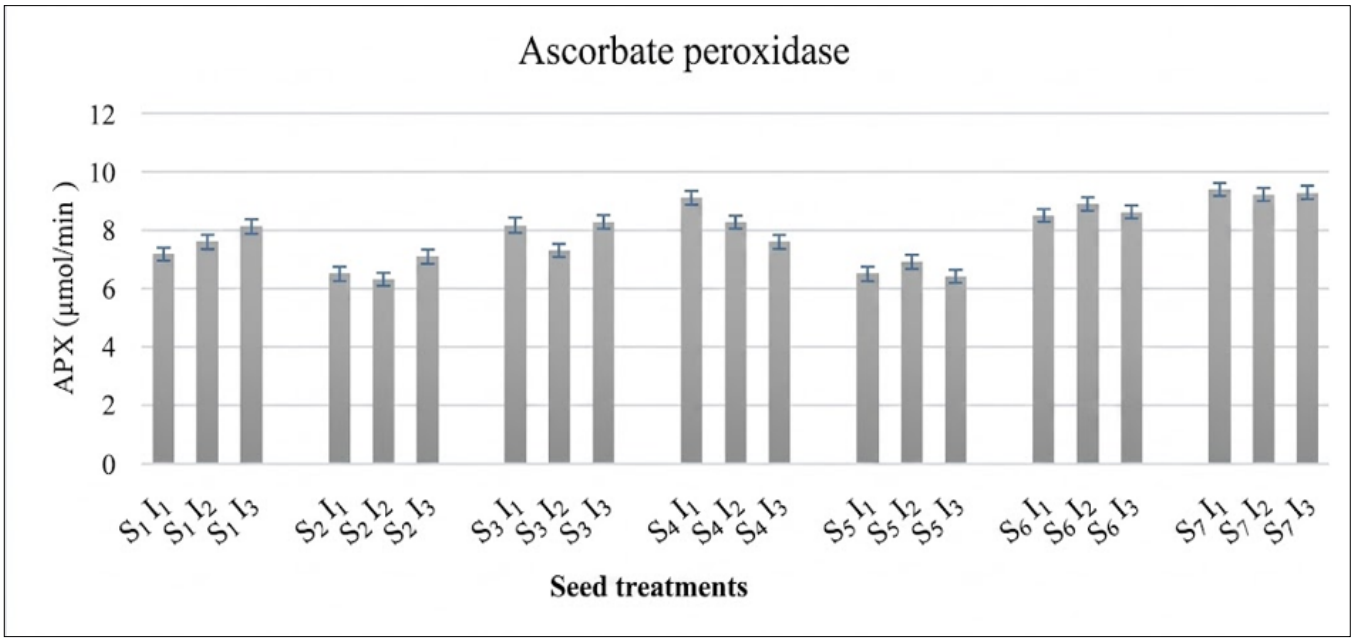


Fig. 4B. Effect of seed treatments and moisture stress on ascorbate peroxidase ($p \leq 0.05$).

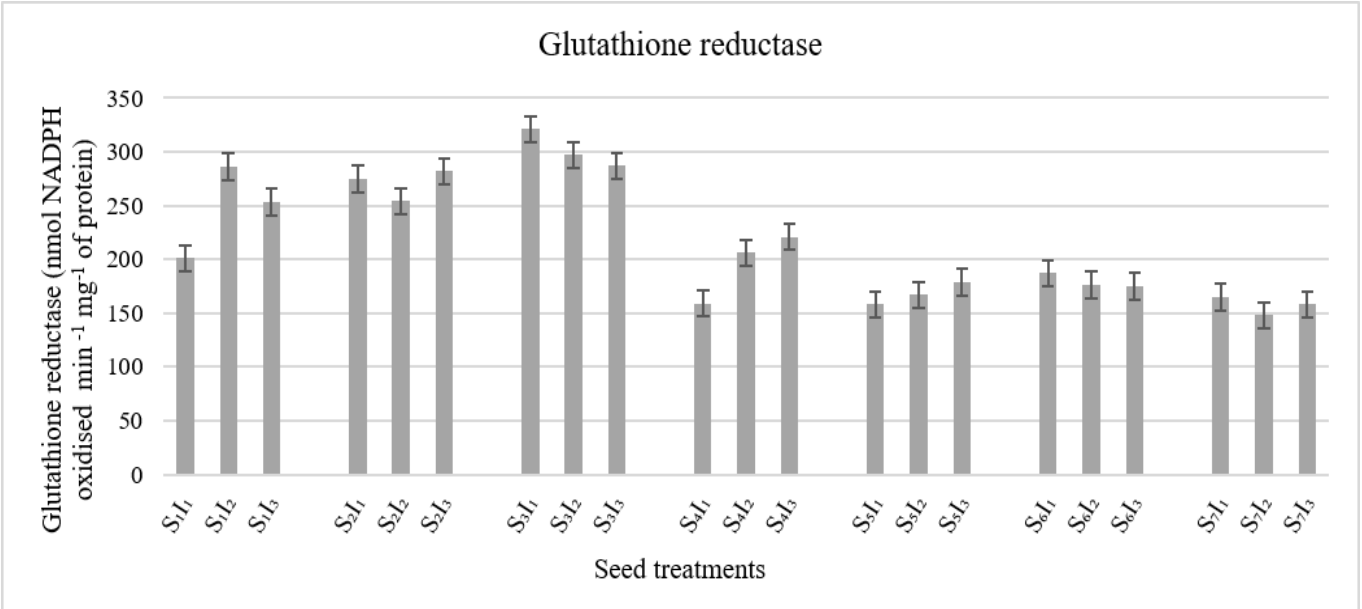


Fig. 4C. Effect of seed treatments and moisture stress on glutathione reductase ($p \leq 0.05$).

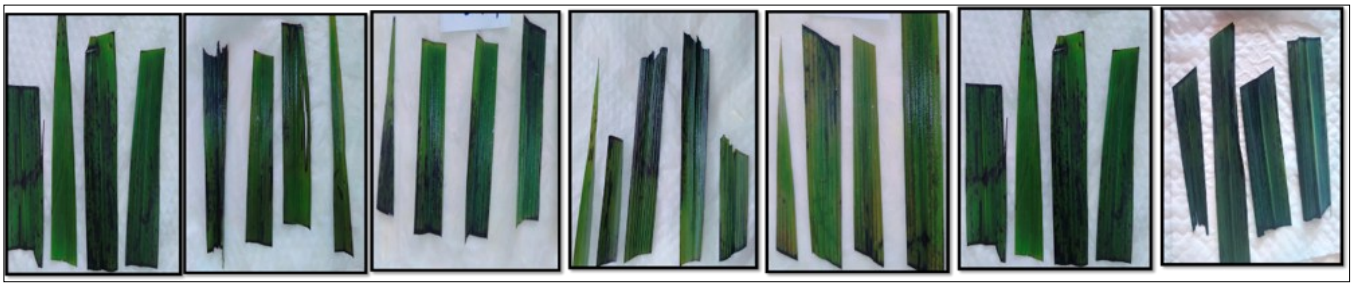


Fig. 5A–G. Nitroblue tetrazolium stained leaves. A: designer; B: *Piriformospora indica*; C: PGPR; D: proline; E: $CaCl_2$; F: hydropriming; G: control.

PGPR: plant growth promoting rhizobacteria.

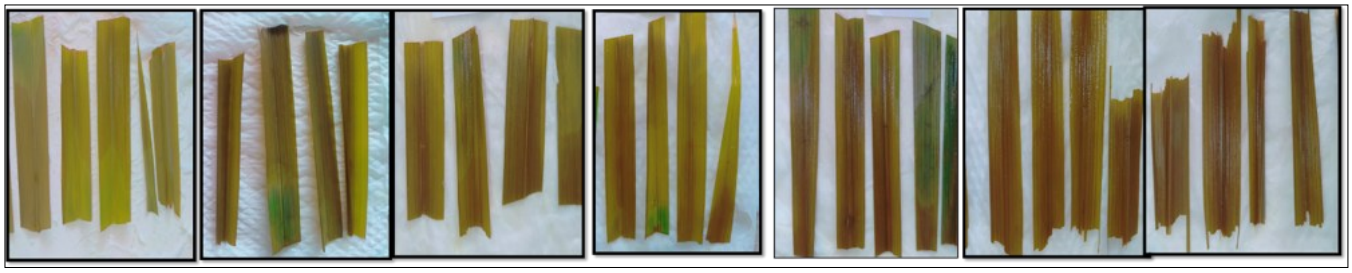


Fig. 6A–G. Nitroblue tetrazolium stained leaves. Nitroblue tetrazolium stained leaves. A: designer; B: *Piriformospora indica*; C: PGPR; D: proline; E: $CaCl_2$; F: hydropriming; G: control.

PGPR: plant growth promoting rhizobacteria.

higher. Overall, a 57 % increase in gross returns was observed with PGPR seed treatment over the control under 50 % FC moisture stress.

In moisture regime 2 stress imposed at MT and PI stages, the highest gross and net returns were obtained with *P. indica* followed by $CaCl_2$ treatment. The increased profitability observed under stress conditions with these seed treatments highlights their effectiveness in mitigating drought impacts. Therefore, adopting such management practices appears more beneficial than cultivating rice without any intervention under similar conditions.

Under non-stress conditions, the highest gross and net returns were achieved with PGPR treatment, followed closely by $CaCl_2$ and designer seed treatments. However, the cost of cultivation for designer seeds was relatively higher due to the inclusion of multiple chemical components, making PGPR and $CaCl_2$ more economically viable options.

Conclusion

Results of the study indicated that seed treatments can be used for managing drought in rice crops. Seed treatment with plant growth promoting rhizobacteria (PGPR) mix-1 was found effective in managing drought under 50 % field capacity (FC) and under moisture stress given at maximum tillering (MT) and panicle initiation (PI) stage. It recorded the highest grain yield, biomass production, net return and gross return. Even under stressed situations the yield obtained from PGPR seed treatment was comparable with irrigated condition. Plant growth promoting rhizobacteria mix-1 treatment not only enhances stress resistance but also imparts disease resistance and enhanced nutrient uptake. Highest grain yield under irrigated conditions was obtained from designer seed treatment. Future research should focus on understanding the long-term physiological and molecular mechanisms involved in drought tolerance induced by different seed treatments. Further studies are needed to evaluate their effects under varying levels of moisture stress and across different rice varieties and agro-climatic conditions. Investigations on the combined application of seed treatments, soil amendments and other breeding techniques, along with their influence on nutrient uptake, root architecture, antioxidant activity and yield stability under field conditions, would help in developing more effective drought management strategies for sustainable rice production.

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Table 6. Effect of seed treatments and moisture stress on economics

Treatments	Gross returns (₹ ha^{-1})			Net returns (₹ ha^{-1})			B : C ratio		
Seed treatments	**I_1**	**I_2**	**I_3**	**I_1**	**I_2**	**I_3**	**I_1**	**I_2**	**I_3**
S1-designer seed	29740.4	32147.6	45461.9	-9391.7	-6584.5	5929.8	0.76	0.83	1.15
S2-*Piriformospora indica* at 10 mL kg^{-1} of seed	30285.7	46329.2	35361.5	-9046.4	7397.1	-4370.5	0.77	1.19	0.89
S3-PGPR mix 1 at 50 g kg^{-1} of seed	46150.7	28786.6	47410.3	7368.6	-9595.5	8228.2	1.19	0.75	1.21
S4-proline 0.1 %	43657.1	30477.9	34377.7	4677.5	-8101.7	-5136.9	1.12	0.79	0.87
S5-$CaCl_2$ 2 %	43763.3	42928.0	45938.4	5034.7	4599.4	6674.8	1.13	1.12	1.17
S6-hydro priming	34813.9	29860.0	38432.7	-3868.2	-8422.1	-784.3	0.9	0.78	0.98
S7-control	30558.8	31774.1	30197.1	-8123.2	-6508.0	-9019.9	0.79	0.83	0.77

PGPR: plant growth promoting rhizobacteria; $CaCl_2$: calcium chloride; B : C ratio: benefit : cost ratio.

Authors' contributions

AS[1] and AS[2] conducted the field experiments, collected the field data, performed all statistical analyses, drafted the manuscript and revised the manuscript. AS[2] also participated in the study design, inspected the study field area, provided advice regarding field conditions, contributed to drafting the manuscript, reviewed and corrected the manuscript. PP approved the study, conducted field visits, provided advice on field conditions and participated in the study design and coordination. All authors read and approved the final manuscript [AS[1] stands for Aswani S and AS[2] for Anitha S].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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

During the preparation of this work the authors used GRAPES KAU in order to do statistical analysis. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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