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RESEARCH ARTICLE

Probiotic assisted drought tolerance in green gram: A novel strategy for sustainable agriculture

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Abstract

Drought stress is a critical factor that limits crop growth and yield. Biopriming has emerged as an effective and eco-friendly method to mitigate drought stress and enhance plant growth. This study evaluated the impact of nodule-associated plant probiotics (NAPP) on green gram seeds through treatments such as biopriming, coating and their combination of biopriming + coating along with hydropriming and uninoculated seeds served as a control in both *in vivo* and pot experiments. The study demonstrated that a combination of 6 mL biopriming + 4 mL coated seeds significantly improved the speed of germination (33%), germination percentage (16%), total seedling length (31%), dry weight (29%), seed vigor index (50%) compared uninoculated seeds. In the pot experiment, seeds treated with the combination of biopriming + coating were planted in different drought conditions *viz.,* severe drought (40% water holding capacity, WHC), moderate drought (70% WHC) and controlled conditions (100% WHC). Combining biopriming + coated seeds showed a higher photosynthetic rate and relative water content, significantly improving plant growth under drought stress and optimal conditions. The biochemical study found that combining biopriming + coated seeds considerably increased proline content, total soluble protein and antioxidant enzymes under drought stress and control conditions. Furthermore, the combination significantly increased yield components, including the number of seeds, pods, 100-seed weight and root nodules, under both control and drought stress conditions. Principal component analysis (PCA) confirmed the modulation of growth, root nodules, antioxidant enzymes and yield components by combining biopriming and coating. It also showed reduced electrical leakage (EL) in green gram under drought stress conditions.

Keywords

antioxidant enzymes; biopriming; drought tolerance; green gram; nodule associated plant probiotics; root nodules; stomatal morphology

Introduction

Biotic and abiotic stress factors significantly impact the agriculture and forestry sectors, leading to economic losses by reducing yield and quality. There is sufficient evidence to suggest that the agricultural industry is adversely affected by abiotic stresses (1, 2). Up to 51-82% of crop yield is lost annually due to abiotic stresses such as extreme temperature, drought, toxicity and lack of plant nutrients worldwide (3). Plants exposed to abiotic stresses change morphology, cellular structure, physiological function, biochemical reactions and molecular responses (2). Moreover, oxidative damage caused by reactive oxygen species (ROS) during drought stress impacts the metabolic functions of crop plants. Drought-induced oxidative damage includes reduced synthesis of photosynthetic pigments, increased accumulation of osmoprotectants like proline, alterations in cell membrane stability and physiological changes such as reduced plant height and smaller leaf area, ultimately reducing decreasing crop yield by 1-30% (4).

Green gram (*Vigna radiata* L.) is a short-duration legume crop grown widely under semiarid and subtropical conditions. Green gram seeds possess antibacterial, antifungal and anticancerous properties, enabling them to neutralize harmful toxins. They are a key ingredient in protein supplements and nutritional formulations (5). India is the major producer of mung bean and it is cultivated in almost all states. The area under cultivation of mung bean in India during 2022-23 was 15.57 lakh ha, with a production of 3.74 million tonnes (6). As a legume, green gram also enhances soil fertility by fixing atmospheric nitrogen through nitrogen-fixing bacteria in root nodules.

Water holding capacity is a critical parameter in evaluating soil moisture availability for plant growth, defined as the maximum amount of water that soil can retain against gravity. It plays a key role in determining the impact of drought stress on crops, as variations in WHC directly influence water accessibility to plants. Water is necessary throughout the phenological growth stages of the crop. However, drought stress during the blooming and seed-filling stages significantly reduces grain productivity (7). Terminal drought stress, characterized by reduced photosynthesis, accelerated leaf senescence and constrained source-sink relationships during the reproductive or grain development stage, significantly reduces grain sink capacity (8).

Several studies have reported that seed priming with bioinoculants can mitigate oxidative damage caused by drought stress and improve crop yield. The Candida glabrata VYP1, *Candida tropicalis* VYW1, *Paenibacillus taichungensis* and *Rhizobium* possess multiple plant growth-promoting traits. These include the production of indole-3-acetic acid (IAA), siderophores, ammonia and polyamines. Additionally, they produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces the growth-inhibiting hormone ethylene. This activity enhances the plant's antioxidant defense system, supporting plant growth (9, 10). Earlier studies reported that non-rhizobial endophytes (NRE) are capable of producing biologically active metabolites and phytohormones such as indole-3 acetic acids (IAA), gibberellins (GA), cytokinins and ACC deaminase (11).

Furthermore, nodule endophytes provide significant benefits to legume crops by obtaining nutrients through nitrogen fixation, phosphate, zinc (Zn) and potassium (K) solubilization (12). Various studies have emphasized the beneficial effects of using a single inoculum for seed priming to enhance pulse crop productivity. However, to the author's knowledge, limited research has investigated the combined effects of rhizobia, fungi, bacteria and yeast on bio-priming and coating, particularly under drought-stress conditions. Therefore, this study evaluated the impact of biopriming and coating with NAPP on the seed and seedling quality of green gram (*Vigna radiata*). This study also assessed the effects of NAPP seed priming on the drought stress tolerance of green gram plants.

Materials and Methods

Microbial consortia and seed material

The Nodule Associated Plant Probiotic (NAPP) microbial consortia comprising *Rhizobium* sp VRE1, *Candida tropicalis* VYW1, *Paenibacillus. staichungensis* TNEB6 and arbuscular mycorrhizal fungi (AMF) were obtained from the Biocatalysts Laboratory, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India. The selection was based on the compatibility and mutualistic relationship between the four microorganisms (9, 10). The consortia were maintained using a standard method (13), which involved culturing each microorganism on its respective nutrient medium: yeast extract mannitol agar for *Rhizobium*, Sabouraud dextrose agar for *Candida tropicalis*, nutrient agar for *Paenibacillus* and a root organ culture system for AMF and desired population was ensured before seed priming and coating (10). Uniform-sized green gram seeds were collected from the National Pulses Research Centre (NPRC), Vamban, Tamil Nadu, India.

Microbial seed treatments

The seeds were surface sterilized with 2% sodium oxychloride (NaOCl) for 5 min and then treated with 80% ethanol for 2 min, followed by rinsing thrice with distilled water. Healthy seeds were subjected to priming and coating with a liquid-based NAPP formulation containing 10^9 CFU/mL. The following seed treatments were tested: uninoculated seeds (T_1), hydro priming (T_2), NAPP- 6 mL priming (T_3), NAPP- 4 mL coating (T_4) and NAPP- 6 mL priming + NAPP- 4 mL coating (T_5). Priming was done for 3 hr with a 1:0.35 (w/v) seed-to-solution ratio and the seeds were shaded for 24 hr as recommended (14). Seeds were coated by gradually adding NAPP inoculum mixture, followed by air drying at 25 °C for 24 hr. The presence of microbes on the primed and coated seeds was confirmed by using scanning electron microscopy (SEM) at the level of 5000X (Model: Quanta 250, Detector: Everhart Thornley) (Fig. 1).

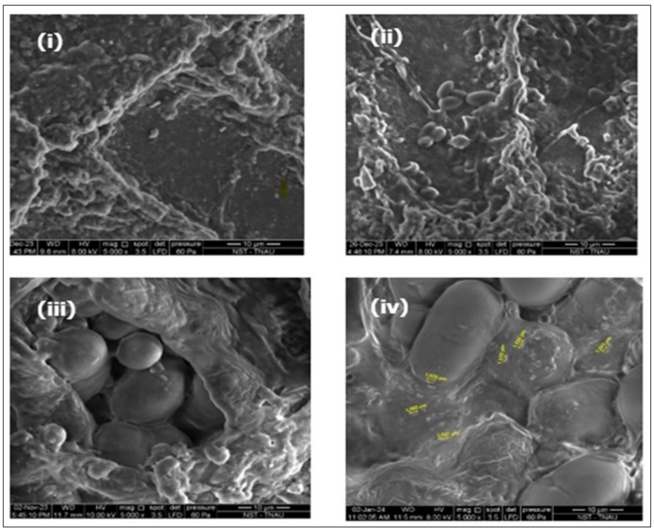

Fig. 1. Scanning electron microscopy (SEM) analysis of nodule-associated plant probiotics in green gram seeds. (i) Control (Seed coat) (ii) Treated (Seed coat) (iii) Control (Cotyledon) (iv.) Treated (Cotyledon).

Physiological parameters of green gram seeds

Treated seeds were estimated for seed quality parameters such as speed of germination, germination percentage, root length (cm), shoot length (cm) and dry matter production (g 10 seedlings^{-1}), following the established protocol (15). The seed vigor index was also determined using the standardized method (16).

Germination percentage (GP) = No. of germinated seedlings/ No. of seeds own x 10
(Eqn.1)

Speed germination = [D1/1] + [D2/2] + [D3/3] + [D4/4] + [D5/5] + [D6/6] + [D7/7] + [D8/8] (Eqn2)

Here, D1, D2, … D8: Number of emerged seedlings and 1, 2, … 8: Number of days after sowing

Seed Vigour Index (SVI) = Average root length + Average hypocotyl length x GP (Eqn3)

Design of the experiment and treatments

The pot experiment was conducted in a greenhouse at the Department of Seed Technology (DSST), TNAU, Coimbatore, India P (latitude 11.0 N and longitude 76.4 E). In January 2024, the experiment involved seeds primed and coated with a liquid-based NAPP formulation before sowing. The following seed treatments were tested: uninoculated seeds (T_1), hydro priming (T_2), NAPP- 6 mL priming (T_3), NAPP- 4 mL coating (T_4) and NAPP- 6 mL priming + NAPP- 4 mL coating (T_5). The pot mixture of red soil, sand and vermicompost in a 3:1:1 ratio was prepared and filled into 10 kg capacity pots. Ten seeds per treatment were sown per pot and 5 were maintained per pot after seedling emergence. Drought stress was applied to planted seedlings at 3 moisture stress levels in a Factorial Randomized Block Design (FRBD) with 5 replications. The moisture stress treatments were as follows: control, 100% WHC, moderate drought- 70% WHC and severe drought- 40% WHC. The plants were grown for 65 days, with moisture stress conditions imposed at the initial and harvest stages. Plant height and biochemical parameters were taken after 30 days of sowing and yield quality parameters were estimated after harvesting. Biochemical analyses were conducted in the laboratories of the DSST and the Department of Crop Physiology, TNAU, Coimbatore, using advanced instrumentation.

Assessment of morphological indices

Morphological parameters were recorded at the flowering and harvesting stage, such as plant height (cm), the number of nodules/plant and yield components, including the number of pods/plants, grains/pod and 100 seed weight.

Analysis of biochemical parameters

Leaf samples were collected after 30 days after sowing (DAS) to determine biochemical parameters such as relative water content (RWC) (17), total soluble protein (18), proline (19) and EL (20).

Photosynthetic pigments and stomatal activity determination

Photosynthetic pigments were measured by homogenizing 0.1 g of fresh leaf sample in 2 mL of 80% acetone, with chlorophyll a, chlorophyll b, total chlorophyll and carotenoids quantified from the 30 DAS leaf samples following the standard procedure (21). The Chl a, Chl b and carotenoid absorbances were measured on a Systronics UV–VIS spectrophotometer at 663, 645 and 470 nm respectively. The following equation calculated the total chlorophyll content:

Total chlorophyll content = (8.02 × OD at 663) - (20.2 × OD at 645) × V/1000 × W (Eqn.4)

Where, OD = optical density (nm), V = final volume (mL) and W = fresh weight of sample (g).

Stomatal morphology was observed using light microscopy (OLYMPUS) with a microscope camera at 10X magnification. A small layer of an adhesive quick fix was applied to the abaxial surface of the leaves and then allowed to dry for 5 min. After that, a thin layer of the leaf surface was placed on a glass slide to observe the activity of the stomata.

Estimation of antioxidant enzymes

30 DAS were used for each evaluation, enzyme measurements were taken 4 times from fully expanded and mature leaves and the average was used for analysis. For estimating antioxidant enzymes, 1 g of fresh leaf sample was homogenized in 10 mL of extraction buffer containing 0.1 M phosphate buffer (pH 7.5), 0.5 mM EDTA, 1 g of polyvinyl pyrrolidone and 100µL of mercaptoethanol. Then, the extract was subsequently centrifuged for 20-25 min at 20000 rpm at 4 °C and the resulting supernatant was used to measure the levels of antioxidant enzymes such as superoxide dismutase (SOD) (22), catalase (CAT) (23) and peroxidase (POD) (24).

Statistical design

All the data were statistically analysed using SPSS v. 21.0 software (Chicago, USA). One-way ANOVA was performed to assess the significance of differences among treatments, followed by Duncan's multiple comparison test for mean separation at $P < 0.05$. The physiological and biochemical data were further analysed using R software's principal component analysis (PCA) to identify patterns and relationships among variables. Bar charts were created and visualized using GraphPad Prism 8 software.

Results and Discussion

This study examined the beneficial effects of seed pretreatment with NAPP on the morphological, physiological and biochemical parameters of green gram seedlings. The NAPP significantly (P≤0.05) improved the physiological quality of green gram seeds compared to uninoculated seeds. Among the various seed treatments, Combination priming and coated seeds (T_5) consistently had superior effects in terms of speed of germination, total seedling growth and seed vigor (Table 1). Combination priming and coated seeds (T_5) exhibited higher speed of germination, germination percentage, root and shoot length, vigor index and dry matter production by 33.33%, 15.58%, 38.66%, 21.64%, 50.98%, 28.87% respectively, over the uninoculated seeds (T_1) (Table 1). Nodule-associated plant probiotics enhance seed germination, vigor index and growth attributes in *Vigna mungo* (9, 10). Priming with *Rhizobium* improved germination and increased seedling length because this strain produces phytohormones such as GA, auxin and cytokinin (10). Auxin, particularly indole-3-acetic acid (IAA), is a crucial phytohormone that promotes cell division, elongation and differentiation, likely contributing to enhanced seed germination and root development observed in the current study (25).

NAPP-treated seeds increased plant growth under control conditions (100% WHC) and improved growth and yield under drought-stress conditions. For instance, under control conditions, NAPP-treated plants (T5) showed a 31.2% increase in plant height and a 46.7% increase in the number of pods per plant. Under severe drought conditions (40% WHC), NAPP-treated plants exhibited a 54.9% improvement in plant height and a 60% increase in pods per plant. These results highlight the efficacy of NAPP in enhancing plant resilience and productivity across varying moisture levels. In the pot experiment, green gram seeds were sown under varying drought stress conditions, such as moderate drought and severe drought conditions, that had significantly decreased morphological traits (e.g., plant height, nodule number), biochemical attributes (e.g., chlorophyll content, total soluble protein) compared to control conditions. Drought stress decreased plant height (18% in severe drought (SD) compared to the control condition. All the seed treatments significantly increased the plant height under control conditions. When plants were under severe drought stress, seeds treated with a combination of NAPP priming + coated seeds (T_5) increased the plant height up to 54.9% compared to uninoculated seeds (Fig. 2A). Days to first flowering were shorter for uninoculated seeds under drought stress than in the control conditions (Fig. 2B); however, this difference was not statistically significant. Early flowering depends on cell expansion and cell growth, which can be affected by drought stress due to excess production of ROS (26). These ROS can impair various physiological processes, leading to reduced growth during the vegetative stage and subsequently affecting flowering and pod formation. However, NAPP enhances plant growth through auxin production and phosphate solubilization by rhizobacteria, which are necessary for plant growth. A similar increase in plant height due to microbial inoculants has been

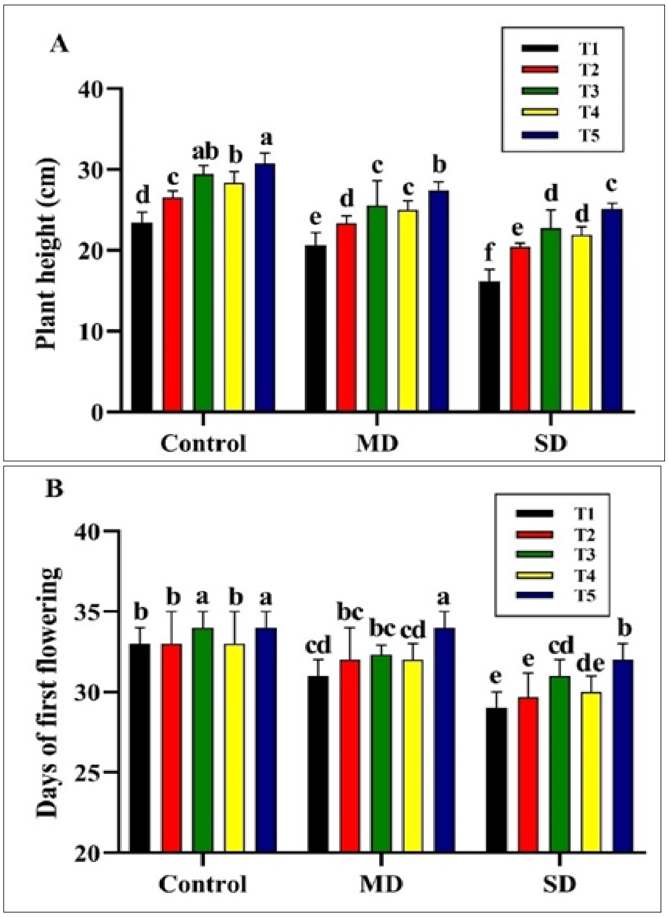

Fig. 2. Effect of nodule-associated plant probiotics on morphological attributes of green gram plants under drought stress (Control, MD- moderate drought, SD- severe drought). Bar graphs were created on data values of the mean ± SE (standard error) of 3 replications, n = 4. Bars with different lowercase letters indicate significant differences at p < 0.05.

Table 1. Effect of nodule-associated plant probiotics on physiological properties of green gram

Treatment	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Seed vigour index	Dry matter production (g 10 seedling^{-1})
T_1	8.4 ± 0.8^c	77 ± 4^d	15.0 ± 0.83^d	13.4 ± 1.00^c	2187 ± 27^e	0.232 ± 0.08^c
T_2	9.8 ± 0.4^b	81 ± 5^c	17.3 ± 1.00^c	13.9 ± 1.10^c	2527 ± 24^d	0.250 ± 0.06^b
T_3	10.9 ± 0.7^a	86 ± 2^{ab}	19.5 ± 1.03^b	15.2 ± 0.69^b	2984 ± 30^b	0.289 ± 0.02^a
T_4	9.5 ± 0.5^b	84 ± 3^{bc}	18.0 ± 0.85^c	14.1 ± 0.78^c	2696 ± 27^c	0.260 ± 0.05^b
T_5	11.2 ± 0.8^a	89 ± 2^a	20.8 ± 0.70^a	16.3 ± 0.91^a	3302 ± 25^a	0.299 ± 0.07^a

Data are means ± standard error of the mean from triplicates and least significant difference P<0.05- Mean with the same uppercase letter are not significant difference. (T1- uninoculated seeds, T2- hydropriming, T3- 6 mL priming, T4- 4 mL coating, T5- 6 mL priming + 4 mL coating) reported in soybean and green gram (27, 28).

Relative water content (RWC) in leaves decreased significantly under drought stress compared to control conditions. Seed treatments with NAPP helped maintain RWC under stress conditions, likely through improved water uptake and retention mechanisms. The combination of biopriming + coated seeds (T5) resulted in (4.6% control), (6.2% moderate drought), (7.4% severe drought) higher RWC than uninoculated seeds (Fig. 3A). Plant growth-promoting bacteria positively influenced the water -holding capacity in plants by regulating stomatal closure mechanisms. These bacteria likely modulate the production of phytohormones, such as abscisic acid (ABA), which play a crucial role in stomatal regulation. Under drought stress, increased ABA synthesis prompts stomatal closure, reducing water loss through transpiration. In this study, plants treated with bacterial inoculants exhibited reduced stomatal conductance, associated with higher relative water content and improved drought tolerance compared to uninoculated plants (29). Plant growth-promoting bacteria (PGPB) can increase plant relative water content by stimulating root growth and producing various plant growth-promoting metabolites, including IAA, phosphate solubilizers, ACC deaminase and siderophores. These metabolites enhance root expansion, enabling plants to absorb more water and nutrients from the soil (30, 31). Consistent with our findings, PGPB has been shown to enhance relative water content and water transport in black gram under drought stress (32).

The total soluble protein content is a measure of RuBP carboxylase activity in leaves and serves as an indicator of photosynthetic efficiency (33). Bioinoculants can enhance soluble protein content by activating and regulating the genes for Rubisco and Rubisco activase, thereby improving CO_2 assimilation during the Calvin cycle (34). The present investigation showed that NAPP biopriming + coated seed recorded a higher total soluble protein content (18.6% in SD) compared to uninoculated seeds (Fig. 3B). Similar findings have been reported, showing that bioinoculants significantly increase the total soluble protein content in tomato plants, thereby enhancing their drought tolerance capacity (35). NAPP had significantly influenced EL (Fig. 3C), whereas a drastic reduction in EL was observed in biopriming + coated seeds (T_5) under stress conditions. The decrease of EL in plants may be attributed to applying stress-tolerant PGPB, which probably reduced membrane permeability under stress conditions (36).

Drought stress resulted in a noticeable reduction in total chlorophyll and carotenoid contents in green gram plants, which decreased after drought stress. Under control conditions, NAPP treatments significantly increased total chlorophyll and carotenoid contents compared to untreated plants. NAPP treatments significantly increased total chlorophyll and carotenoid contents compared to untreated plants. Under severe drought stress, plants treated with

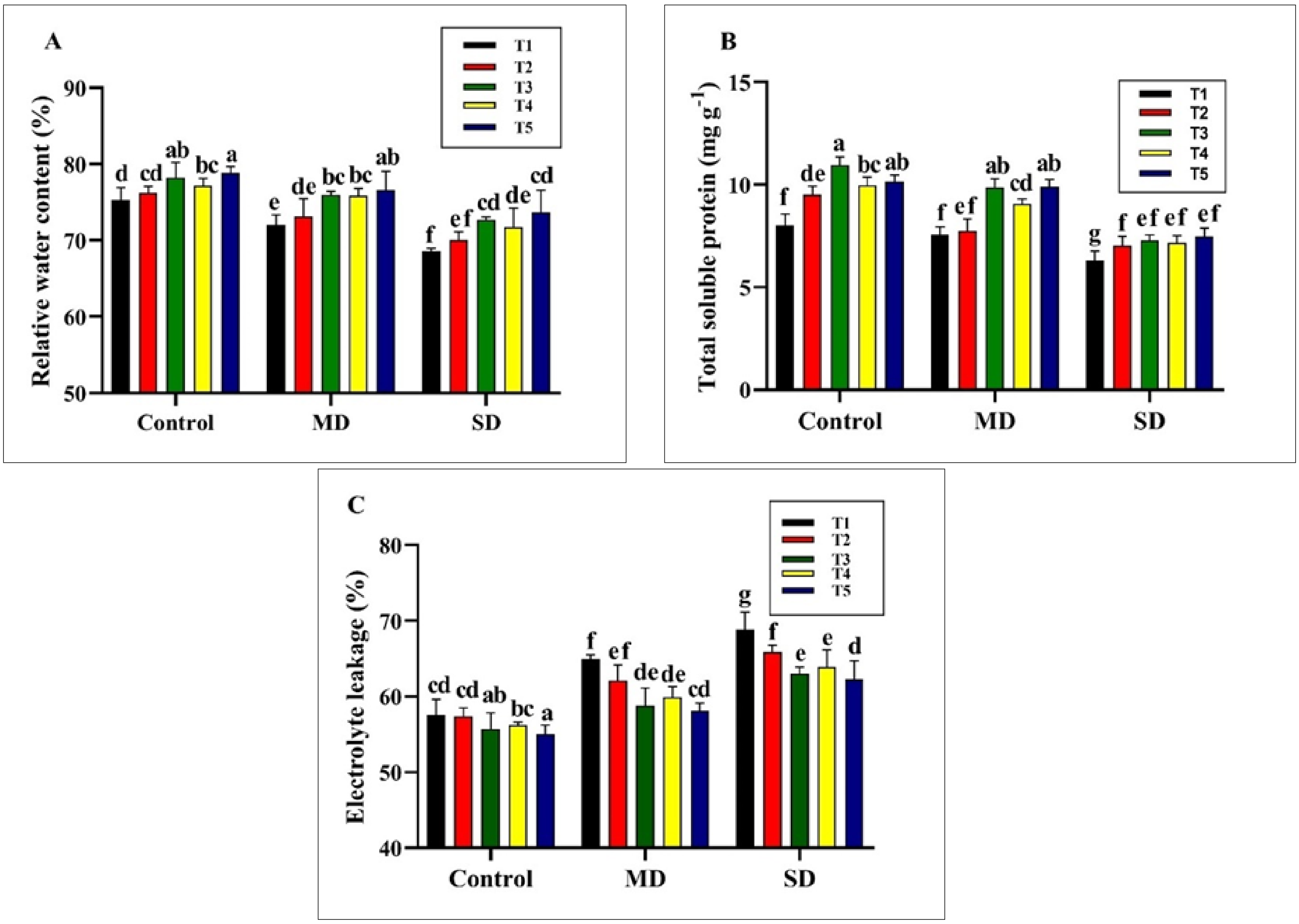

Fig. 3. Effect of nodule-associated plant probiotics on (A) relative water content, (B) total soluble protein and (C) electrolyte leakage of green gram plants under drought stress (Control, MD- moderate drought, SD- severe drought). Bar graphs were created on data values of the mean ± SE (standard error) of 3 replications, n = 4. Bars with different lowercase letters indicate significant differences at $p < 0.05$.

priming + coated seeds (T_5) showed a 46.4% increase in total chlorophyll and 113,3% increase in carotenoid levels compared to uninoculated seeds (Fig. 4A, B). When considering leaf stomatal activity, a maximum number of stomata opened under the control condition compared to stress conditions. Plants treated with priming + coating (T5) exhibited a higher number of open and semi-open stomata compared to uninoculated seeds (Fig. 5). Similar results have been reported in soybean (37) and maize (38), where biopriming treatments improved stomatal activity under stress conditions. Drought stress may decrease the synthesis of chlorophyll and carotenoids due to the breakdown of enzymes responsible for synthesizing leaf pigments. Similar findings in black gram have shown improved total chlorophyll and carotenoid contents under stress conditions following NAPP treatment (10, 39, 40). Besides, plants have an innate drought avoidance mechanism by regulating the chlorophyll content, which helps to mitigate the over-excitation of photosynthetic pigments and reduces the production of ROS (41). Drought stress may induce stomatal closure, leading to a reduced rate of transpiration, which negatively impacts both photosynthesis and plant biomass production (42).

Proline content in leaves significantly increased at all drought stress conditions (severe and moderate stress) compared to non-stressed controls. This result implies that a combination of priming + coating seeds (T_5) had higher levels of proline content under moderate drought (28.9%) and severe drought (37.5%) in comparison to uninoculated seeds (Fig. 4C). These findings suggest that the seedlings were able to alleviate drought stress by maintaining osmotic balance and minimizing oxidative stress damage. Consistent with our findings reported in chickpea (36, 40), reported higher proline contents in wheat subjected to drought stress. 1-pyrroline-5-carboxylate synthetase (P5CS) plays a crucial role in the initial proline production stage under stress conditions; therefore, the elevated proline levels may enhance stress tolerance (43).

Plants exposed to moderate drought and severe drought conditions exhibited significantly higher levels of antioxidant enzymes, including SOD, CAT and POD, compared to control conditions. Among the seed treatments, significant increases in antioxidant enzyme activities were recorded for priming + coated seeds (T5). Under severe drought stress, CAT, POD and SOD activities are 25.2%, 13.9% and 45.7% respectively, compared to uninoculated seeds (Fig. 6). Antioxidant enzymes are essential for plants to confer tolerance against oxidative stress by regulating pathways influenced by malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) (44, 45). Plants have evolved antioxidant defense systems to mitigate oxidative stress, protecting them from ROS's harmful impacts and enhancing stress tolerance.

Drought stress is commonly linked to oxidative damage caused by the excessive production of ROS. These ROS include superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals (.OH) and hydrogen peroxide (H_2O_2), which negatively affect plant growth (46). Under abiotic stress conditions, the accumulation of excessive ROS leads to lipid peroxidation and the production of MDA, which subsequently damages cell membranes, photosynthetic system and chlorophyll biosynthesis (47). Various cellular organelles like mitochondria and chloroplasts commonly produce antioxidant enzymes such as CAT, POD and SOD.

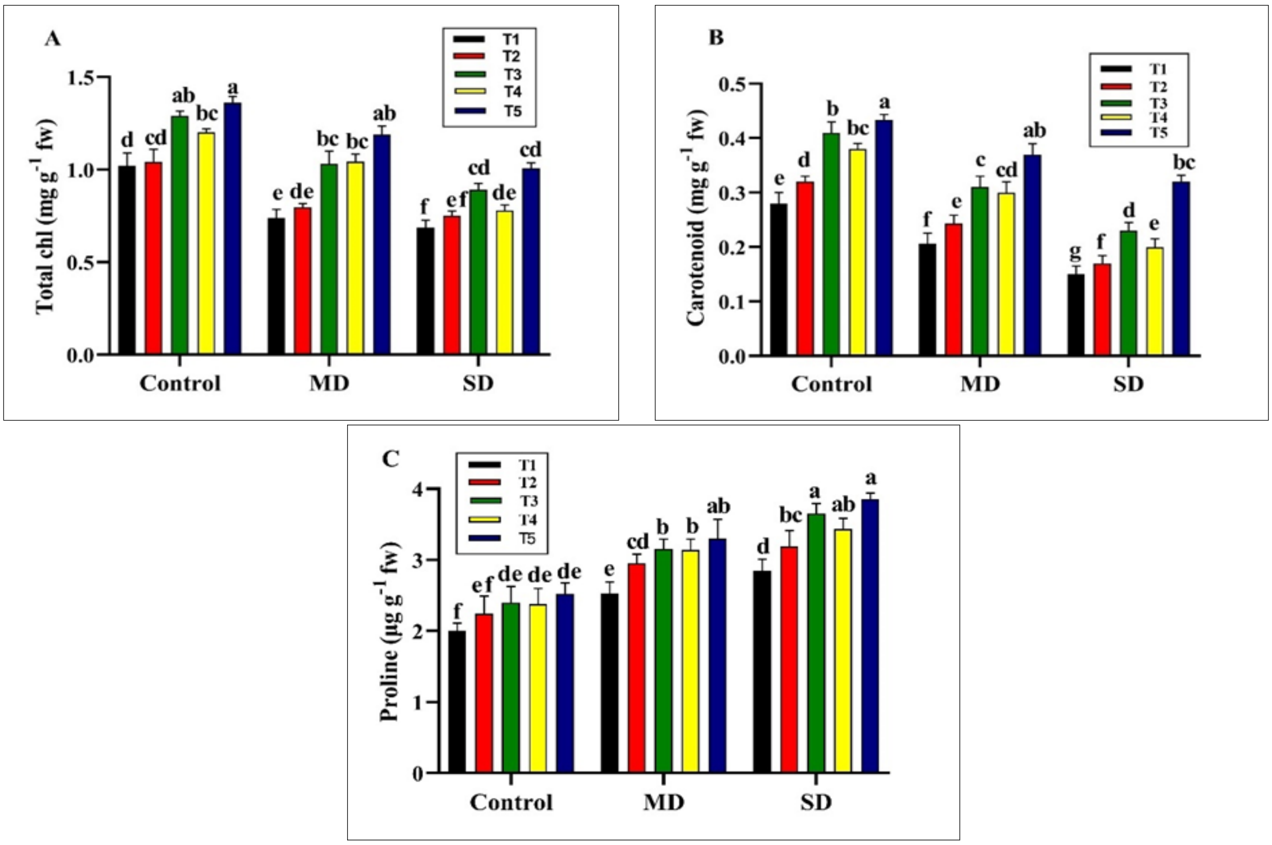

Fig. 4. Effect of nodule-associated plant probiotics on (A) total chl, (B) carotenoid and (C) proline of green gram plants under drought stress. (Control, MD- moderate drought, SD- severe drought). Bar graphs were created on data values of the mean ± SE (standard error) of 3 replications, n = 4. Bars with different lowercase letters indicate significant differences at $p < 0.05$.

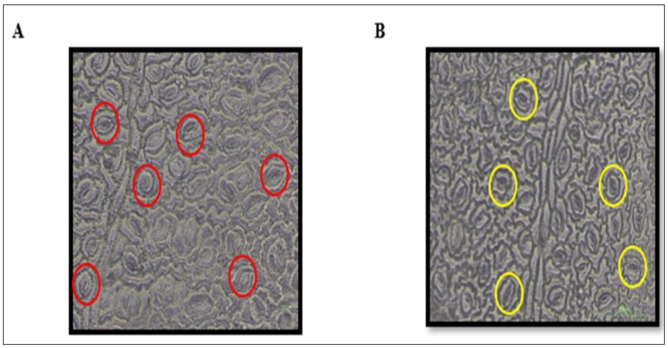

Fig. 5. Influence of microbial consortia on stomatal morphology under severe drought conditions (A- uninoculated plants, B- NAPP priming + coated seed plants).

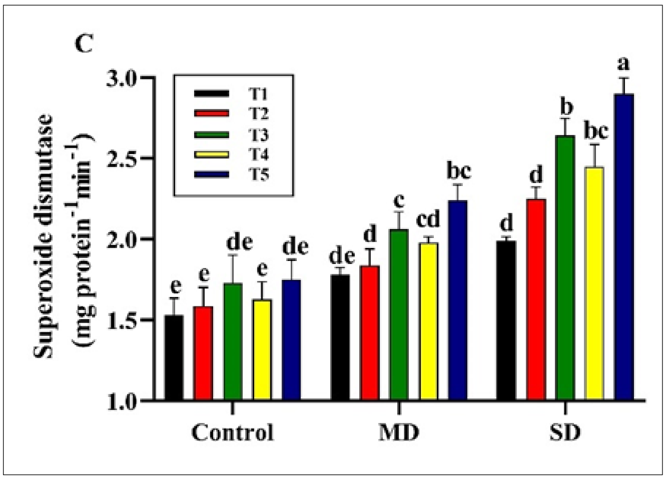

Fig. 6. Effect of nodule-associated plant probiotics on (A) catalase, (B) peroxidase and (C) superoxide dismutase activity in green gram plants under drought stress. (Control, MD- moderate drought, SD- severe drought). Bar graphs were created on data values of the mean ± SE (standard error) of 3 replications, n = 4. Bars with different lowercase letters indicate significant differences at p < 0.05.

These enzymes play indispensable roles in the antioxidant defense of biological systems (48). Hence, it is evidenced that NAPP seed treatment reduces cellular oxidative damage by inducing the enzyme amino cyclopropane carboxylate. This enzyme enhances the plant antioxidant defense system by reducing the synthesis of ethylene and ROS under stress conditions (49).

Drought stress significantly influences yield-related traits such as pods/plant, number of seeds/plant and 100 seed weight (P< 0.05). Compared to uninoculated seeds, priming + coated seeds (T5) significantly increased the number of pods/plant (18 and 16), number of seeds/pods (9 and 8), 100 seed weight (3.408 g and 3.270 g) under moderate and severe drought stress conditions respectively. All the treatments increased yield traits under control conditions compared with stress conditions (Fig. 7). Under control conditions, plants from priming + coated seeds showed more nodules than uninoculated seeds. When plants were exposed to MD and SD stress, NAPP priming + coated plants showed maximum root nodules as 15 in VBN4 and 14 in CO8, compared to uninoculated seeds (Fig. 7D). Water shortage during the flowering stage leads to increased pollen abortion, resulting in a reduction in yield components such as the number of pods and seeds per plant (50). However, the reduction in 100-seed weight suggests that the plants under water deficit conditions allocated more dry matter toward maintaining survival and vegetative growth rather than seed development. This higher partitioning of dry matter reflects a strategic resource allocation by the plant to cope with stress, prioritizing root and shoot growth over reproductive structures. Drought stress significantly

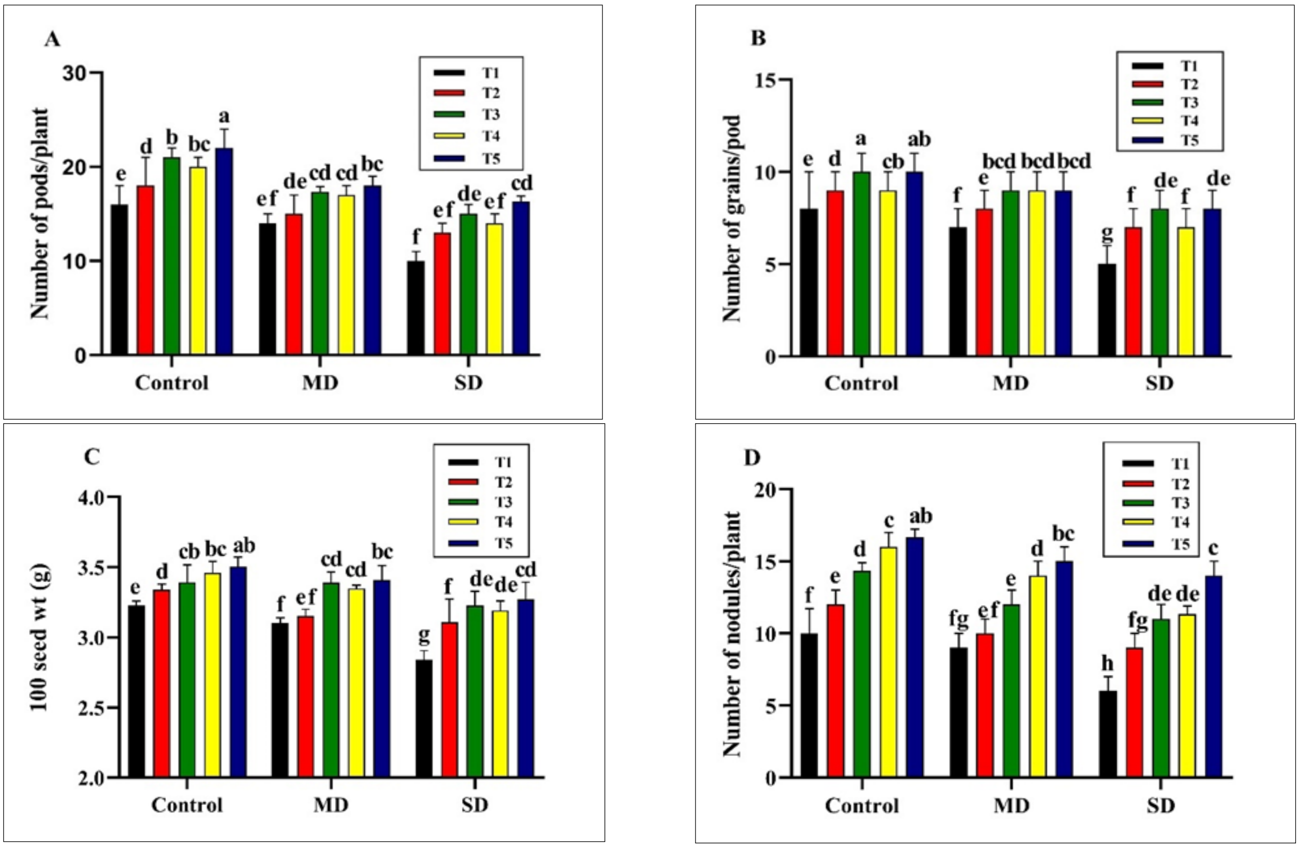

Fig. 7. Effect of nodule-associated plant probiotics on yield components and number of nodules in green gram plants under drought stress (Control, MD- moderate drought, SD- severe drought). Bar graphs were created on data values of the mean ± SE (standard error) of 3 replications, n = 4. Bars with different lowercase letters indicate significant differences at p < 0.05.

reduced the number and weight of nodules, leghemoglobin content and nitrate reductase activity, decreasing nitrogen content. Nodule-associated plant probiotics increased nitrogen fixation capabilities and effectively mitigated the adverse impacts of drought stress. However, NAPP increased phosphate solubilization, promoted plant growth, stimulated nodulation and suppressed disease, contributing to increased yield in the green gram (40, 51). In a parallel study, AMF inoculation with *Sesbania sesban* increased the number and size of nodules, leghemoglobin content and nitrogenase activity, leading to improved nitrogen uptake and assimilation (52).

Principal component analysis (PCA) was conducted to evaluate the relationships between various treatments and variables (Fig. 8). The first principal component (PC1) accounted for 93.2% of the total variability. In comparison, the second principal component (PC2) contributed an additional 4.6%, explaining most of the observed variation. Notably, plant height (PH), days to first flowering (DFF), relative water content (RWC), total soluble protein (TSP), total chlorophyll (Total chl), carotenoids (CAR), proline (PRO), CAT, POD, SOD, number of nodules (NN), pods per plant (PP), germination percentage (GP) and seed weight (SW) were significantly higher in primed and coated seeds (T5) compared to uninoculated seeds (T1). Conversely, electrolyte leakage (EL) levels were significantly lower in T5 than in T1, indicating reduced cellular damage and enhanced membrane stability in the primed and coated seeds. The PCA results validated our findings, showing that plants grown from priming + coated seeds exhibited a stronger positive correlation with plant growth traits under drought stress than uninoculated seeds. The improved plant development under abiotic stress conditions can be attributed to endophytic fungi releasing growth-promoting substances such as GAs and IAA, which support plant resilience and growth (51). The growth and drought tolerance observed in NAPP priming + NAPP coated may result from increased ACCD Deaminase (1-Aminocyclopropane-1-Carboxylate Deaminase) activity and enhanced IAA production, contributing to drought resilience as shown in black gram (9, 10). Other studies supported our study, which reported that NAPP *Rhizobium* sp VRE1 + *C. tropicalis* VYW1+ *P. staichungensis* TNEB6 + AMF induces drought stress tolerance (4, 27, 28).

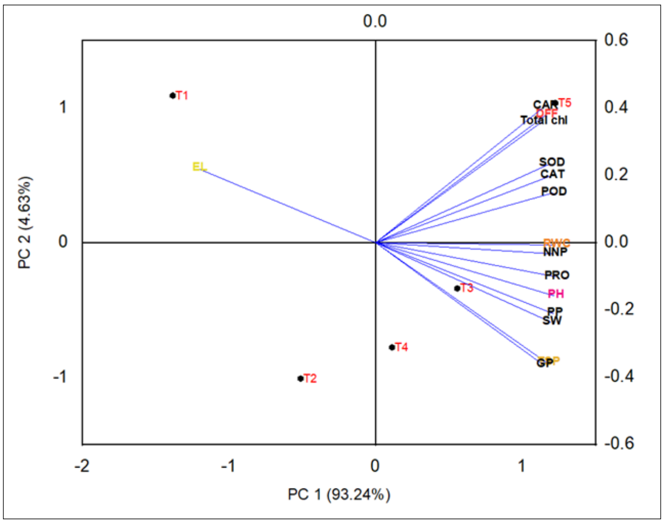

Fig. 8. Principal component analysis (PCA) for identifying variation trends in treatment responses.

Conclusion

Drought stresses significantly impact the morphological traits (e.g., plant height, nodule number), biochemical attributes (e.g., chlorophyll content, antioxidant enzymes) and yield traits (e.g., pod and seed number) of green gram. Our study demonstrated that green gram plants grown from NAPP-treated seeds showed increased growth, total chlorophyll and relative water content under drought stress. NAPP treatment also effectively regulated osmoprotectants, enhancing plant tolerance to drought. Furthermore, NAPP increased enzymatic antioxidants such as CAT, POD and SOD and reduced EL in drought stress conditions. Additionally, priming + coating with NAPP improved the number of nodules, pods and seed yield compared to uninoculated seeds. Therefore, treating green gram seeds with NAPP before sowing could be an effective bioagent to enhance productivity and suppress diseases by promoting plant health and resilience. These findings suggest that microbial consortia-based seed treatments represent an effective, economical and eco-friendly strategy to mitigate drought stress, with potential applications for improving productivity in other drought-affected crops.

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Authors' contributions

VM carried out the experiment and wrote the original draft. KS supervision, validation, reviewed and edited the manuscript. VV performed data validation. AT analysis and interpretation of results. US supplied the necessary resources. TM and CV reviewed and edited the manuscript. SM was in charge of visualization. AA analysis and interpretation of results. SK carried out the experiment. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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