PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 1406-1414
https://doi.org/10.14719/pst.4603

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Enhancing plant resilience and drought stress in green gram through seed priming with nodule-associated plant probiotics

Vinoth Kumar Muniyappan[1], K Sundaralingam[1], U Sivakumar[2], V Vijaya Geetha[3*], R Jerlin[1], N Shobana[2], V Babu Rajendra Prasad[4] & T Murugeshwari[1]

[1] Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641003, India

[2] Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

[3] Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Tindivanam 604 002, India

[4] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

*Email: geetha_seed@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY

Received: 09 August 2024
Accepted: 19 September 2024

Available online
Version 1.0 : 14 November 2024
Version 2.0 : 18 November 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Muniyappan VK, Sundaralingam K, Sivakumar U, Geetha VV, Jerlin R, Shobana N, Prasad VBR, Murugeshwari T. Enhancing plant resilience and drought stress in green gram through seed priming with nodule-associated plant probiotics. Plant Science Today. 2024; 11(4): 1406-1414. https://doi.org/10.14719/pst.4603

Abstract

Drought stress is a critical environmental stress that hinders plant growth and development. Most pulses are grown in rainfed ecosystems, significantly affected during drought conditions. To address this predicament in this study, a liquid microbial consortium of nodule-associated plant probiotics was used for seed priming of Greengram to assess its efficacy in alleviating drought stress and improving seed quality attributes. Applying an optimum dose of plant probiotics at the appropriate stage enhances plant productivity, improving tolerance to stresses and reducing dependence on harmful agrochemicals. To optimize the dose, two green gram seeds viz., VBN4 and CO8, were subjected to various concentrations of nodule-associated plant probiotics (NAPP), namely, 2, 4, 6 and 8%, along with the control and hydropriming. In the present study, 2% NAPP improved the physiological and biochemical parameters of the green gram, such as germination, seed vigour index, protein content, dehydrogenase activity and α-amylase in both varieties. The primed seeds were further evaluated under moisture stress conditions by exposing them to various concentration levels of Polyethylene Glycol 6000 ranging from -2 to -6 bar. The experiment revealed that increasing the concentration of PEG 6000 above in -4 bar reduced germination and seedling vigour in both green gram varieties. However, green gram seeds primed with 2% NAPP showed greater moisture stress resistance than nonprimed seeds. The highest activity of stress-related enzymes such as catalase, peroxidase and super oxidase dismutase triggered by NAPP in green gram plants was observed to have potential for drought stress management. This study highlights the importance of NAPP as a potential seed priming agent for improving seed germination and vigour under moisture stress.

Keywords

germination; seed vigour index; dehydrogenase activity; catalase; superoxide dismutase

Introduction

In typical agriculture, chemical fertilizers and pesticides increase production and yield. However, the extravagant use of chemicals harms ecological balance, crop productivity and food safety and is considered the primary cause of land and water pollution. Over the past few years,

sustainable agriculture has gained more attention, which promotes organic approaches in the context of preserving environmental quality and ensuring soil health. A crucial aspect of sustainable agriculture is the interaction between microorganisms and plants (1). Therefore, agricultural inputs and practices based on microbes could benefit soil fertility and plant health.

In India, pulse production is uncertain due to the evolution of abiotic stress factors (e.g., high-temperature moisture and salinity). It is cultivated mainly as a rainfed crop and drought during the critical growth stage affects productivity due to heavy flower drop and the incidence of pests and diseases. In various crops, water scarcity adversely affects seed germination and seedling vigour (2). A lack of effective seed production strategies, inadequate post-harvest handling operations, farmers' awareness of suitable varieties and the nonavailability of quality seeds are the major factors for low-quality seed production. The quality of seeds is a critical factor in sustainable agriculture, which produces vigorous seedlings under adverse conditions. Pulses have low productivity because of using low-quality seeds under rainfed conditions and improper crop management (3). Seed priming is a pre-sowing treatment that improves uniform germination and overall plant growth, even under water deficit conditions. Moreover, seed priming reduces the imbibition period and enhances the regulation of enzymes such as lipase, protease, α-amylase and DNA repair during imbibition in preparation for seed germination (4).

Seed biopriming is the application of beneficial biological agents to seeds, such as phytohormones and beneficial microorganisms, such as bacteria, fungi and yeast that increase germination and protect seeds from environmental conditions. This novel technique increases the speed of germination (SP), total seedling length, germination rate (GR) and vigour index (VI) without harming the ecosystem. It helps to improve soil fertility by fixing nitrogen from the atmosphere, solubilizing insoluble nutrients, decomposing plant residues and stimulating plant growth (5).

Growing evidence suggests that using a consortium of microbes during the inoculation process could increase plant productivity compared with using a single microbe (6). However, the microbial consortium significantly promoted plant growth and improved plant resilience under adverse environmental conditions. Studies have been reported on plant growth-promoting bacterium-induced stress resilience in pea and rice (7,8). Green gram (*Vigna radiata* L.) is one of the most important legume crops in the world and is cultivated mainly in developing countries. Dry and sprouted seeds are highly used because of their high protein content (24-27%), essential amino acids, fibres, minerals and vitamins (9). Soil fertility is improved by fixing atmospheric nitrogen by forming symbiotic associations with rhizobia, essential for significant crop germination and used as green manure. Therefore, it enhances soil fertility and productivity, which promotes cropping systems. Indian agriculture depends primarily on two monsoons, specifically the Southwest Monsoon and the Northeast Monsoon. 35-45% of the cropping area is under drought conditions due to the scarcity of monsoon rains (10). Therefore, identifying the beneficial microbes that contribute to the tolerance of crop plants to drought is crucial.

Rhizobium sp. VRE1+ *Candida tropicalis* VYW1 influences plant growth-promoting traits such as indole acetic acid, ammonia, 1-aminocyclopropane-1-carboxylate, siderophore and polyamine production (11). Furthermore, the multifunctional plant growth-promoting traits of *Paenibacillus taichungensis* TNEB6+ AMF play essential roles in nodulation, plant growth and yield enhancement in Blackgram (12,13). In this context, a microbial consortium was formulated with three NAPP (*Rhizobium* sp VRE1 + *Candida tropicalis* VYW1+ *Paenibacillu staichungensis* TNEB6+ and Arbuscular Mycorrhizal Fungi (AMF). The formulated consortium was tested with two green gram varieties, VBN4 and CO8, using a biopriming approach under water stress conditions and compared with hydro-primed and untreated seeds. With various concentrations of Nodule Associated Plant Probiotics, as well as hydroprimed and control seeds, to simulate drought stress conditions. The effect of stress was evaluated based on seed germination, the growth potential of seedlings and specific enzyme activities. Significant differences were observed among primed, hydro-primed and nonprimed seeds.

Materials and Methods

Source of seeds and bioinoculants

Certified seeds green gram varieties VBN4 and CO8 were purchased from the National Pulses Research Centre, Vamban, Tamil Nadu and used for this experiment. The microbial consortium (*Rhizobium* sp. VRE1 + *C. tropicalis* VYW1 + *P. taichungensis* TNEB6 + AMF) used in this experiment was carefully crafted by the Department of Agricultural Microbiology of Tamil Nadu Agricultural University, Coimbatore, India based on the compatibility and mutualistic interactions among the four microorganisms (11-13). Seed quality assessment-related experiments were carried out by the Department of Seed Science and Technology, TNAU, Coimbatore.

Biopriming of green gram seeds with microbial consortia

The seeds were surface sterilized with 2% NaOCl for 5 min and then treated with 80% ethanol for 2 min, followed by rinsing three times with distilled water. The seeds were primed with a liquid-based NAPP formulation containing 10^9 CFU per mL at different concentrations. The treatment details were as follows: control dry seeds (T_1), hydro priming (T_2), priming with 2% NAPP (T_3), 4% NAPP (T_4), 6% NAPP (T_5) and 8% NAPP (T_6) for three h at a 1:0.35 seed-to-solution ratio and shade-dried for 24 h to maintain the original moisture content. The priming efficiency was assessed based on the number of colonies that appeared. The presence of microbes on the primed seeds was determined by scanning electron microscopy (SEM) (Fig. 3). The highest percentage of viable bacterial cells in the bioprimed seeds was selected to further assess seed quality parameters.

Effect of bio agent concentration on seedling growth and vigour

The efficacy of the microbe was determined by scanning electron microscopy using the optimal concentration of each microbial consortium. Various physiological parameters, such as percentage of germination, germination speed, total seedling length (cm), shoot length (cm), root length (cm) and dry matter (g/10 seedlings), were measured according to the protocol of (14) and seed vigour index (15).

Germination percentage (GP) = (Eqn.1)

Number of seeds germinated/Number of seeds sown x 100

Speed of germination = (Eqn.2)

[G1/1]+[G2/2]+G3/3]+[G4/4]+[G5/5]+G6/6]+[G7/7]+[G8/8]

Here, G1, G2, … G8: Number of emerged seedlings and 1, 2, … 8: Number of days after sowing

Seed Vigor Index (SVI-I)= (Eqn.3)

Average root length + Average hypocotyl length x GP

Seed Vigor Index (SVI-II)= (Eqn.4)

Total Dry Matter Production x GP

Determination of biochemical parameters

The protein content was determined using a standard method (16) with an absorbance spectrophotometer at 580 nm. A digital electrical conductivity meter (Elico type CM-82) with a cell constant of 1.0 was used to determine the electrical conductivity (EC) (17). Moreover, dehydrogenase enzyme activity was determined in treated seed samples (3 g) using tetrazolium chloride (18). The α-amylase activity was determined using a standard method (19) and the absorbance was measured at 670 nm using a spectrophotometer (UV-VIS Double Beam Spectrophotometer).

Effect of nodule-associated plant probiotics on seedling growth and vigour under drought stress conditions

The best-performing treatment (T_3) and green gram VBN4 and CO8 control seeds were studied under low osmotic potential conditions (-2, -4 and -6 bar). The osmotic solutions were prepared by using polyethene glycol 6000. The following relationship was used to create the desired osmotic potential for drought stress as follows:

$\Psi s =$ (-1.12x10-2)xC-(1.18x10-4)xC+(2.67x10-4)xCxT+(8.39x10-7)xCxT

where Ψs is the osmotic pressure (Bar), C is the concentration of polyethene glycol 6000 (g kg^{-1} H_2O) and T is the temperature (°C) (20). After that, the germination percentage was evaluated according to the ISTA seed testing protocols (14).

Determination of enzyme activity

Primed and control seeds were sown under different moisture stress viz., -2, -4 and -6 bar conditions. Each treatment took 2 grams of plant sample on the eighth day after sowing. Treatment wise collected plant tissues were subjected to assess the superoxide dismutase (21), peroxidase (22) and catalase (23) activity.

Statistical design

All the data are expressed by triplicate samples. For the statistical analysis of the data, analysis of variance was performed using SPSS (version 22, IBM Inc., Chicago, IL, USA) and the means were separated using the least significant difference (LSD) test ($P < 0.05$). The physiological and biochemical data were also subjected to principal component analysis (PCA) using R software along with Microsoft Excel.

Results

Effects of nodule associated plant probiotics on Physiological traits

The experiment results revealed a significant increase ($P < 0.05$) in seedling growth for the VBN4 and CO8 varieties of green gram seeds subjected to different treatments compared with the untreated seeds. Among the various concentrations of biopriming agents used, T_3 and T_4 consistently exhibited better performance in terms of seedling growth, vigour and other physiological parameters. Seed germination was greatest in the T_3 treatment (85%), followed by the T_4 treatment (84%) compared with the untreated control (77%). T_3 (2%) significantly increased the total length of the seedlings (34.2 cm), the mean length of the roots (19.2 cm) and the length of the shoots (15.0 cm) compared with untreated seeds. A greater vigour index (2907) was observed in the T_3 treatment, followed by the T_4 treatment (2772), than in the untreated control (2204). The dry matter production was significantly greater in the T_3 treatment (0.283 g/10 seedlings), followed by the T_4 treatment (0.271 g/10 seedlings), than in the untreated control (0.230 g/10 seedlings) (Table 1). Similarly, for CO8 green gram seeds, treatment with different concentrations (T_3 to T_6) of the microbial consortia significantly improved seedling growth characteristics, viz., germination, root length and shoot length, compared with those of untreated seeds. Total seedling growth significantly improved ($P < 0.05$) with microbial treatment (Table 2). However, increasing the NAPP concentration affected seed germination and other seedling parameters.

Effect of nodule-associated plant probiotics on Biochemical Changes

The protein content of VBN4 green gram seeds significantly differed among the treatments. The bioprimed seeds of T_3 recorded the highest percentage of protein content (20.90%), followed by those of T_4 (20.75%) and the untreated control (Fig. 1A). The electrical conductivity of T_3 was 0.214 dSm^{-1}, which was lower than untreated control (0.289 dSm^{-1}) (Fig. 1B). T_3 showed an increase in the DA level (0.724 OD value/620 nm), followed by T_4 (0.697 OD value/620 nm) compared to the untreated control. Dehydrogenase activity slightly increased with increasing NAPP concentration and ranged between 0.597 and 0.724

Table 1. Effect of nodule-associated plant probiotics on the growth attributes of green gram VBN4 seeds

Treatments	SG	G (%)	RL (cm)	SL (cm)	TSL (cm)	SVI - I	SVI- II	DMP	AB (%)	DS (%)
T_1	8.1±2.0d	77±4c	15.4±0.25c	13.2±1.7d	28.6±3.0e	2204±33d	17.7±1.5e	0.230±0.09d	10±2b	13±3c
T_2	9.9±0.7b	80±3b	17.2±0.29b	13.8±1.4cd	31.0±2.5d	2478±31bc	20.2±1.3d	0.253±0.07c	8±2ab	12±1c
T_3	10.7±1.5a	85±2a	19.2±0.01a	15.0±1.2a	34.2±2.1a	2907±27a	24.1±1.8a	0.283±0.04a	6±1a	9±2a
T_4	10.5±0.9a	84±3a	18.5±0.39a	14.5± 1.5b	33.0±2.4b	2772±40a	23.1±1.9b	0.271±0.04b	6±1a	10±2ab
T_5	10.1±1.3c	81±2b	18.3±0.14a	13.9±1.0bc	32.2±2.0c	2608±38b	21.4±2.0c	0.264±0.08bc	8±2ab	10±1ab
T_6	9.8±1.6c	79±5b	17.0±0.35b	13.5±1.6cd	30.5±2.7d	2410±46c	20.5±3.5d	0.260±0.07bc	9±3b	13±2c

(SG-speed of germination, G- germination, RL- root length, SL- shoot length, TSL- total seedling length, SVI- seed vigour index, DMP- dry matter production (g 10 seedling^{-1}), AB- abnormal, DS- dead seeds). The data are presented as the means ± standard errors of the means from triplicate samples, with P<0.05 indicating that the means with separate letters were significantly different. (T_1- Control, T_2- Hydropriming, T_3- 2% NAPP, T_4- 4% NAPP, T_5- 6% NAPP, T_6- 8% NAPP).

Table 2. Effect of nodule-associated plant probiotics on the growth attributes of green gram CO8 seeds

Treatments	SG	G (%)	RL (cm)	SL (cm)	TSL (cm)	SVI - I	SVI- II	DMP	AB (%)	DS (%)
T_1	9.2±1.6e	93±4c	17.3±2.3e	15.5±2.5d	32.8±4.1c	3050±21f	23.0±1.9c	0.247±0.06c	4±1c	3±1
T_2	11.0±1.9cd	95±2bc	19.1±1.6d	16.6±1.8c	35.7±3.6b	3396±16d	25.7±2.4b	0.270±0.05b	3±1b	2±1
T_3	12.1±1.2a	98±2a	21.3±1.3a*	17.7±3.0a	39.1±3.2a	3832±18a	28.3±2.8a	0.289±0.08a	2±1a	0
T_4	11.7±1.3ab	96±4ab	20.6±2.1b	17.3±1.9ab	38.1±2.7a	3655±24b	26.6±3.5b	0.277±0.09ab	4±2c	0
T_5	11.4±1.1bc	96±3bc	20.1±2.0c	16.9±2.0bc	37.1±3.7ab	3557±19c	26.3±1.9b	0.274±0.07ab	4±1c	0
T_6	10.5±1.8d	95±2c	18.8±1.4d	16.5±1.1c	35.6±4.2b	3373±28e	25.6±2.9b	0.269±0.09b	4±2c	2±0

(SG-speed of germination, G- germination, RL- root length, SL- shoot length, TSL- total seedling length, SVI- seed vigour index, DMP- dry matter production (g 10 seedling^{-1}), AB- abnormal, DS- dead seeds). The data are presented as the means ± standard errors of the means from triplicate samples, with P<0.05 indicating that the means with separate letters are significantly different. (T_1- Control, T_2- Hydropriming, T_3- 2% NAPP, T_4- 4% NAPP, T_5- 6% NAPP, T_6- 8% NAPP).

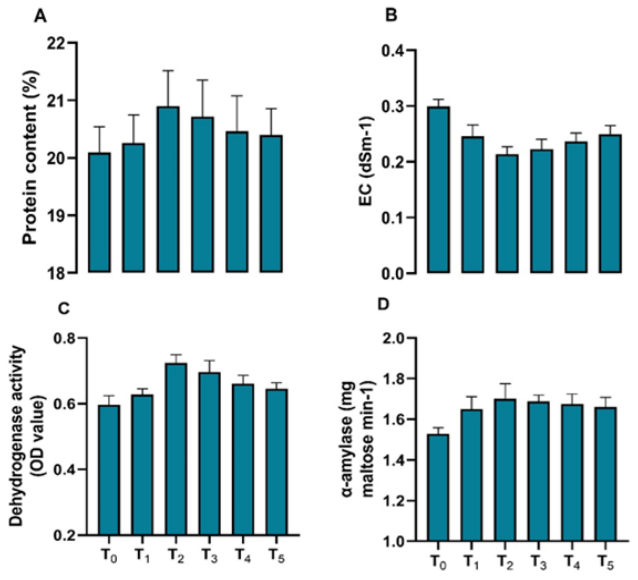

Fig. 1. Protein content (A), electrical conductivity (B), dehydrogenase activity (C) and α-amylase activity (D) of green gram VBN4 bioprimed with various concentrations of microbial consortia.(T_1- Control, T_2- Hydropriming,

OD/620 nm (Fig. 1C). However, biopriming of CO8 green gram seeds did not significantly affect the protein content between treatments (Fig. 2A). EC of the CO8 variety was lower in the bioprimed seeds in T_3(0.176 dSm^{-1}) followed by T_4 (0.181 dSm^{-1}) than in the control (0.192 dSm^{-1}) (Fig. 2B). For dehydrogenase activity, T_3 resulted in increased level of dehydrogenase activity (0.813 OD value/620 nm) compared with that of T_1 (0.767 OD value/620 nm) (Fig. 2C). The results revealed that the level of EC found in the bioprimed seeds decreased, whereas the dehydrogenase enzyme increased. The α-amylase activity was measured in pregerminated seeds, T_3 (1.691 mg maltose min^{-1}) was followed by T_4 (1.688 mg maltose min^{-1}) concerning the control (1.529 mg maltose min^{-1}) (Fig. 1D). Similar results were observed for CO8 α-amylase activity, T_3 had the maximum level of α-amylase activity (1.719 mg maltose min^{-1}) followed by T_4 (1.714 mg maltose min^{-1}) compared with untreated control seeds (1.701 mg maltose min^{-1}) (Fig. 2D). The results revealed that the amount of α-amylase activity in the bioprimed seeds increased, whereas the germination percentage increased.

Scanning electron microscopy (SEM)

Scanning electron microscopy revealed the presence of microbes in the seed coat of the bioprimed seeds. The results also confirmed the movement of bacteria from the seed to the radicle and the whole root system (Fig. 3).

Principal component analysis

Principal component analysis was used to determine the impact of nodule-associated plant probiotics treatment on the physiological and biochemical changes of the seeds. Principal component analysis of the VBN4 and CO8

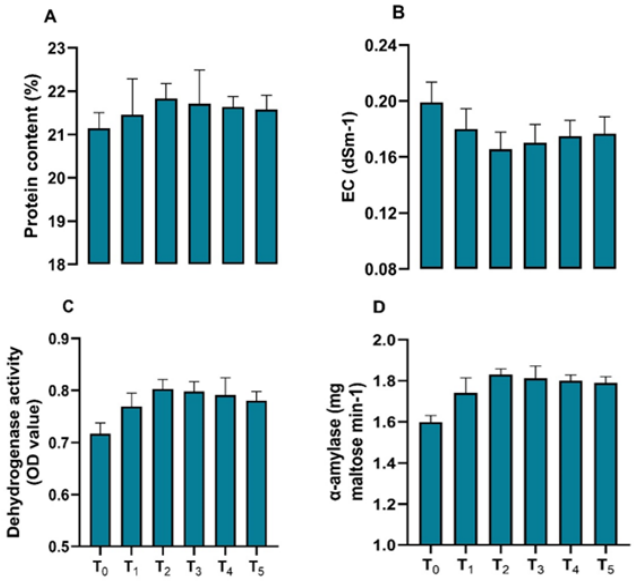

Fig. 2. Protein content (A), electrical conductivity (B), dehydrogenase activity (C) and α-amylase activity (D) of CO8 green gram seeds bioprimed with various concentrations of microbial consortia. (T_1- Control, T_2- Hydro-

varieties (Fig. 4A, B) revealed two principal components and the different NAPP concentration indices were represented by PC1 and PC2, which accounted for 98.8% of the total variation (Fig. 4A). For VBN4 variety, principal component 1 (PC1, X-axis) accounted for 94.9% of the variation and principal component 2 (PC2, Y-axis) accounted for 3.9% of the variation and the variety of CO8 accounted for 98.9% of the total variation (Fig. 4B). For CO8 variety, (PC1, X-axis) accounted for 94.9% of the variation and PC2 (Y-axis) accounted for 3.88% of the variation. PCA of the physiological and biochemical parameters of the green gram VBN4 and CO8 varieties revealed that germination percentage, vigour index, protein content, dehydrogenase activity and α-amylase were more significant in T_3 than in T1, whereas the changes in electrical conductivity were reversed in T_3; i.e., when the protein content, α-amylase and dehydrogenase activity decreased, whereas electrical conductivity has increased. Hence, the results showed that among the NAPP treatments, T3 significantly increased the biochemical properties of the seeds.

Effect of Nodule associated plant probiotics on seed germination under moisture stress conditions

Greengram seedling growth was significantly affected by moisture stress at various concentrations of PEG 6000 (-2, -4 and -6 bar). The results of this study revealed that the response of the seeds varied according to the level of moisture stress. With increasing stress levels (PEG 6000), the germination speed, germination rate and total seedling length of both green gram varieties gradually decreased. At a moisture stress level of -6 bar, VBN4 and CO8-treated seeds exhibited a more significant percentage of germination compared with control plants under conditions of -6 moisture stress. Compared with VBN4, the CO8 variety had a more substantial percentage of germination with higher concentrations of PEG stress (Fig. 5a). The results showed that compared with the control seeds, the primed seeds could withstand germination and seedling growth up to -6 bar moisture stress.

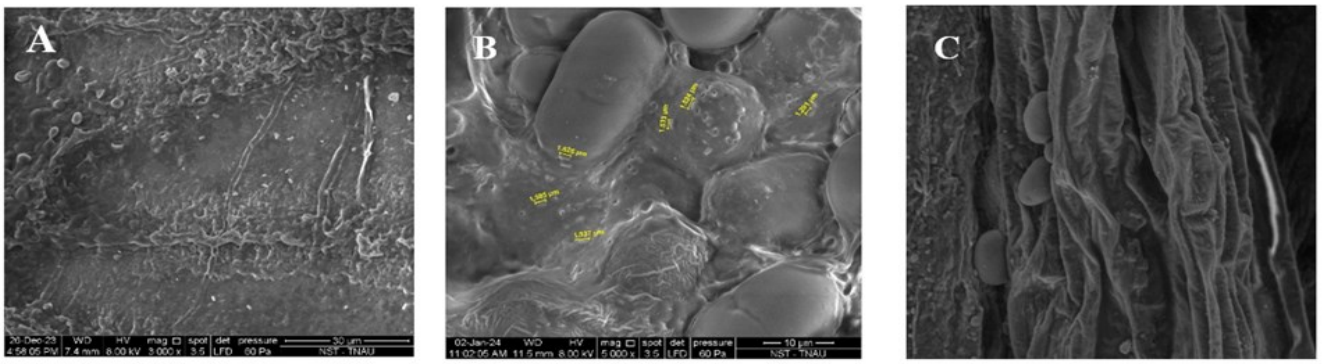

Fig. 3. Scanning electron microscopy (SEM) analysis of plant probiotics associated with nodules. (A) seed coat, (B) cotyledon and (C) radicle

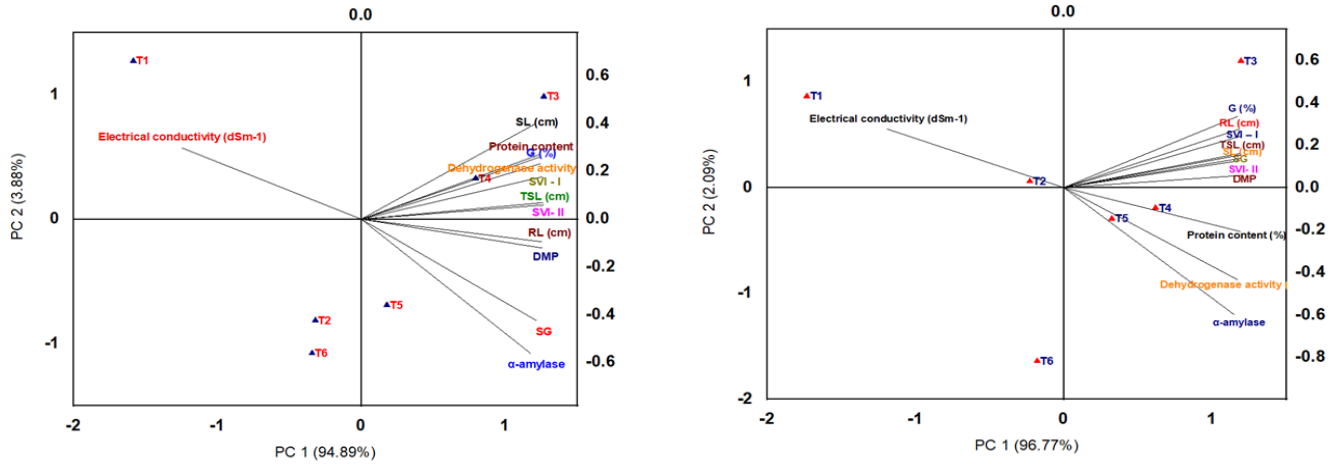

Fig. 4. Principal component analysis showing the impact of treatments on variables of the VBN4 (A) and CO8 (B) varieties. (T_1- Control, T_2- Hydropriming, T_3- 2% NAPP, T_4- 4% NAPP, T_5- 6% NAPP, T_6- 8% NAPP).

Effect of Nodule associated plant probiotics on antioxidant enzymes under conditions of moisture stress

Catalase and peroxidase activity was observed in the green gram plants treated with probiotics at different moisture stress levels. Bioprimed seeds showed greater catalase and peroxidase activity (Fig. 5b, c) in response to moisture stress. The enzyme activity of the NAPP-treated seeds increased to -4 bar of moisture stress. The enzyme activity then decreased with increasing stress levels. The highest catalase and peroxidase activities were observed in VBN4 bioprimed seeds (2.59 µM H_2O_2 reduced min^{-1} mg of $protein^{-1}$ and 0.78 OD value min^{-10}) compared with the control (0.99 reduced min^{-1} mg of $protein^{-1}$ and 0.35 OD value min^{-10}) under moisture stress conditions of -4 bar. Similarly, the green gram variety of CO8 recorded the highest catalase and peroxidase activity in the bioprimed seeds (2.80 µM H_2O_2 reduced min^{-1} mg of $protein^{-1}$ and 1.09 OD value min^{-10}, respectively) compared with the control seeds under the condition of moisture stress of -4 bar.

Moisture stress increased the superoxide dismutase activity of both varieties (Fig. 5d). SOD activity was more significant in bioprimed VBN4 and CO8 seeds (2.29 mg of $protein^{-1}$ and 2.45 mg of $protein^{-1}$, respectively) than in untreated control seeds under -4 bar moisture stress. Among the varieties, CO8 NAPP-treated plants exhibited more catalase, peroxidase and superoxide dismutase activities than VBN4 treated plants under moisture stress conditions.

Discussion

Enhancing plant tolerance to stress conditions is a crucial task for sustainable agriculture. Despite being well established and proven, the benefits of plant growth bacteria include improving yield, quality, and resistance to crop stress. Globally, 50-80% of crop yield is lost annually because of extreme temperature, drought, deficiency and toxicity to plant nutrients (24). Among various abiotic stresses, drought stress occurs when the soil's available moisture content decreases to the point where transpiration by the plants exceeds water absorption. Drought stress results in reduced germination and retarded growth, thereby leading to poor overall crop establishment. Beneficial microbes are the most feasible, reliable and long-term option for managing abiotic and biotic stress and their effects on plant growth, yield and productivity (25).

Using seeds formulated with potentially beneficial microorganisms is an attractive ecological technique for improving the seed germination percentage and plant growth under unfavourable environmental conditions (26, 27). The use of a bioinoculant as a priming agent increases the accumulation of rhizosphere stimuli, aids in the cycling of biochemical nutrients, fosters enzyme activity and aids in improved translocation and crop performance in response to changing climatic conditions. Furthermore, the biopriming of seeds with microbial consortia facilitates seed germination and plant growth, depending on the

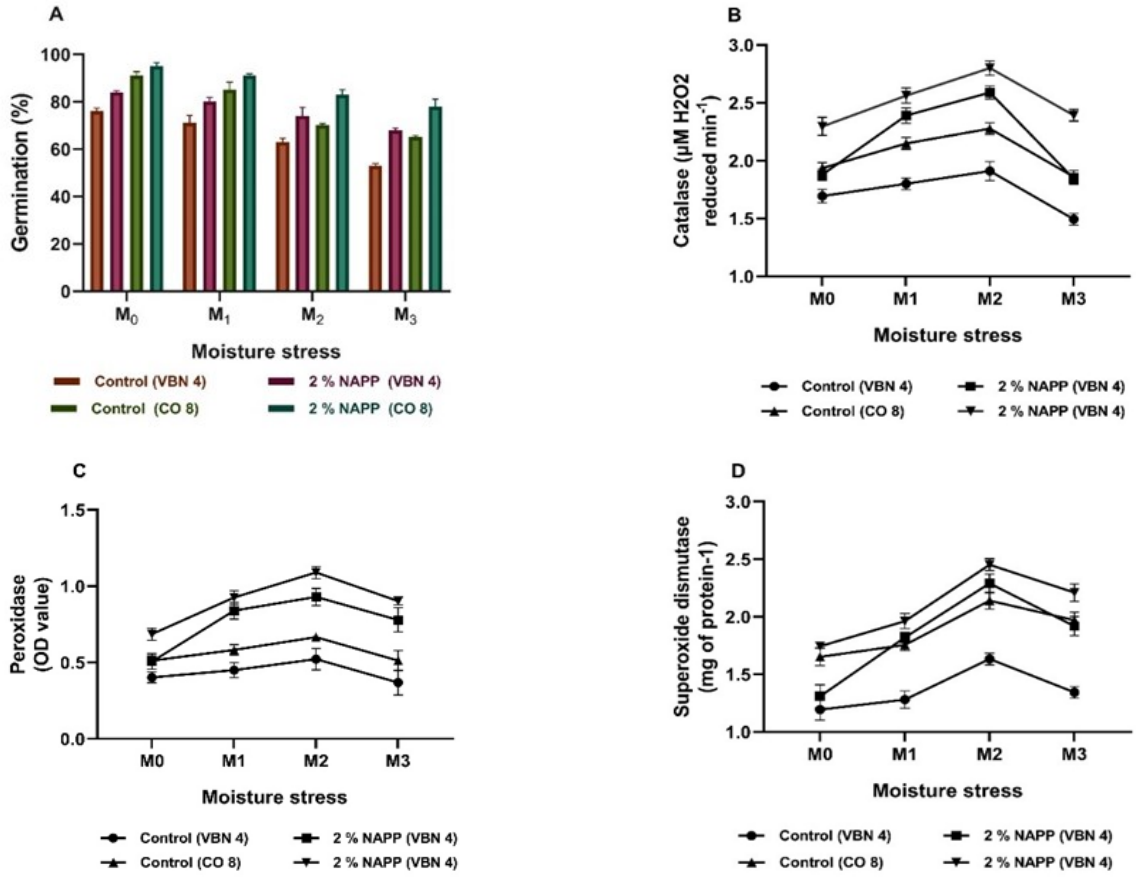

Fig. 5. Effect of plant probiotics on germination (%), catalase activity (µM H_2O_2 reduced min^{-1} mg of $protein^{-1}$), peroxidase activity (OD value) and superoxide dismutase activity (mg of $protein^{-1}$) in the green gram varieties of VBN4 and CO8. The data are presented as the means ± standard errors of the means from triplicate experiments. (M_0- without moisture stress, M_1= -2 bar, M_2= -4 bar, M_3= -6 bar).

concentration of microbial cells used for priming. Selecting an optimal concentration is crucial for preventing seeds from experiencing any stress and ensuring the overall impact of biopriming (28). Hence, a standardization experiment was carried out to determine the optimum concentration of microbial consortia required for biopriming.

Interestingly, the present studies revealed that 2% and 4% NAPP increased the seed quality parameters compared with the control. These gibberellins trigger the amylase enzyme, which increases starch assimilation in seeds and promotes early germination (29-31).

Bioprimed seeds exhibit a more significant percentage of germination because the production of microbial seed leachates provides a source of carbon and nitrogen during the initial days. Subsequently, the translocation of quantum and nature of photosynthates in the form of root exudates determines the proliferation of microbial inoculants (32). This could be the possible reason for the improved germination of the bioprimed seeds (NAPP) compared with the germination of the control seeds. Numerous researchers have reported that biopriming with microbial consortia improves seed germination in various crops, including black gram (33, 34).

In the present study, the bioprimed seeds' physiological parameters, such as germination speed, germination percentage, shoot length, seed vigour index and dry matter production, increased due to biopriming. However, bioinoculants at lower concentrations have a more significant effect than those at higher concentrations (34). This may be because bioinoculants at lower concentrations induce a mild stress response in plants, which may enhance their growth and defence mechanisms. Higher concentrations might overwhelm the plant, leading to adverse effects (35).

The interaction between microbes and plants produces phytohormones and hydrolyzing enzymes, facilitates the mobilization of nutrients from the endosperm to the embryo and contributes to increased dry matter production. Many microorganisms can produce phytohormones, which regulate plant growth and development. Auxin is one of the most critical phytohormones for plant growth and development. IAA is vital for plant cell division, extension, and differentiation, and it enhances xylem formation, seed germination and root growth. Additionally, it promotes adventitious and lateral roots and the formation of pigments, enhances photosynthesis and mediates responses to fluorescence, light and gravity (36).

The increased production of hydrolytic enzymes such as protease and amylase by nodule-associated plant probiotics increases the germination percentage. The essential enzyme known as amylase is crucial for hydrolyzing food reserves and aiding in the utilization of starch-based reserves. The early germination of the embryo is caused by this enzyme hydrolyzing the starch reserves and providing sugars. According to (34), the nutrients or metabolites produced by seed and microorganism consortia could increase the amount of nutrients available for their cell multiplication and expansion, improving seedling growth. The protein content was slightly more significant in the bioprimed seeds than in the control seeds.

A reliable indicator of seed vigour and viability is believed to be the electrical conductivity of the seed leachate. The electrical conductivity of primed seeds was lower than that of the control. The production of energy and synthesis of proteins during germination depends on the dehydrogenase enzyme. The viability of the seeds was evaluated based on colour changes in living tissue.

The activity level of the dehydrogenase enzyme system is closely related to the respiration and viability of the seeds when exposed to a 2,3,5-triphenyl tetrazolium chloride solution. The dehydrogenase enzyme activity is an outstanding and reliable metabolic marker for assessing the viability of seeds (37). However, biopriming with NAPP slightly changed the amount of dehydrogenase in the primed seeds. Our findings are consistent with previous studies on maize seeds (38).

The present investigation revealed that moisture stress significantly affected seed quality traits such as germination, total seedling length and vigour index of untreated seeds at moisture levels ranging from -2 to -6 bar. However, the bioprimed seeds resisted induced moisture stress up to -4 bar. These results align with those of (39), who reported that a green gram affected plant growth under moisture stress. Growth-promoting bacteria aid in plant root system development and increase plant nutrient uptake, water-holding capacity and soil structure (40).

Abiotic stress causes cells to accumulate reactive oxygen species. ROS such as hydrogen peroxide, superoxide and hydroxyl radicals are produced in mitochondria and chloroplasts due to disturbances in the electron transport cycle. These ROS are highly reactive with macromolecules like proteins and membrane lipids, thereby causing damage to the cell membrane (41). The antioxidant enzymes are superoxide dismutase, peroxidase and catalase, which metabolize oxygen-free radicals, thereby counteracting the generated stress-free radicals. The study revealed that catalase, peroxidase and superoxidase activity increased significantly with moisture stress in bioprimed plants compared with nonprimed seeds, which aligns with previous findings (42). The use of probiotics can also modify the antioxidant defence system based on the enzymatic activities of POD and SOD, increasing tolerance to the ROS produced under moisture-stress conditions. Microorganisms withstand moisture stress conditions by increasing reactive oxygen species scavengers in host plants, which acts as a protective mechanism for stress-exposed plants by detoxifying ROS, including hydrogen peroxide (H_2O_2), singlet oxygen ($_1O^2$) and hydrogen radical (•OH) intermediates of oxygen in mitochondria and chloroplasts (43).

Conclusion

Biopriming with nodule-associated plant probiotics significantly increased the germination of green gram seeds. Among the various concentrations of NAPP, 2% contributed positively to germination and increased the total seedling length, seed vigour index and antioxidant enzyme activity with reduced electrical conductivity of the seeds. However, the bioprimed seeds could tolerate abiotic stress (moisture stress) better than the unprimed seeds because of their increased vigour and germination. From this study, it can be concluded that seed priming with beneficial microorganisms is a practical and eco-friendly approach for improving seed quality and minimizing the side effects of typical crop production practices on the environment. Furthermore, identifying the ability of beneficial plant microbes to withstand moisture stress at the molecular level will demonstrate the benefits of seed priming with beneficial microbes in improving stress resilience for a wide range of crops.

Acknowledgements

The authors are thankful to Tamil Nadu Agricultural University for the scholarship provided to the first author and the facilities to conduct the research.

Authors' contributions

VM experimented and wrote the original draft. KS supervised, validated, and reviewed and edited the manuscript. US supplied the necessary resources, such as VV analysis and interpretation of results. RJ was in charge of visualization. NS analysis and interpretation of results. VB Data validation. TM reviewed and edited the manuscript. 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.

References

1. Raman J, Kim JS, Choi KR, Eun H, et al. Application of lactic acid bacteria (LAB) in sustainable agriculture: advantages and limitations. International Journal of Molecular Sciences. 2022;23 (14). https://doi.org/10.3390/ijms23147784.
2. Mostafavi K, Geive HS, Dadresan M, Zarabi M. Effects of drought stress on germination indices of corn hybrids (*Zea mays* L.). International Journal of Agri Science. 2011; 1(1): 10-18.
3. Narayanan GS, Prakash M, Kumar VR. Effect of integrated seed treatments on growth, seed yield and quality parameters in black gram [*Vigna mungo* (L.) Hepper]. Indian Journal of Agricultural Research. 2017;51(6):556-61. https://doi.org/10.18805/IJARe.A-4106
4. Hussain S, Zheng M, Khan F, Khaliq A, Fahad S, et al. Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. Scientific Reports. 2015;5(1). https://doi.org/10.1038/srep08101
5. Kazimierczak R, Srednicka-Tober D, Hallmann E, Kopczyńska K, Zarzynska K. The impact of organic vs. conventional agricultural practices on selected quality features of eight potato cultivars. Agronomy. 2019;9(12). https://doi.org/10.3390/agronomy9120799.
6. Sarkar D, Singh S, Parihar M, Rakshit A. Seed biopriming with microbial inoculants: A tailored approach to improve crop performance, nutritional security and agricultural sustainability for smallholder farmers. Current Research in Environmental Sustainability. 2021;3. https://doi.org/10.1016/j.crsust.2021.100093.
7. Patel S, Sayyed RZ, Saraf M. Bacterial determinants and plant defense induction: their role as biocontrol agents in sustainable agriculture. Plant, Soil and Microbes: Volume 2: Mechanisms and Molecular Interactions. 2016:187-04. https://doi.org/10.1007/978-3-319-29573-2_9.
8. Narayanasamy S, Thangappan S, Uthandi S. Plant growth-promoting Bacillus sp. cahoots moisture stress alleviation in rice genotypes by triggering antioxidant defense system. Microbiological Research. 2020;239. https://doi.org/10.1016/j.micres.2020.1 26518.
9. Khattak GS, Ashraf M, Elahi T, Abbas G. Selection for large seed size at the seedling stage in mungbean (*Vigna radiata* (L.) Wilczek). Breeding Science. 2003;53(2):141-3. https://doi.org/10.1270/jsbbs .53.141
10. IMD. Annual Climate Summary-2022 National Climate Centre, India Meteorological Department. 2022;24.
11. Geetha Thanuja K, Annadurai B, Thankappan S, Uthandi S. Non-rhizobial endophytic (NRE) yeasts assist nodulation of Rhizobium in root nodules of blackgram (*Vigna mungo* L.). Archives of Microbiology. 2020;202:2739-49. https://doi.org/10.1007/s00203-020-019 83-z.
12. Raja SR, Uthandi S. Non-rhizobial nodule associated bacteria (NAB) from blackgram (*Vigna mungo* L.) and their possible role in plant growth promotion. Madras Agricultural Journal. 2019;106(1-3):1. https://doi.org/10.29321/MAJ.2019.000291.
13. Annadurai B, Kennedy ZJ, Uthandi S. Drought tolerant Rhizobium sp. VRE1 induced osmotic stress tolerance, seed germination and seedling vigor in blackgram (*Vigna mungo* L.). International Journal of Ecology and Environmental Sciences. 2020;2(3):37-42.
14. ISTA. International rules for seed testing. The International Seed Testing Association-ISTA, Bassersdorf, Switzerland. 2019;19-8.
15. AbdulBaki AA, Anderson JD. (Vigor determination in soybean seed by multiple criteria. Crop Sci. 1973;13:630-3.https://doi.org/10.2135/cropsci1973.0011183X60013x.
16. Ali-Khan ST, Youngs CG. Variation in protein content of field peas. Canadian Journal of Plant Science. 1973;53(1):37-41. https://doi.org/10.4141/cjps73-005.
17. Presley JT. Relationship of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Disease Reporter. 1958; 42(7), 852.
18. Kittock DL, Law AG. Relationship of seedling vigor to respiration and tetrazolium chloride reduction by germinating wheat seeds 1. Agronomy Journal. 1968;60(3):286-8. https:// doi.org/10.2134/agronj 1968.00021962006000030012x.
19. Paul AK, Mukherji S, Sircar SM. Metabolic changes in rice seeds during storage. Indian Journal of Agricultural Science. 1970; 40 (12):1031-1036.
20. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant physiology. 1973;51(5):914-6. https://doi.org/10.1104/pp.51.5.914.

21. Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. Plant physiology. 1977;59(2):309-14. https://doi.org/10.1104/pp.59.2.309.

22. Aebi H. Catalase in vitro. Meth. Enzymol. 1984, 105, 121-26. https://doi.org/10.1016 /S00 76-6879(84)05016-3.

23. Malik CP, Singh MP. Peroxidase, Plant Enzymology and Histoenzymology Assay. (1980). Kalyani Publishers. New Delhi. https://doi.org/10.1016/j.colsurfb.2007.01.004.

24. Oshunsanya SO, Nwosu NJ, Li Y. Abiotic stress in agricultural crops under climatic conditions. Sustainable agriculture, forest and environmental management. 2019:71-100. https://doi.org /10.100 7/978-981-13-6830-1_3.

25. Kaushal M, Wani SP. Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agriculture, Ecosystems and Environment. 2016;231:68-78. https://doi.org/10.1016/j.agee.2016.06.031.

26. Singh A, Banerjee A, Roychoudhury A. Seed priming with calcium compounds abrogate fluoride-induced oxidative stress by upregulating defence pathways in an indica rice variety. Protoplasma. 2020;257:767-82. https://doi.org/10.1007/s00709-019-01460-5.

27. Lastochkina O, Garshina D, Ivanov S, Yuldashev R, et al. Seed priming with endophytic Bacillus subtilis modulates physiological responses of two different *Triticum aestivum* L. cultivars under drought stress. Plants. 2020;9(12). https://doi.org/10.3390/plants9121810

28. Chin JM, Lim YY, Ting AS. Biopriming vegetable seeds with metal -tolerant biocontrol agents to enhance tolerance to biotic and abiotic stress. Biological Control. 2022;174. https://doi.org/10.1016/j.biocontrol.2022.105035.

29. Fincher GB. Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annu. Rev. Plant Physiol. Plant Mol Biol 1989; 40: 305-45. https://doi.org/10.1146/annurev.pp.40.060189.001513

30. Gubler F, Kalla R, Roberts JK, Jacobsen JV. Gibberellin-regulated expression of a myb gene in barley aleurone cells: evidence for Myb transactivation of a high-pI alpha-amylase gene promoter. The Plant Cell. 1995;7(11):1879-91. https://doi.org/10.1105/tpc.7.11.1879

31. Kaneko M, Itoh H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M. The α-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. Plant Physiology. 2002;128(4):1264-70. https://doi.org/10.1104/pp.010785

32. Sharma P, Bhatt A, Jyoti B. Effect of seed bio-priming with microbial inoculants on plant growth, yield and yield contributing characters in soybean [*Glycine max* (L.) Merril]. International Journal of Economic Plants. 2018;5:053-8.

33. Raja K, Sivasubramaniam K, Anandham R. Seed infusion with liquid microbial consortia for improving germination and vigour in blackgram [*Vigna mungo* (L.) Hepper]. Legume Research-An International Journal. 2019;42(3):334-40. https://doi.org/10.18805/LR-3948.

34. Monisha S.Studies on harnessing the potential of bioinoculants for the management of abiotic and biotic stress in black gram [*Vigna mungo* (L.) Hepper] Ph.D. (Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore;2023.

35. Benmrid B, Ghoulam C, Zeroual Y, Kouisni L, Bargaz A. Bioinoculants as a means of increasing crop tolerance to drought and phosphorus deficiency in legume-cereal intercropping systems. Communications Biology. 2023;6 (1):1016. https://doi.org/10.1038/s42003-023-05399-5

36. Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. Cold Spring Harbor perspectives in biology. 2011;3 (4). https://doi.org/10.1101/cshperspect.a001438

37. Muniyappan VK, Tamilmani E, Desikan R, Ranganathan U. Influence of groundnut seed viability on biodiesel feedstock quality. Industrial Crops and Products. 2019;140. https://doi.org/10.1016/j.indcrop.2019.111697.

38. Revathi R, Vanangamudi K. Biopriming of maize hybrid seeds with biocontrol agents to improve germination and vigour. Madras Agricultural Journal. 2014;101(1/3),59-65.

39. Chaudhary S, Priya M, Jha UC, Pratap A, Hanumantha Rao B, Singh I, et al. Approaches toward developing heat and drought tolerance in mungbean. Developing climate resilient grain and forage legumes. 2022:205-34.

40. Patten CL, Glick BR. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Applied and environmental microbiology. 2002;68(8):3795-801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002.

41. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. International Journal of Molecular Sciences. 2021;22(9). https://doi.org/10.3390/ijms22094642.

42. Piri R, Moradi A, Balouchi H, Salehi A. Improvement of cumin (*Cuminum cyminum*) seed performance under drought stress by seed coating and biopriming. Scientia Horticulturae. 2019;257. https://doi.org/10.1016/j.scienta.2019.108667.

43. Abdelaal K, AlKahtani M, Attia K, Hafez Y, et al. The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. Biology. 2021;10(6):520.https://doi.org/10.3390/biology10060520