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Effect of bioinoculant seed coating on alleviation of drought stress in blackgram (*Vigna mungo* L.)

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Abstract
A study was undertaken in the Department of Seed Science and Technology, Tamil Nadu Agricultural University to evaluate the performance of microbial seed coating under water stress condition in blackgram. Seeds were coated with bioinoculants *viz.*, *Rhizobium japanicum* (10g/kg of seeds), *Phosphobacteria* (10g/kg of seeds), *Trichoderma harzianum* (4g/kg of seeds), *Bacillus subtillis* (10g/kg of seeds) individually and in consortium and the seeds were shade dried. The performance of coated seeds in terms of germination and other seedling quality parameters were evaluated under water stress condition at -4, -5 and -6 bar created by PEG 6000 solution in the laboratory. The results revealed that *Rhizobium japanicum, Phosphobacteria, Trichoderma harzianum* and *Bacillus subtillis* could able to tolerate water stress conditions of -4, -5, and -6 bars and increased the germination percentage, seedling length, dry matter production and vigour index. Therefore, microbial seed coating could be a successful presowing seed treatment to alleviate water stress in blackgram.

Keywords: Blackgram, water stress, bioinoculants, seed coating, seed germination, seedling vigour

Introduction

India is the largest producer and consumer of pulses in the world, and the blackgram cultivation accounts for around 19% of India's total pulse growing area and 23% of the country's total pulse production. India produced 24.5 lakh tonnes of blackgram from 4.6 million hectares of land with an average productivity of 533 kg/ha during 2020–2021 (agricoop.nic.in). Legume crops are susceptible to a number of abiotic threats, among which drought being one of the main yield limiting factors (Micheletto *et al.,* 2007) [10] which reduced the field emergence, plant growth, photosynthetic rate, stomatal conductance, pollen sterility, seed set, pod numbers and seed yield (Nadeem *et al*., 2019) [12]. Seed quality is also affected by forming shriveled seeds which leads to poor germination. Under situations of extreme drought stress, the production of photosynthetic pigments was slowed, and cell membrane stability decreased. A sudden increase in ethylene production under abiotic stress has adverse effects, which causes senescence (Glick *et al*., 1998) [5]. Reactive oxygen species (ROS) produced during drought stress cause a decrease in plant growth and development by affecting the plant physiological and biochemical events.

Negative effects of water stress are reduced through bioinoculants seed treatment by nitrogen fixation, phosphorus solubilization, phytohormone synthesis, pathogen suppression, and production of antioxidant enzymes. Bioinoculants like *Trichoderma harzianum, Rhizobium japanicum, Bacillus subtillis* and *Phosphobacteria* regulate phytohormones such as gibberellins, auxins, cytokinins, ABA, and ethylene. During water stress, more ABA and ethylene are produced, which results in senescence and shedding of leaves, flowers and pods. An essential enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase produced by Plant Growth Promoting Rhizobacteria (PGPR) controls the production of ethylene by metabolising ACC into α-ketobutyrate and ammonia under water stress conditions (Glick *et al.,*1998) [5]. Free radical accumulation causes damage to cell membranes and cellular machinery which can be eliminated by antioxidant enzymes including catalase (CAT), peroxidase (POD), and Superoxide dismutase (SOD) under water stress condition (Moreno -Galvan *et al.,* 2020) [11]. Seed coating is an effective method for application of inoculants which successfully transports the bioinoculants into the rhizosphere of the plants (Pedrini *et al.,* 2017) [15] and coating has been recommended as a viable approach for inoculating various agricultural seeds (Jetiyanon *et al.,* 2008) [7]. Based on this background, an experiment was conducted to increase the germination and seedling vigour of blackgram through bioinoculant seed coating under water stress condition.

Materials and Methods
Freshly harvested blackgramVamban 8 seeds were obtained from Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu. The commercial formulation of *Bacillus subtillis* and *phosphobacteria* culture were procured from Department of Plant Pathology and Department of Agricultural Microbiology respectively, Tamil Nadu Agricultural University (TNAU), Coimbatore. The *Rhizobium japanicum* and *Trichoderma harzianum* were obtained from the Green Life Biotech Laboratory, Coimbatore. The experiment was conducted by adopting Factorial Completely Randomized Block Design (FCRD) in four replications.
Blackgram seeds were first coated with the adhesive Carboxymethyl Cellulose at 1% followed by the seeds were coated with bioinoculants *viz.*, *Rhizobium japanicum* (10g/kg of seeds), *Phosphobacteria* (10g/kg of seeds), *Trichoderma harzianum* (4g/kg of seeds), *Bacillus subtillis* (10g/kg of seeds) individually and in consortium then the coated seeds were shade dried. The performance of coated seeds in terms of germination and other seedling quality parameters were evaluated under water stress conditionat -4, -5 and -6 bar created by PEG 6000 solution in the laboratory.

Treatment details
T_0: Untreated seeds (control)
T_1: *Rhizobium japanicum* (10g/kg)
T_2: *Phosphobacteria* (10g/kg)
T_3: *Trichoderma harzianum* (4g/kg)
T_4: *Bacillus subtillis* (10g/kg)
T_5: *T. harzianum* (4g/kg) + *R. japanicum* (10g/kg)
T_6: *B. subtillis* (10g/kg) + *R. japanicum* (10g/kg)
T_7: *T. harzianum* (4g/kg) + *Phosphobacteria* (10g/kg)
T_8: *B. subtillis* (10g/kg) + *Phosphobacteria* (10g/kg)
T_9: *T. harzianum* (4g/kg) + *R. japanicum* (10g/kg) + *Phosphobacteria* (10g/kg)
T_{10}: *B. subtillis* (10g/kg) + *R. japanicum* (10g/kg) + *Phosphobacteria* (10g/kg)
T_{11}: *B. subtillis* (10g/kg) + *T. harzianum* (4g/kg) + *R. japanicum* (10g/kg) + *Phosphobacteria* (10g/kg)

(Treatments T_5 – T_{11}: each inoculants coated with 10g/kg except *Trichoderma harzianum* 4g/kg)
Water stress was created by moistening the germination paper with different concentrations of PEG 6000 solution *viz.*, -4, -5 and -6 bar and germination test was conducted with 400 seeds in four replications each with 100 seeds in all the water stress conditions. Germination test was conducted at a temperature of 25 ± 1 °C and a relative humidity of 95 ± 2% and germination per cent was calculated using normal seedlings as indicated in ISTA regulations after seven days of incubation (ISTA, 2013) [6]. Four replicates of 100 seeds each were placed in petri plates with PEG 6000 solution at -4, -5 and -6 bars to assess the speed of germination. Daily counts of seeds that germinated based on radical protrusion were made until the final count on the seventh day. The following formula, provided by (Maguire, 1962) [9], was used to compute the speed of germination and was worked out in numbers.

$$\text{Speed of germination} = \frac{X_1}{Y_1} \quad \frac{X_2\text{-}X_1}{Y_2} \quad \frac{X_n\text{-}X_n\text{-}1}{Y_n}$$

Where
X_1- Number of seeds germinated at first count
X_2- Number of seeds germinated at second count
X_n- Number of seeds germinated on n^{th} count
Y_1-Number of days from sowing to first count
Y_2 -Number of days from sowing to second count
Y_n- Number of days from sowing to n^{th} count

Root length and shoot length (cm) was measured by using ten normal seedlings. Root length was measured from the collar region to the tip of the primary root and measured the shoot length from the collar region to the growing tip of the shoot and the mean value was calculated and expressed in cm. After the seedling length measurement, the ten normal seedlings were shade dried for 24 hours and dried in hot air oven at 80^0 C for another 24 hours and measured the dry weight of the seedlings. Seedling vigour index was calculated as per the method suggested by Abdul-Baki and Anderson (1973) [1] and the mean values were expressed in whole number.
Seedling vigourindex II= Germination (%) x Dry matter production

Statistical analysis
The data were analyzed statistically using Agdata and Agres software and the critical differences were calculated at 5% probability level (Panse and Sukhatme., 1967) [14].

Results and Discussion
The various microbial inoculant coated seeds performed better than the control seeds under -4, -5 and -6 bar water stress condition created by using PEG 6000. Control seeds without water stress recorded maximum seed quality parameters than coated seeds sown in water stress condition. A highly significant difference was found in *Trichoderma harzianum* followed by *Trichoderma harzianum* + *Rhizobium japanicum* + *Phosphobacteria* coated seeds over the control seeds in terms of maximum speed of germination (5.80, 4.77, 3.57), germination (87%, 87%, 80%) (Table 1), root length (17.1 cm, 15.7 cm, 10.2 cm) (Table 2), shoot length (17.3 cm, 16.1 cm, 12.3 cm) (Table 2), dry matter production (0.238g, 0.232g,0.196g) and vigour index (2917, 2692,1746) (Table 3) at -4, -5 and -6 bar, which was on par with *Trichoderma harzianum* + *Rhizobium japanicum* + *Phosphobacteria* in terms of speed of germination (5.01, 4.69, 3.08), germination (85%, 85%, 79%) (Table 1), root length (16.9cm, 15.2cm, 10.1cm) (Table 2), shoot length (17.1cm, 15.9cm, 9.8cm) (Table 2), dry matter production (0.295g, 0.217g, 0.193g) and vigour index (2897, 2405, 1860) (Table 3) over control seeds in terms of speed of germination (2.57, 1.66, 0.99), germination (43%, 32%, 21%) (Table 1), root length (6.2cm, 6.4cm, 5.1cm) (Table 2), shoot length (9.8cm, 9.3cm, 5.2cm) (Table 2), dry matter production (0.166g, 0.153g, 0.106g) (Table 3) and vigour index (683, 534, 216) (Table 3)).With increasing the water stress level, the seed germination percentage and all seedling vigour parameters were decreased rapidly in both inoculated and non-inoculated control seeds. The same observations were recorded by Niu *et al*., (2018) [13] in foxtail millet. The seeds treated with *Pseudomonas fluorescens* DR7, *Pseudomonas fluorescens* DR11, *Enterobacter hormaechei* DR16, and *Pseudomonas migulae*

DR35 recorded the maximum germination under drought stress, between -0.30 MPa and -1.03 MPa.

Blackgram seeds coated with *T. viride*, recorded the maximum germination of 97.3% and plant dry mass of 18.88 g (Leo Daniel *et al.*, 2011) [8]. *Pseudomonas fluorescens* Pf 2, Pf 4, Pf 6, and Pf 28 and three isolates of *Trichoderma harzianum* TH 08, IRRI 1 and TH 26 treated tomato seed showed similar results *viz.*, maximum shoot and root length (Anbazhagan *et al.*, 2020) [2]. According to Annadurai *et al.*, (2020) [3], blackgram seeds coated with *Rhizobium sp.* VRE1 alleviated the effect of water stress by recording maximum seed germination. Aliasgharzad *et al.*, (2006) [4] reported that soybean seeds inoculated with *G. etunicatum* and *B. japonicum*, had positive influence on seedling dry weights, higher relative water content and low water potential showed the drought avoidance and fundamental mechanism of plant-microbe relationship. Piri *et al.*, (2019) [16] proved that inoculating blackgram seed with *Pseudomonas fluorescence* CHA0 under all osmotic potential conditions (0, -3 and -6 bar) produced the maximum seedling emergence percentage. Roscha *et al.*, (2019) [17] reported that under severe water deficit conditions, seeds inoculated with *R. irregularis* took 4 days for final seedling emergence compared to about 7 days for seeds without treatment. According to Vurukonda *et al.*, (2016) [18] PGPBs accumulate antioxidants and osmoprotectants which can enhance root development under stress condition. Seed treatment with *Azospirillum* species improved the root growth and promoted lateral root formation during drought by producing indole acetic acid (Zahir *et al.*, 2009) [19].

Table 1: Effect of seed coating with bioinoculants on speed of germination and germination in blackgram under different water stress condition

Treatments	Speed of germination					Germination (%)				
	control	-4 bar	-5 bar	-6 bar	Mean	control	-4 bar	-5 bar	-6 bar	Mean
Control	5.30	2.57	1.66	0.99	2.63	84	43	32	21	45
Rhizobium japannicum (10g/kg)	8.40	4.90	4.30	3.57	5.29	87	76	68	57	72
Phosphobacteria (10g/kg)	8.40	3.89	3.13	2.50	4.48	89	76	67	57	72
Trichoderma harzianum (4g/kg)	9.43	5.80	4.77	3.57	5.89	91	87	87	80	86
Bacillus subtillis (10g/kg)	8.80	4.33	3.91	2.67	4.93	85	76	71	57	71
T. harzianum (4g/kg) + *R. japannicum* (10g/kg)	8.17	5.63	4.50	3.10	5.35	91	69	63	56	70
B. subtillis (10g/kg) + *R. japannicum* (10g/kg)	8.53	4.11	3.17	2.40	4.55	89	60	57	51	64
T. harzianum (4g/kg) + *Phosphobacteria* (10g/kg)	9.10	4.60	4.17	3.50	5.34	91	72	65	57	71
B. subtillis (10g/kg) + *Phosphobacteria* (10g/kg)	8.97	4.00	3.30	2.60	4.72	85	69	63	57	69
T. harzianum (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	9.53	5.01	4.69	3.08	5.58	87	85	85	79	84
B. subtillis (10g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	8.63	4.14	3.72	2.74	4.81	88	85	79	76	82
B. subtillis (10g/kg) + *T. harzianum* (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	9.21	4.14	3.31	2.39	4.76	87	69	64	63	71
Mean	8.54	4.43	3.72	2.76	4.86	88	72	67	59	71
	T	P	T X P			T	P	T X P		
S.Ed	0.09	0.05	0.18			1.677	0.968	3.355		
CD (P=0.05)	0.18	0.10	NS			3.330	1.922	NS		

Table 2: Effect of seed coating with bioinoculants on root length and shoot length in blackgram under different water stress condition

Treatments	Root length (cm)					Shoot length (cm)				
	control	-4 bar	-5 bar	-6 bar	Mean	control	-4 bar	-5 bar	-6 bar	Mean
Control	15.9	6.2	6.4	5.1	8.4	16.8	9.8	9.3	5.2	10.3
Rhizobium japannicum (10g/kg)	17.1	12.9	11.7	9.3	12.7	17.5	14.1	12.9	9.8	13.5
Phosphobacteria (10g/kg)	17.6	14.8	12.1	9.6	13.5	17.5	15.2	14.2	9.6	14.1
Trichoderma harzianum (4g/kg)	17.4	17.1	15.7	10.2	15.1	19.1	17.3	16.1	12.3	16.2
Bacillus subtillis (10g/kg)	17.9	14.3	12.7	9.8	13.7	17.1	15.1	12.8	9.9	13.7
T. harzianum (4g/kg) + *R. japannicum* (10g/kg)	17.4	15.1	12.7	9.8	13.8	17.5	15.3	12.7	9.7	13.7
B. subtillis (10g/kg) + *R. japannicum* (10g/kg)	17.3	14.4	11.9	9.7	13.3	17.3	14.1	12.1	9.5	13.2
T. harzianum (4g/kg) + *Phosphobacteria* (10g/kg)	17.8	13.7	11.2	9.3	13.1	17.7	14.2	12.7	9.8	13.6
B. subtillis (10g/kg) + *Phosphobacteria* (10g/kg)	17.7	15.5	13.1	8.4	13.6	16.6	15.1	13.1	11.2	14.2
T. harzianum (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	17.8	16.9	15.2	10.1	15.1	19.6	17.1	15.9	9.8	15.6
B. subtillis (10g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	17.2	16.6	14.3	10.6	14.7	20.2	17.6	15.8	13.8	16.8
B. subtillis (10g/kg) + *T. harzianum* (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	17.8	15.1	12.2	8.9	13.5	16.8	15.6	11.9	8.9	13.1
Mean	17.4	14.4	12.4	9.2	13.3	17.8	14.9	13.3	10.1	14.1
	T	P	T X P			T	P	T X P		
S.Ed	0.335	0.193	0.670			0.326	0.188	0.653		
CD (P=0.05)	0.665	0.384	S			0.648	0.374	NS		

Table 3: Effect of seed coating with bioinoculants on drymatter production and vigour index in blackgram under different water stress condition

Treatments	Drymatter Production (g / 10 seedlings)					Vigour Index				
	control	-4 bar	-5 bar	-6 bar	Mean	control	-4 bar	-5 bar	-6 bar	Mean
Control	0.286	0.166	0.153	0.106	0.178	2760	683	534	216	1049
Rhizobium japannicum (10g/kg)	0.286	0.238	0.220	0.157	0.225	2991	2005	1679	1093	1942
Phosphobacteria (10g/kg)	0.289	0.217	0.176	0.144	0.207	3121	2634	1990	1293	2260
Trichoderma harzianum (4g/kg)	0.336	0.286	0.232	0.196	0.263	3349	2917	2692	1746	2676
Bacillus subtillis (10g/kg)	0.291	0.238	0.202	0.180	0.228	2974	2263	1800	1110	2037
T. harzianum (4g/kg) + *R. japannicum* (10g/kg)	0.291	0.301	0.208	0.172	0.243	3171	2093	1569	1093	1982
B. subtillis (10g/kg) + *R. japannicum* (10g/kg)	0.291	0.254	0.226	0.193	0.241	3048	1707	1383	979	1779
T. harzianum (4g/kg) + *Phosphobacteria* (10g/kg)	0.288	0.251	0.228	0.189	0.239	3228	2027	1561	1098	1979
B. subtillis (10g/kg) + *Phosphobacteria* (10g/kg)	0.282	0.220	0.170	0.162	0.209	2939	2095	1647	1058	1935
T. harzianum (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	0.308	0.295	0.217	0.193	0.253	3294	2897	2405	1860	2614
B. subtillis (10g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	0.309	0.310	0.224	0.155	0.249	3237	2917	2686	1596	2609
B. subtillis (10g/kg) + *T. harzianum* (4g/kg) + *R. japannicum* (10g/kg) + *Phosphobacteria* (10g/kg)	0.287	0.228	0.208	0.156	0.220	3009	2064	1570	1145	1947
Mean	0.220	0.250	0.205	0.167	0.229	3093	2192	1793	1191	2067
	T	P	T X P			T	P	T X P		
SEd	0.00496	0.00286	0.00992			64.575	37.282	129.151		
CD (P=0.05)	0.00984	0.00568	NS			128.181	74.005	NS		

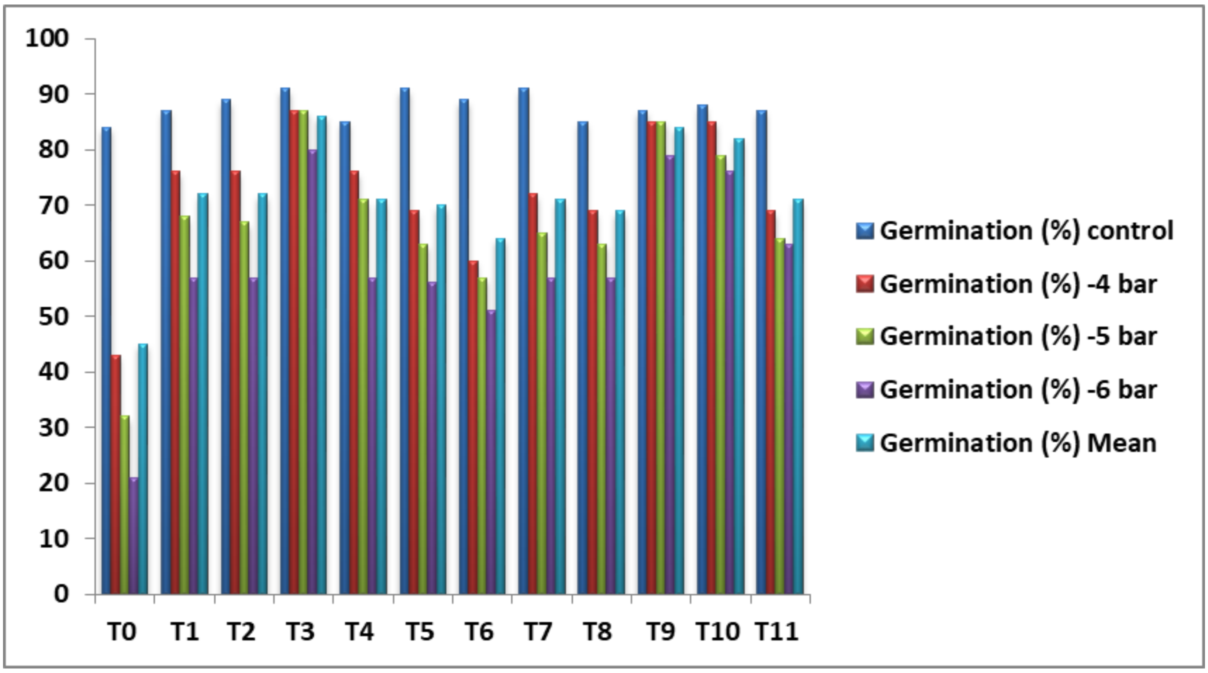

Fig 1: Effect of bioinoculants coated blackgram seeds on germination under different water stress condition

T_0 :Control
T_1 :*Rhizobium japannicum*
T_2 :*Phosphobacteria*
T_3 :*Trichoderma harzianum*
T_4 :*Bacillus subtillis*
T_5 :*T. harzianum + R. japannicum*
T_6 :*B. subtillis+ R. japannicum*
T_7 :*T. harzianum+ Phosphobacteria*
T_8 :*B. subtillis+ Phosphobacteria*
T_9 :*T. harzianum + R. japannicum + Phosphobacteria*
T_{10}:*B. subtillis+ R. japannicum + Phosphobacteria*
T_{11}:*B. subtillis+ T. harzianum+ R. japannicum + Phosphobacteria*

Conclusion
The results indicated that blackgram seeds coated with bioinoculant *Trichoderma harzianum* (10g/kg) individually or in combination with *Trichoderma harzianum* (4g/kg) + *Rhizobium japanicum* (10g/kg) + *Phosphobacteria* (10g/kg) each increased the seed germination percentage and seedling vigour under water stress condition.

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