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Performance of Bioprimed Chilli Seed under Moisture Stress Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Laboratory experiment was carried out using 'PKM 1' chilli (*Capsicum annuum* L.) seed to standardize bioprimed seeds under moisture stress condition inorder to improve seed germination and seedling vigour. To induce the moisture stress, optimize the different concentration of water holdings capacity viz., 80, 60, 40 and 20% were used for best bioprimed seed treatment (biocontrol agents (*Pseudomonas fluorescens* 60% 12 h, *Trichoderma viride* 60% 9 h) and liquid biofertilizers (*Azospirillum* 10% 9 h and Phosphobacteria 15% 9h)) along with hydroprimed seed and control seed. Seed bioprimed with *Pseudomonas fluorescens* 60% for 12 h improved the germination percentage (82), root length (14.2 cm), shoot length (6.2 cm), dry matter production (0.0489 g 10 seedlings^{-1}) and vigour index (1673) compared to control seed. Seed biopriming with *Pseudomonas fluorescens* 60% for 12 h can be adopted to improve seed germination and seedling vigour under moisture stress condition upto 20%.

Keywords: Biocontrol agents; liquid biofertilizers; germination; vigour.

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1. INTRODUCTION

Low crop productivity faced by Indian Agriculture is mainly because of poor soil health and polluted environment. Though the high quality seeds are used for sowing in the field, it undergoes several stresses during the emergence and establishment leading to poor survival and reduced plant stand. Drought is a major limitation for crop production in rainfed ecosystems. It is not simply the lack of water that lowers yield potential, but also the timing and duration of drought stress related to phenological processes [1].

One possibility to increase plant water acquisition and/or drought tolerance is to use beneficial microorganisms as inoculants. Priming generally induces faster and more uniform seed germination especially in adverse physical conditions of many crop species [2]. The seed germination and seedling growth are modified by factors like salt stress (Abdel-Ghani, 2009) and water holding capacity of soil [3].

Moisture stress is a critical environmental factor that restricts seed germination. Moisture stress during the earlier phase of seed germination affects the field emergence, seedling establishment ultimately the yield of the crop [4]. In order to overcome these stresses encountered during seed germination in the field, several authors have recommended seed priming such as hydropriming, halopriming, osmopriming and solid matric priming [5-8].

Seed priming is an efficient method for increasing seed vigour and synchronization of germination, as well as the growth of seedlings of many crops under stressful conditions and it play a positive role in the tolerance to abiotic stresses. Priming is seed invigoration treatments, which consist of a controlled imbibitions of the seeds followed by dehydration back to their initial water content [9]. This treatment could enable the crop to establish under initial moisture stress condition and established vigourous seedling also give higher yield under terminal moisture stress condition [10].

Biopriming is a process of biological seed treatment that refers combination of seed hydration (Physiological aspect of disease control) and inoculation (Biological aspect of disease control) of seed with beneficial organism to protect seed. It is an ecological approach using selected fungal antagonists against the soil and seed borne pathogens. Biological seed treatments may provide an alternative to chemical control. Seed may be planted moist or dried for storage. The addition of microbial bicontrol agents during biopriming allows for colonization of the seed prior to planting and adds new dimension to seed priming treatment [11]. It is accepted that microorganism such as AM fungi and plant growth-promoting rhizobacteria (PGPR) such as *Azospirillum brasilense, Pseudomonas and Bacillus,* are very effective in enhancing the ability of plants to become established and to cope with stress Bases on these above views, this experiment was conducted to analyse the suitable biopriming seed treatment under moisture stress condition in chilli.

2. MATERIALS AND METHODS

Genetically-pure, fresh seed of the chilli (*Capsicum annuum* L.) cultivar 'PKM 1' were obtained from the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu and used in this study. The bio-control agents, *T. viride* (Tv1) and *P. fluorescens* (Pf1) were obtained from the Department of Plant Pathology, TNAU and liquid biofertilizers (Azospirillum and Phosphobacteria) were obtained from the Department of Agricultural microbiology, TNAU, Coimbatore.

Seeds (n = 400) were bio-primed separately using 40, 60, or 80% (w/v) *T. viride* or *P. fluorescens* for 3, 6, 9 or 12 h, in parallel with conventional hydro-priming in distilled water. Unprimed seed served as the controls. Seeds (all 400) were soaked in an equal volume of each concentration of each bio-control agent. After soaking, the seeds were removed from each solution and shade dried at room temperature to measure selected seed quality parameters. The whole experiment was carried out with four replications in a completely randomised factorial design. The standarised best bioprimed seed treatment were used for moisture stress study.

A known quantity (weight basis) of sand was taken in an aluminium tray. Then, known quantity of water was added till the media reached saturation and weighed again. The difference in weight and the total quantity of water added to reach saturation was noted. This ratio of sand and water was taken as 100% moisture holding capacity and accordingly, 80, 60, 40 and 20% of water was added to sand media to create 80, 60,

40 and 20% water holding capacities, respectively. Four replicates of 100 seeds from each treatment were sown in each of the above water holding capacity and kept in the germination room and at the end of 14 days, the following observations were made. The following observations were recorded for stress tests conducted.

2.1 Germination

The germination test was conducted by using paper (between paper) medium. Four replicates of 100 seeds each were germinated in a germination room maintained at 25±2°C temperature and 90±3% RH. At the end of fourteenth day of sowing, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage.

2.2 Root Length

At the time of germination count, ten normal seedlings were selected at random from each replication and used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root. The mean values were calculated and expressed in centimetre.

2.3 Shoot Length

The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the point of attachment of seed to tip of the leaf and the mean values were expressed in centimetre.

2.4 Drymatter Production

The ten normal seedlings were placed in a paper cover and dried in shade for 24h and then, they were kept in an oven maintained at 103±2°C for 16±1h. The dried seedlings were weighed and the mean values were expressed in g 10 seedlings^{-1}.

2.5 Vigour Index

Vigour index values were computed using the following formulae and the mean values were expressed in whole number.

Vigour index = Germination percentage x Total seedling length (cm).

2.6 Statistical Analysis

The data obtained from different experiments were analysed for the 'F' test of significance following the methods described by [12] Panse and Sukhatme (1985). Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test is non-significant, it was indicated by the letters NS.

3. RESULTS AND DISCUSSION

The performance of bioprimed seed under different water holding capacities namely 20, 40, 60 and 80% along with non primed seed revealed that the bioprimed seed with *Pseudomonas fluorescens* 60% 12h enhanced the germination in all the water holding capacities. The percentages of germination (55, 82, 75 and 48) under 20, 40, 60 and 80% water holding capacities when compared with nonprimed seed (40, 62, 51 and 40), respectively (Table 1). This indicated that *Pseudomonas fluorescens* bioprimed seed could able to withstand extreme conditions like low and high moisture levels. The high germination recorded at low moisture (20% WHC) stress condition could be attributed to the faster germination rate of bioprimed seed because of more metabolic and cell expansion activities.

Bradford [9] who stated that maize seeds bioprimed with *Pseudomonas fluorescens* 80% for 12h recorded higher germination percentage and seedling vigour under high moisture level of 80%. The results of the present study are in agreement with the findings of [13] who stated that rice seeds bioprimed with *Pseudomonas fluorescens* 60% for 12h recorded higher germination per cent and seedling vigour under high moisture level of 20%. The results are also in confirmation with the findings of [14] in tomato, brinjal and chilli; [15] in carrot and onion; [16] in mustard and radish; [17] in bhendi and beetroot and [3] in bittergourd and ashgourd. The results are also in concordant with [18] who stated that positive interactions were developed under drought conditions between *Pseudomonas putida* or *Bacillus megaterium* and AM fungi in stimulating plant growth and drought tolerance.

In the case of root length and shoot length, seed bioprimed with *Pseudomonas fluorescens* 60% 12h recorded the root length of 9.3,14.2,12.9,

8.0 cm and shoot length of 2.5,6.2,4.1,2.0 cm, respectively whereas non primed seed recorded the root length of 6.9, 11.7, 9.3, 5.5 cm and shoot length of 1.9,3.2,2.3,1.0 cm under 20, 40, 60 and 80% water holding capacities, respectively.

Table 1. Effect of bioprimed seed on germination (%) under moisture stress conditions

Bio-priming treatments (T)	Water holding capacities (%)				Mean
	20	40	60	80	
Nonprimed seed	40 (39.23)	65 (53.73)	51 (45.57)	40 (39.23)	48 (43.85)
Hydropriming 6 h	40 (39.23)	65 (53.73)	56 (48.45)	40 (39.23)	50 (45.00)
Azospirillum 10% 9 h	40 (39.23)	69 (56.17)	60 (50.77)	41 (39.82)	53 (46.72)
Phosphobacteria 15% 9 h	49 (44.43)	78 (52.03)	67 (54.94)	44 (41.55)	60 (50.77)
Trichoderma viride 60% 9 h	45 (42.13)	71 (57.42)	62 (51.94)	40 (39.23)	55 (47.87)
Pseudomonas fluoresces 60% 12 h	55 (48.72)	82 (54.90)	75 (60.00)	48 (43.85)	65 (53.73)
Mean	45 (42.13)	71 (57.42)	62 (51.94)	42 (40.40)	
	T	M	T*M		
SEd	0.08	0.10	0.21		
CD (P=0.05)	0.18	0.20	0.44		

(Figures in parentheses indicate arcsine values)

Table 2. Effect of bioprimed seed on root length (cm) under moisture stress conditions

Bio-priming treatments (T)	Water holding capacities (%)				Mean
	20	40	60	80	
Nonprimed seed	6.9	11.7	9.3	5.5	8.4
Hydropriming 6 h	7.3	12.0	10.1	6.2	8.9
Azospirillum 10% 9 h	7.8	12.5	10.9	6.8	9.5
Phosphobacteria 15% 9 h	8.9	13.7	12.0	7.7	10.6
Trichoderma viride 60% 9 h	8.5	12.8	11.3	7.2	10.0
Pseudomonas fluoresces 60% 12 h	9.3	14.2	12.9	8.0	11.1
Mean	8.1	12.8	11.1	6.9	
	T	M	T*M		
SEd	0.05	0.03	0.09		
CD (P=0.05)	0.09	0.07	0.11		

Table 3. Effect of bioprimed seed on shoot length (cm) under moisture stress conditions

Bio-priming treatments (T)	Water holding capacities (%)				Mean
	20	40	60	80	
Nonprimed seed	1.9	3.2	2.3	1.0	2.1
Hydropriming 6 h	2.0	4.1	2.6	1.0	2.4
Azospirillum 10% 9 h	2.0	4.9	3.0	1.2	2.8
Phosphobacteria 15% 9 h	2.2	5.8	3.7	1.8	3.4
Trichoderma viride 60% 9 h	2.0	5.5	3.3	1.4	3.1
Pseudomonas fluoresces 60% 12 h	2.5	6.2	4.1	2.0	3.7
Mean	2.1	5.0	3.2	1.4	
	T	M	T*M		
SEd	0.04	0.02	0.06		
CD (P=0.05)	0.09	0.05	0.13		

Table 4. Effect of bioprimed seed on drymatter production (g 10 seedlings^{-1}) under moisture stress conditions

Bio-priming treatments (T)	Water holding capacities (%)				Mean
	20	40	60	80	
Nonprimed seed	0.0220	0.0414	0.0310	0.0112	0.0264
Hydropriming 6 h	0.0225	0.0425	0.0321	0.0115	0.0272
Azospirillum 10% 9 h	0.0238	0.0432	0.0335	0.0120	0.0281
Phosphobacteria 15% 9 h	0.0274	0.0465	0.0488	0.0131	0.0340
Trichoderma viride 60% 9 h	0.0261	0.0447	0.0361	0.0127	0.0299
Pseudomonas fluoresces 60% 12 h	0.0301	0.0489	0.0313	0.0137	0.0310
Mean	0.0253	0.0445	0.0355	0.0124	
	T	M	T*M		
SEd	0.00015	0.00012	0.00030		
CD (P=0.05)	0.00030	0.00024	0.00060		

Table 5. Effect of bioprimed seed on vigour index under moisture stress conditions

Bio-priming treatments (T)	Water holding capacities (%)				Mean
	20	40	60	80	
Nonprimed seed	352	924	592	260	532
Hydropriming 6 h	372	1047	711	288	604
Azospirillum 10% 9 h	392	1201	834	328	689
Phosphobacteria 15% 9 h	544	1521	1052	418	884
Trichoderma viride 60% 9 h	473	1299	905	344	755
Pseudomonas fluorescens 60% 12 h	649	1673	1275	480	1019
Mean	464	1277	895	353	
	T	M	T*M		
SEd	6.0	4.9	10.1		
CD (P=0.05)	12.23	10.25	20.3		

The results indicated that even under high moisture regimes (80% WHC), the *Pseudomonas fluorescens* 60% 12 h showed superior performance which could be attributed to the higher cell division and expansion of bioprimed seed as well as more production of growth promoting substances. The results pertaining to drymatter production and vigour indices followed the same trend as that of root and shoot length (Table 5 above). The results of the present study are in agreement with the findings of [13] and [19] in hybrid maize, and [10] in rice.

Seed priming has improved seed germination and seedling establishment under extreme drought conditions The positive role of priming on abiotic stress tolerance might be due to the improved antioxidant production [20]. Antioxidants are natural defensive elements in seed that scavenge excessive reactive oxygen species (ROS). In priming the excessive ROS were scavenged during early imbibition process and it play an essential role in ensuring successful germination, especially under stress conditions [21].

With regards to PGPR strains, application of PGPR can enhance phytohormones content of seed under moisture stress condition [22]. Phytohormones plays critical role in regulating plant growth and its response to stress. *Pseudomonas fluorescens* treatment had improved phytohormonal characters under water deficit condition. The *P. fluorescens* protect plants from drought stress [23] and significantly promote the seedling growth under stress condition. Application of *Pseudomonades sp*. under water stress improved the antioxidant [24].

4. CONCLUSION

It is summarized from the study that seeds of chilli bioprimed with *Pseudomonas fluorescens* 60% 12h could able to tolerate low (20% WHC) and high moisture (80% WHC) regimes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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