

Original Research Article

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Osmotolerant Rhizobacteria Improve Seedling Vigour and Plant Growth of Mustard under Water Scarcity

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ABSTRACT

Drought has major impacts on Indian agriculture and it is known to reduce seed germination and plant development by influencing physiological and biochemical functions of plants. Imparting drought tolerance through microorganisms is an eco-friendly and cost-effective method. In the present investigation, seven bacterial strains isolated from drought stressed habitats were tested for their osmotolerance and ability to help mustard to withstand drought stress. Strains NSRSSH-1 followed by MRD-17 and MKS-6 found to grow well in 30% PEG 6000 induced osmotic stress compared to other strains. Drastic reduction in growth was observed in 40% PEG 6000 in all strains. These strains were studied for mustard seed germination promotion under osmotic stress condition. Inoculation with MRD-17, MKS-6, MCL-1 and MMS-5 recorded more than 90 % percent germination at 48 h and highest seedling fresh weight was observed in seedlings inoculated with MRD-17 followed by MKS-6 and MCL-1. Strains MRD-17 and MKS-6 selected for further experiments. Bacterial strains MKS-6 and MRD-17 were further tested for growth promotion under water deficit stress. Seedling length and vigour (*in vitro*) were enhanced under both control and osmotic stress condition due to inoculation with MKS-6 and MRD-17. At both sterile and unsterile conditions (*in vivo*), shoot and root dry weight at 15 and 30 DAS were also improved upon inoculation with MRD-17 and MKS-6 under control as well as under drought stress condition. Rhizobacteria MKS-6 and MRD-17 found to have ability to induce drought tolerance in mustard and can be used to develop bioinoculants for mustard drought stress alleviation.

Keywords

Osmotolerance,
Mustard,
Germination, Plant
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Introduction

Drought is an extreme climate event that develops slowly followed by increase in intensity and duration which leads to major eco-hydrological and socio-economic impacts (Kim *et al.*, 2019). Drought is expected to become more severe and common in the

coming decades as a result of global warming. Indian agriculture heavily relies on country's climate. Failure in monsoons leads to water shortage and reduction in crop growth and production. Extreme stress conditions limit plant growth and development. All stages of plant growth were affected by drought (Fahad *et al.*, 2014). Drought stress reduces seed

germination and plant development by influencing various physiological and biochemical functions of plants.

Mustard is one of the most important oilseed crops in the world and especially in India, primarily grown as an edible oil seed crop. The oil content of mustard seeds varies from 28.6 % to 45.7 %. Seed residue after oil extraction is used as cattle feed and fertilizer ((Jensen *et al.*, 1996). Nearly 37% of the total rapeseed mustard area is rain-fed in India, where the crop is prone to be affected by drought which results in acute yield losses. All stages of plant growth were affected by drought stress including seed germination, early stages of germination, vegetative and reproductive stages of mustard (Ghobadi *et al.*, 2006). Reduction in plant height, number of primary branches and secondary branches per plant, thousand seed weight and seed yield were observed as a result of drought stress in mustard. Under drought condition, yield reductions of 17-94 % have been reported in mustard (Chauhan *et al.*, 2007).

Recent studies indicate that microorganisms can also help the crops to cope with drought stresses which are an eco-friendly and cost-effective method. Meena *et al.*, (2017), reported that bacteria belonging to different genera including *Bacillus*, *Pseudomonas*, *Rhizobium*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Enterobacter*, *etc.* have been reported to provide tolerance to host plants and also improve their growth under drought. Enhancement in germination of seeds, growth of seedlings and plant biomass upon inoculation with rhizobacteria under drought stress conditions were observed in mustard, pearl millet and cluster bean (Bandeppa *et al.*, 2015; Manjunatha *et al.*, 2017; Rathi *et al.*, 2018a). The last two decades have witnessed many reports on the utilization of such microbes for induction of tolerance against drought stress.

Kavamura *et al.*, (2013), reported that plant growth promoting rhizobacteria (PGPR) obtained from extreme environmental conditions may protect plants from the harmful effects of drought stress and increases crop productivity in arid or semi-arid areas. Hence, identification and development of eco-friendly strategies using microbes that can improve plant growth under water deficit stress are an immediate need in agricultural systems. Considering these facts in view, the present study was carried out to screen and identify the potential osmotolerant bacterial strains. The influence of these bacterial strains on germination of seeds and early stages of plant growth under osmotic stress condition were also studied.

Materials and Methods

Growth and maintenance of rhizobacteria

For the present study, bacteria isolated from mustard, cluster bean and pearl millet grown under drought conditions, available in the germplasm of Division of Microbiology, IARI, New Delhi were used. Table 1 has the list of rhizobacterial strains used in the study. Nutrient agar medium and slants and storage at 4 °C was used for the growth and maintenance of these bacterial cultures. Subculturing was done as and when needed.

Screening of bacterial strains

Effect of osmotic stress on rhizobacterial growth

Nutrient broth supplemented with 30 and 40 % PEG 6000 in culture tubes were used for the determination of the influence of osmotic stress on rhizobacterial growth. Inoculum of 2 %, 24 h old bacterial cultures were inoculated and incubated for 48h at 28 ± 2 °C on a shaker at 200 rpm. After 48 h, turbidity of the cultures was measured as a determinant of

bacterial growth using a spectrophotometer at 660 nm.

Effect on seed germination

Mustard seeds of variety Pusa Karishma LES-39 (Drought-susceptible) obtained from Division of Genetics, IARI, New Delhi was used for the experiment. Mustard seeds were surface sterilized with 0.1 % HgCl₂ solution for 2 min, then washed several times thoroughly with sterile water to remove traces of mercuric chloride. The seeds were inoculated with one day old cultures of bacterial strains for 60 min. After inoculation the seeds were placed on petri plates containing 0.8 % agar with 20 % PEG 6000 as osmotic stress treatment and 0.8 % agar as no stress condition. Uninoculated controls and replications were maintained for each treatment. The plates were incubated for germination 48 h at 25 ± 2° C. Observations such as seed germination percentage and fresh weight of seedlings were recorded after 48 h if germination to screen the potential plant growth promotion by rhizobacterial strains.

Influence of osmotolerant bacteria on seed germination and plant growth

Two plant growth promoting and osmotolerant bacterial isolates were selected and further analysed under *in-vitro* and *in-vivo* conditions for their potential in drought stress alleviation.

Influence on germination of seeds and vigor

Surface sterilized mustard seeds were inoculated with one day old cultures of MRD-17 and MKS-6, placed on petri plates containing 0.8% agar as control and 0.8% agar with 20% PEG 6000 as osmotic stress. The plates were incubated at 25 ± 2° C for 72 h and observations on seedling length and

vigour index were taken to determine the influence of osmotolerant rhizobacteria on seed germination and seedling characteristics. Seedling vigour index = SVI = Germination percentage × Seedling length (Fig. 3).

Influence on early stages of plant growth

The study was conducted under both sterile and unsterile condition. Surface sterilized mustard seeds were inoculated with MRD-17 and MKS-6, and sown in four-inch pots containing 500 g soil. For maintaining sterile condition, experiment was conducted in sterile soil, by autoclaving the pots with soil at 120°C, 15 psi for 4 h and by maintaining the moisture content using sterile water. Field capacity was maintained in control treatments and water deficit stress was imposed after 7 days of germination in stress treatments by maintaining 50 % field capacity. Moisture level in pots was maintained by gravimetric method. Shoot and root dry weight were recorded at 15th and 30th days after sowing.

Statistical analysis

Data on screening of bacterial strains was subjected to one factor analysis and data generated on most of the other parameters was subjected to two factor analysis using OPSTAT statistical software. Duncan's multiple range test (DMRT) at $p < 0.05$ was performed for comparison of treatment means.

Results and Discussion

Effect of osmotic stress induced by PEG 6000 on the growth of bacterial strains was observed (Table 2). Even though reduction in growth was observed under osmotic stress, all bacterial strains were observed to grow in 30 and 40 % PEG 6000 concentration. In control treatment, MRD-17, NSRSSS-1, NAD-7 and MKS-6 recorded highest growth. Nearly 47-

69 % reduction in growth was recorded due to the supplementation of media with 30% PEG 6000. Highest growth was recorded for NSRSSS-1 followed by MRD-17 and MKS-6. Although, the strains were observed to grow in presence of 40% PEG 6000, growth was drastically reduced. Bacteria isolated from extreme environments are known to possess tolerance to those environments. As these bacterial strains were from drought stressed environment, all strains had tolerance to PEG 6000 induced osmotic stress. Malakar *et al.*, (2014), also reported reduction in bacterial growth under PEG 6000 induced osmotic stress. Less reduction in growth under

osmotic stress compared to control indicates its osmotolerance ability (Bandeppa *et al.*, 2015; Manjunatha *et al.*, 2017). Potential of these bacterial strains in improving the germination of mustard seeds (*invitro*) under osmotic stress condition were analysed (Table 3). Even though, inoculation with MRD-17, MMS-5, MKS-6 and MCL-1 recorded more than 90 % seed germination at 48 h, the treatments were found to be statistically on par. Inoculation with bacterial strains significantly improved the fresh weight of seedlings. Highest seedling fresh weight was observed in seedlings inoculated with MRD-17 followed by MKS-6 and MCL-1.

Table.1 Bacterial isolates used for the study

Sl. No.	Bacterial isolates
1	MRD-17
2	MMS-5
3	MKS-6
4	NAD-7
5	NSRSSS-1
6	MCL-1
7	MKS-1

Table.2 Effect of osmotic stress on bacterial isolates growth

Sl. No.	PGPB	Growth obtained (in O.D. at 660 nm)		
		Control ²	30 % PEG 6000	40 % PEG 6000
1	MRD-17	2.135	0.893	0.307
2	MMS-5	1.494	0.590	0.152
3	MKS-6	1.878	0.711	0.271
4	NAD-7	1.942	0.658	0.292
5	NSRSSS-1	2.120	1.103	0.353
6	MCL-1	1.496	0.466	0.111
7	MKS-1	1.402	0.430	0.065
SE (m) ±		0.084	0.047	0.021
CD @ 5%		0.257	0.144	0.063

Values are means of three replications

¹Observations at 48 h after incubation at 28 ± 2°C in 200 rpm orbital shaker.

²Nutrient broth was used for the growth of bacteria

Table.3 Effect of bacterial inoculation and osmotic stress on seed germination

Sl. No	Treatments	Control		Osmotic stress ²	
		Germination percentage	Fresh weight (mg) seedling ⁻¹	Germination percentage	Fresh weight (mg) seedling ⁻¹
1	Uninoculated	88.89	12.24	31.11	5.18
2	MRD-17	95.56	18.04	75.56	11.38
3	MMS-5	97.78	14.47	62.22	8.56
4	MKS-6	93.33	17.13	73.33	10.29
5	NAD-7	86.67	15.18	64.44	8.71
6	NSRSSH-1	86.67	14.64	55.56	7.36
7	MCL-1	95.56	16.02	51.11	6.89
8	MKS-1	88.89	13.98	53.33	7.64
SE (m) ±		4.158	1.075	4.303	0.949
CD @ 5%		N/A	3.251	13.011	2.870

Values are means of three replications.

¹Observations were taken at 48 h of germination

²Osmotic stress was induced by supplementing 0.8% agar added with 20% PEG 6000

Table.4 Anova for various seedling characteristics as the effect of osmotolerant bacteria inoculation under control and osmotic stress condition

Sl. No	Treatments	Factor (A)		Factor (B)		Factor (A X B)	
		SE (m) ±	CD @ 5%	SE (m) ±	CD @ 5%	SE (m) ±	CD @ 5%
1	Seedling length	0.102	0.317	0.125	0.388	0.176	0.549
2	Seedling vigour	12.101	37.699	14.820	46.171	20.959	65.296

Table.5 Effect of inoculation with rhizobacteria and osmotic stress on early stages of plant growth under sterile condition

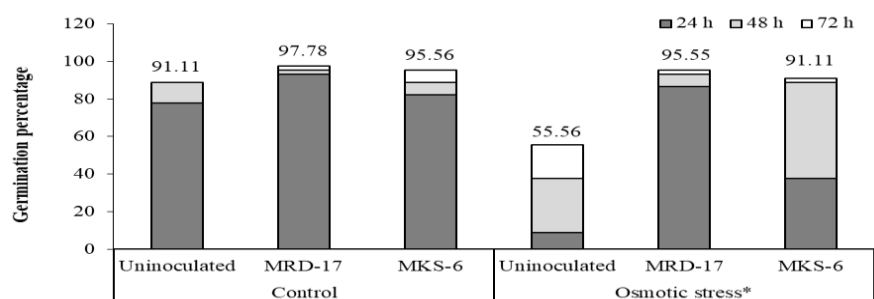
Treatments		Shoot dry weight (mg plant ⁻¹)				Root dry weight (mg plant ⁻¹)			
		15 DAS		30 DAS		15 DAS		30 DAS	
Control (Field capacity)	Uninoculated	11.33	64.28	6.97	19.48				
	MRD-17	17.96	170.41	25.59	49.31				
	MKS-6	19.1	127.84	16.91	43.23				
Water deficit stress (50 % Filed capacity)	Uninoculated	4.99	28.75	4.49	5.83				
	MRD-17	11.01	87.26	8.06	22.2				
	MKS-6	9	70.35	8.73	18.37				
		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Factor (Water deficit Stress)		0.220	0.685	1.320	4.112	0.379	1.181	0.566	1.764
Factor (Inoculation)		0.269	0.839	1.617	5.037	0.464	1.446	0.693	2.160
Factor (water deficit stress X Inoculation)		0.381	1.187	2.286	7.123	0.656	2.045	0.981	3.055

Values are means of three replications

Table.6 Effect of inoculation with rhizobacteria and osmotic stress on early stages of plant growth under unsterile condition

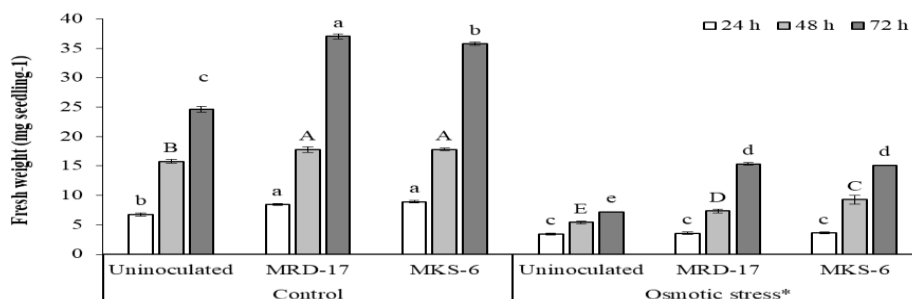
Treatments		Shoot dry weight (mg plant ⁻¹)				Root dry weight (mg plant ⁻¹)			
		15 DAS		30 DAS		15 DAS		30 DAS	
Control (Field capacity)	Uninoculated	6.37	43.7	4.54	8.06				
	MRD-17	11.38	63.23	9.06	15.12				
	MKS-6	9.31	57.13	8.35	14.36				
Water deficit stress (50 % Field capacity)	Uninoculated	4.88	30.95	3.28	6.55				
	MRD-17	8.75	51.72	5.1	11.68				
	MKS-6	7.88	48.16	5.04	13.39				
		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
Factor (Water deficit Stress)		0.130	0.404	0.481	1.500	0.106	0.331	0.081	0.253
Factor (Inoculation)		0.159	0.494	0.590	1.837	0.130	0.405	0.099	0.310
Factor (water deficit stress X Inoculation)		0.224	0.699	0.834	N/A	0.184	0.573	0.141	0.438
Values are means of three replications.									

Fig.1 Effect of selected bacterial isolates on germination percentage at 24, 48 and 72 h under control and osmotic stress condition



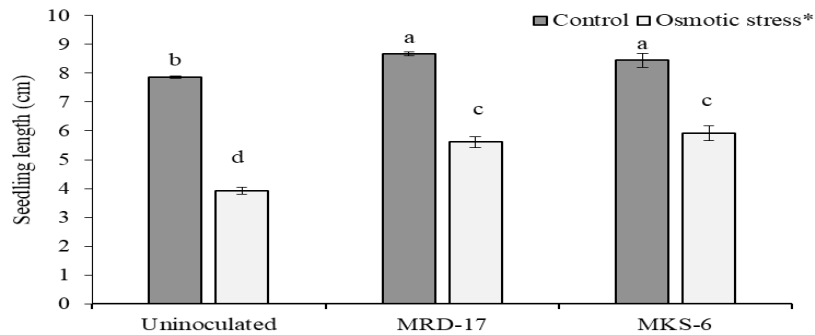
Values are the means of 3 replications and numbers in end of the columns indicate the germination percentage of each treatment at 72 h. *Osmotic stress induced by 20% PEG 6000

Fig.2 Effect of selected bacterial isolates on fresh weight of seedlings under osmotic stress condition at 24, 48 and 72 h of germination



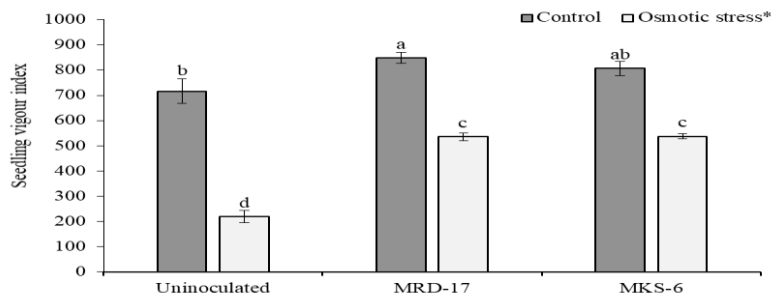
Values are means of three replications. Error bars represent SE. Data followed by different letters are significantly different at the specified time at P < 0.05. *Osmotic stress induced by 20% PEG 6000

Fig.3 Effect of inoculation with the osmotolerant bacterial isolates on the length of seedlings at 72 h under osmotic stress condition



Values are means of three set of 15 replications. Error bars represent SE. Data followed by different letters are significantly different at $P < 0.05$. *Osmotic stress induced by 20% PEG 6000

Fig.4 Effect of inoculation with the osmotolerant bacterial isolates on the length of seedlings at 72 h under osmotic stress condition



Values are means of three replications. Error bars represent SE. Data followed by different letters are significantly different at $P < 0.05$. *Osmotic stress induced by 20% PEG 6000

Osmotic stress drastically reduced the percent germination and fresh weight of seedlings. Inoculation with rhizobacteria enhanced the seed germination percentage (1.65 to 2.42-fold) and seedling fresh weight (1.33 to 2.20-fold) under osmotic stress condition. Highest enhancements were observed in seedlings inoculated with MRD-17 followed by MKS-6. Similar observations were also recorded by Delshadi *et al.*, (2017). Germination rate and vigour was improved in *Onobrychis sativa* upon inoculation with *Azotobacter* and *Pseudomonas* but there was no effect on germination percentage. In our study, even though NSRSSS-1 was more osmotolerant, its seed germination promoting potential was less compared to MRD-17 and MKS-6. Hence,

bacterial strains MKS-6 and MRD-17 were selected for further experiments.

Selected bacterial strains MRD-17 and MKS-6 were further tested for their ability to enhance seedling vigour in petri plates (*invitro*) and plant growth (*invivo*) under water deficit stress condition under pot experiment. Observations were subjected to two-way anova and analysed (Table 4). Inoculation with MRD-17 and MKS-6 led to enhancement in seedling length (Fig 1) under control (one-fold) as well as osmotic stress condition (1.4 to 1.5-fold). Even though the values of seedlings inoculated with both the osmotolerant bacteria were statistically on par, MRD-17 recorded highest seedling

length under control condition and MKS-6 recorded under osmotic stress condition. Significant enhancement in seedling vigour index was observed in seedlings inoculated with these osmotolerant bacteria (Fig 2). Nearly one-fold increase under control and 2.5-fold increase under osmotic stress condition was observed in seedling vigour index due to inoculation.

Many reports available on the effect of inoculation with rhizobacteria on the improvement of seed germination under osmotic stress condition in many crops. Seed priming with plant growth-promoting microbial inoculants significantly promoted the seed germination and fresh weight under moisture stress (Kalita *et al.*, 2015). Nezarat and Gholami (2009) also observed similar significant enhancement in seed germination and seedling vigour of maize due to the inoculation with PGPR cultures *Pseudomonas putida*, *P. fluorescence*, *Azospirillum lipoferum* and *A. brasilense*. Inoculation with *Pseudomonas aeruginosa* strain BHUPSB02, *Bacillus subtilis* strain BHUPSB13, *P. putida* strain BHUPSB04, *Bacillus boronophilus* strain BHUPSB19 and *Paenibacillus polymyxa* strain BHUPSB17 were reported to increase the length and dry weight of 21 days old seedlings, even though there was no significant improvement in germination percentage (Yadav *et al.*, 2010).

Potential of MKS-6 and MRD-17 in the alleviation of water deficit stress of mustard plants were tested in pot experiments under sterile and unsterile condition. Under both sterile and unsterile conditions, significant increase in shoot and root dry weight was observed due to inoculation with MRD-17 followed by MKS-6 under control and osmotic stress condition. Zhu *et al.*, (2014) also confirmed the influence of plant growth promoting rhizobacteria in increasing the shoot and root dry weight, shoot morphology,

biomass, water use efficiency and photosynthetic rate in wheat under water deficit stress condition. Significant enhancement in plant height, biomass and relative water content was observed under drought stress conditions in jujube plants inoculated with *Pseudomonas lini* and *Serratia plymuthica* (Zhang *et al.*, 2020).

Influence of inoculation was clearly observed in sterile condition compared to unsterile condition. Under sterile condition (Table 5), nearly 36-72% increase at 15 DAS and 50-62% increase at 30 DAS was observed in shoot and root dry weight due to inoculation under control condition. Similarly, inoculation led to 45-55% increase at 15 DAS and 59-73% increase at 30 DAS in shoot and root dry weight under drought stress condition. Under unsterile condition (Table 6), 24-44% enhancement in shoot and 44-50% increase in root dry weight were observed in seedlings inoculated with MRD-17 and MKS-6 during 15th and 30 DAS in control condition. Inoculation with osmotolerant bacterial strains MRD-17 and MKS-6 led to significant enhancement up to 36-44% in shoot and 35-51% in root dry weight under water deficit stress condition. In unsterile soil, the positive impact on plant growth was observed to be less prominent than sterile soil. It may be due to the competition given by native soil microbes to bioinoculants. Oats inoculated with plant growth promoting *Bacillus* strains also reported similar results (Hashmi *et al.*, 2019). Higher benefits of inoculation in terms of root biomass and shoot biomass were reduced in wheat and Amaranthus under sterile condition as compared to unsterile soil (Nair *et al.*, 2007; Mahmood *et al.*, 2014).

Rhizobacteria are known to improve water use efficiency of inoculated plants under abiotic stress conditions (Mayak *et al.*, 2004). The present investigation confirmed the effect

of bacterial strains MKS-6 and MRD-17 in imparting drought stress tolerance in mustard. These strains have the potential to be developed into bio inoculants for imparting drought stress tolerance to mustard.

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