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Interactive effect of rhizobacteria and drought stress on physiological attributes of mustard

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ABSTRACT

Drought stress is a major abiotic stress in mustard and its effects can be negated by the application of soil microbes. Screening of rhizobacteria for their drought stress alleviation led to the selection of two isolates namely NAD-7 and MKS-6. The experiment was conducted at ICAR-IARI during 2019-20. Plants inoculated with these microbes exhibited superior performance in terms of biomass and yield. The selected isolates were further evaluated for their effect on physiological and biochemical attributes during vegetative and reproductive stages. The stress was imposed during 35 (vegetative) and 50 DAS (reproductive) for 15 days duration and left for recovery. The drought stress exhibited significant reduction in RWC (23%), MSI (16%) and total chlorophyll content (27%) as compared to irrigated plant during vegetative stage. Similar trend was observed during reproductive stage too. Inoculation with isolates NAD-7 and MKS-6 attenuated the harmful effects of stress as shown by the improved RWC (78%), MSI (7%) and total chlorophyll (28-32,%, respectively) in contrast to uninoculated plants. Results showed that imposed stress significantly affected the parameters during both stages, but rhizobacterial inoculations attenuated the harmful effects of stress. Similar improvements were also recorded during recovery for both stages. These findings show that isolates NAD-7 and MKS-6 can improve plant physiological attributes and help plant overcome the deleterious effects of water deficit stress.

Keywords: Chlorophyll content, Growth stages, Osmotolerant bacteria, Water deficit stress

Drought is one of the predominant abiotic stresses affecting plant growth and productivity, especially in arid and semiarid regions across the globe. Drought stress led to yield losses of more than 50% of many agricultural crops worldwide (Wang *et al*. 2003). Of the total land area, 64% of the land is affected by drought and this may have severe consequences on productivity of crops (Meena *et al*. 2017). Drought stress has severe impact on plant's physiology such as growth, membrane stability, chlorophyll content and water status of plants. The detrimental effects on membrane integrity and chlorophyll content of leaf are associated with the accumulation of reactive oxygen species (Khan *et al*. 2019). Many approaches are being explored to alleviate the deleterious effect of drought on plants. Among these, exploration of microorganisms, particularly, plant growth promoting rhizobacteria (PGPRs) is considered one of the most efficient, cost-effective and eco-friendly approach to cope with effect of drought stress on plants (Paul *et al*. 2017). Studies have indicated the potential of PGPRs in improving drought stress tolerance in many agricultural crops (Tiwari *et al*. 2016, Khan and Bano 2019). They have reported that inoculation significantly improved plant water status, membrane integrity, root and shoot biomass over uninoculated control plants in presence of water deficit stress. Inoculation with *Burkholderia phytofirmans* PsJN resulted in improved root and shoot growth, chlorophyll content, relative water content and membrane integrity which consequently enhanced yield of wheat under drought stress condition (Naveed *et al*. 2014). It was also observed in a study by Danish *et al*. (2020) that inoculation with PGPR significantly improved photosynthetic pigment and biomass of maize exposed to drought stress.

Hence in the present investigation, we screened osmotolerant rhizobacteria for their effect on growth and yield of mustard under water deficit stress conditions. The osmotolerant rhizobacteria were further evaluated for their effect on physiological responses and chlorophyll content during vegetative and reproductive stages of plant growth in mustard subjected to water deficit stress.

MATERIALS AND METHODS

Bacterial cultures and growth conditions: Eleven osmotolerant rhizobacterial cultures obtained from the

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germplasm of Division of Microbiology, ICAR-IARI, New Delhi were used for the present study. The cultures were grown on nutrient agar slants. The stock cultures were maintained on agar slants and refrigerated at 4ºC. Tubes containing nutrient broth were inoculated with rhizobacterial cultures, and incubated at 28±2ºC in an orbital shaker for 48 h.

Screening for water deficit stress alleviation in mustard: The screening of all eleven rhizobacterial isolates for water deficit stress alleviation was carried out in drought sensitive mustard (*Brassica juncea* L.) var. Pusa Karishma LES-39 (Chauhan *et al*. 2011) under controlled condition of National Phytotron facility, ICAR-IARI, New Delhi (June to September 2019). Prior to sowing, mustard seeds were kept for priming with 48 hr old rhizobacterial cultures for 1 hr. The seeds were sown in plastic pots filled with unsterilized soil. Water deficit stress was imposed 30 days after sowing (DAS) for 30 days. The uninoculated plants exposed to field capacity (FC) served as absolute control, and the uninoculated plants exposed to 50% FC served as control. The inoculated plants were maintained at 50% FC. Following stress treatment, plants were maintained at field capacity till harvest. Sampling in triplicates for shoot, root fresh and dry weight was carried out at 60 DAS. At the time of harvest yield data was recorded in triplicate. Based on the parameters studied, two best performing bacterial isolates were selected for further studies.

Pot experiment in net house: To evaluate the effect of the selected isolates on the physiological attributes and chlorophyll content under water deficit stress, a pot experiment was carried out at ICAR-IARI, New Delhi (October 2019 to January 2020). Mustard seeds were inoculated with 48 h old rhizobacterial strains NAD 7 and MKS 6 (~10^7 CFU/mL) before sowing for 1 h. The seeds were sown in earthen pots filled with unsterilized soil. Plants were maintained at two moisture levels, field capacity (irrigated, 100% FC) and short-term water deficit stress (Water deficit stress, 50% FC). In the experiment, one set of plants was exposed to short duration stress of 15 days during the vegetative stage (35 DAS), while the other set of plants was exposed to short duration stress of 15 days during the reproductive stage (50 DAS) of the crop. Following stress treatment, plants were irrigated till harvest. Sampling in triplicates for relative water content (RWC), membrane stability index (MSI) and total chlorophyll content were carried out after 5 days of stress (5 DOS), 15 days of stress (15 DOS) and 5 days after rewatering (5 DAR) during the above mentioned growth stages of the crop.

Physiologcal attributes: Relative water content was measured by the method of Barrs and Weatherly (1962). Membrane stability index was estimated according to the method of Sairam (1994). Total chlorophyll content was determined by the method of Hiscox and Israelstam (1979). Leaf discs were and 50 mg were transferred to test tubes containing 5 ml Dimethyl Sulphoxide and incubated for 4 h. The absorbance of the extract was taken at 645 and 663 nm using spectrophometer against DMSO blank.

Agronomic traits: Data on root and shoot dry weight was recorded at the end of stress imposition. Yield was taken at harvestable maturity and data was recorded.

Statistical analysis: The statistical analysis was done by Analysis of variance (ANOVA) and Duncan multiple range test (DMRT) using the agricolae package (Mendiburu 2016) of RStudio 1.0.141 (RStudio Team 2015).

RESULTS AND DISCUSSION

Screening of osmotolerant rhizobacteria for stress alleviation: Eleven osmotolerant rhizobacterial isolates were screened for drought stress alleviation in mustard. In this study all the rhizobacterial isolates were evaluated for their effect on growth and yield of mustard under drought stress condition.

Root and shoot biomass at stress: Root and shoot biomass was significantly reduced on exposure to water deficit stress (Table 1). Significant improvement in shoot fresh weight was recorded in all inoculated plants. The highest shoot fresh and dry weight was recorded in NAD-7 inoculated plant (Table 1). Inoculation with NAD-7 isolate also led to similar enhancement in root fresh and dry weight (Table 1). Maximum root fresh and dry weight was recorded due to inoculation with isolate NAD-7. Root and shoot biomasses are considered as important selection traits for plant drought tolerance. In fact, significant positive correlations between plant biomass and improved drought stress tolerance have been depicted in several studies (Gontia-Mishra *et al*. 2016, Manjunatha *et al*. 2017). The

Table 1 Effect of the selected osmotolerant rhizobacteria on plant growth and yield under water deficit stress conditions

Treatment*	Shoot FW (g)	Root FW (mg)	Shoot DW (g)	Root DW (mg)	Yield (g)
100% FC**	12.09	266.67	1.61	92.00	2.25
50% FC	3.07	39.00	0.30	19.33	1.61
MKS-1	7.00	105.00	0.70	40.67	1.68
MMS-3	5.41	76.00	0.47	22.00	1.64
MMS-5	7.57	106.00	0.68	38.33	1.82
MCL-1	5.74	111.33	0.53	40.33	1.65
KPSR-2	8.46	126.33	0.79	40.33	1.85
MKS-6	9.85	178.67	0.89	61.67	2.14
NAD-7	10.44	219.67	1.03	69.67	2.20
MKR-2	8.58	130.00	0.77	51.33	1.99
NSRSSS-1	9.09	131.67	0.88	52.00	2.07
NAAR-1	7.19	89.67	0.70	42.33	1.73
MRD-17	9.41	117.67	0.84	40.33	2.10
SE (m)±	0.654	17.741	0.089	6.701	0.114
CD (P =0.05)	1.911	51.859	0.259	19.588	0.332

* In all the treatments soil was maintained at 50% field capacity (FC) except the absolute control, **Absolute control without inoculum treatment

present investigation reveals that the root and shoot biomass was severely affected in presence of water deficit stress. But, rhizobacterial inoculations led to significant enhancement in the plant biomass over uninoculated plants under water deficit stress conditions.

Response on yield: Water scarcity has severe impact on plant growth and development, which eventually affects yield. The yield obtained due to inoculation with isolates MKS-6, NAD-7, MKR-2, NSRSSS-1 and MRD-17 were statistically at par with absolute control with the highest value in isolate NAD-7 inoculated plant (Table 1). According to our observations, isolates NAD-7 and MKS-6 performed better in all the parameters studied under screening. Hence, these two isolates were used for further studies.

Effect of rhizobacteria on plant physiological responses under water deficit stress: The effect of inoculation on plant physiological parameters was assessed under water deficit stress during vegetative and reproductive stages of growth. Leaf relative water content (RWC) and membrane stability index (MSI) are considered as important traits to assess plant drought tolerance (Farag *et al*. 2019). RWC of leaf was significantly reduced in uninoculated plants exposed to 5 days (13%) and 15 days (23%) of water deficit stress (Fig 1A and B) as compared to their corresponding irrigated control during both growth stages. Inoculation with isolates NAD-7 and MKS-6 significantly increased RWC under 15 DOS (7 and 8%, respectively). But, there was no beneficial effect of inoculation on RWC under 5 DOS during both the growth stages. Studies have indicated that higher level of leaf RWC corresponds to increase in drought tolerance (Dash *et al*. 2017, Bangar *et al*. 2019). Tiwari *et al*. (2016) showed that drought stress significantly reduced relative water content in chickpea. However, inoculation with rhizobacteria *Pseudomonas putida* MTCC5279 significantly improved RWC indicating that rhizobacteria help to maintain better plant water status. These findings are in agreement with our results.

During vegetative stage, membrane stability index (MSI) was significantly reduced when the uninoculated plants were exposed to water deficit stress (Fig 1C). Significant improvement in MSI was recorded in only plants inoculated with isolate MKS-6 under 5 DOS (7%), however, under 15 DOS, significant improvement was observed due to inoculation with both the isolates NAD-7 and MKS-6 (7 and 7%, respectively). Under reproductive stage, significant increase in MSI was recorded in both the inoculated plants during 5 DOS (Fig 1D). But, under 15 DOS, MSI obtained in inoculated plants were at par with uninoculated plants. In both the growth stages, recovery of MSI was achieved in uninoculated as well as inoculated plants upon rewatering. Bangar *et al*. (2019) reported that plants with high MSI were correlated with better drought tolerance. The decrease in MSI upon exposure to drought stress is probably due to accumulation of reactive oxygen species, which cause increase in the content of malondialdehyde and electrolyte leakage (Bandeppa *et al*. 2019). However, inoculation with rhizobacteria resulted

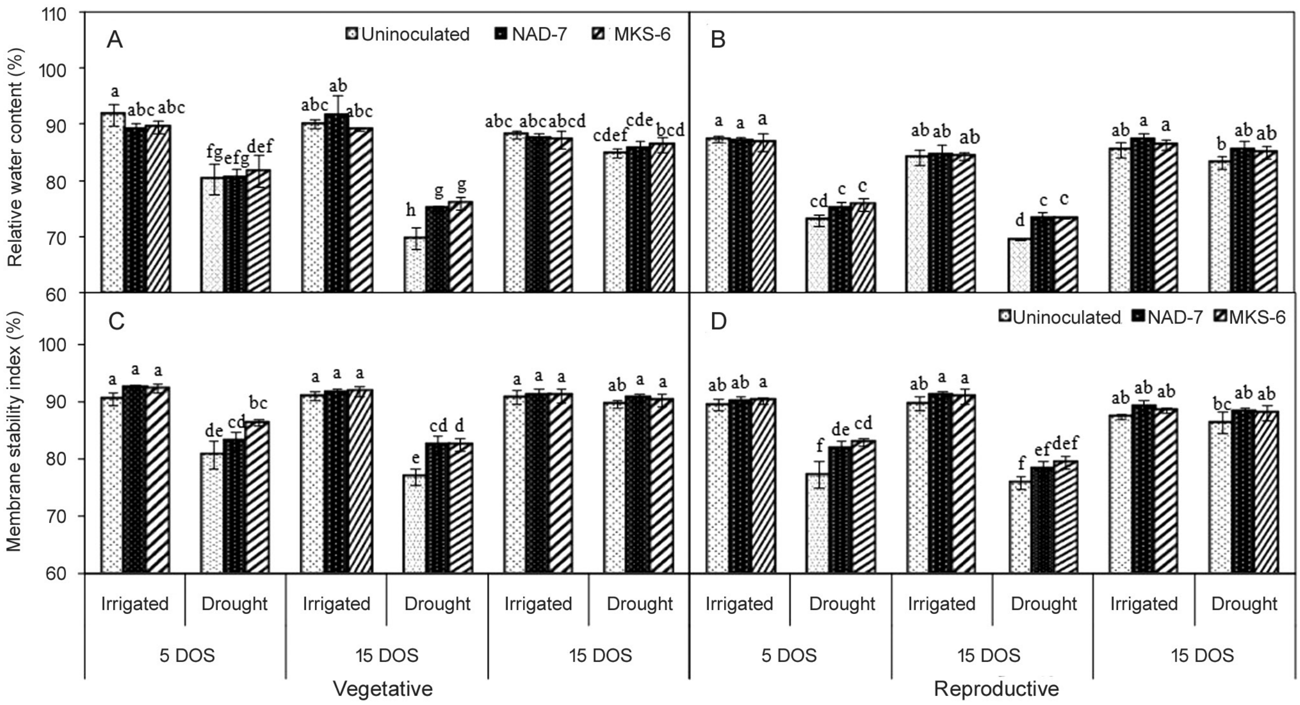

Fig 1 Effect of osmotolerant rhizobacterial inoculation at 5 days of stress (DOS), 15 days of stress (DOS) and 5 days after rewatering (DAR). Relative water content during (A) vegetative stage and (B) reproductive stage ; membrane stability index during (C) vegetative stage and (D) reproductive stage . Data represent mean of three replication. Different letters on the graph show significant difference (P < 0.05) in mean values.

Table 2 Effect of osmotolerant rhizobacterial inoculation on total chlorophyll content

Drought stress	Treatment	Total chlorophyll content (mg g^{-1} DW)					
		Vegetative stage			Reproductive stage		
		5 DOS	15 DOS	5 DAR	5 DOS	15 DOS	5 DAR
Field capacity	Uninoculated control	7.19	6.89	7.49	5.56	6.70	6.21
	NAD 7	9.94	9.78	8.21	7.58	8.01	7.96
	MKS 6	7.96	8.34	7.58	7.73	8.27	6.77
50% field capacity	Uninoculated control	5.52	5.02	8.18	4.34	5.09	7.65
	NAD 7	6.33	7.38	10.71	5.21	6.09	10.00
	MKS 6	6.68	6.99	10.86	6.08	6.18	9.41
SE (m)$\pm$		0.439	0.161	0.274	0.399	0.23	0.238
CD (P =0.05)		1.366	0.503	0.853	1.244	0.715	0.742

DOS- Days of stress; DAR- Days after rewatering

in significant decrease in malondialdehyde content and electrolyte leakage. Thus, it indicated that rhizobacterial inoculation increased cell membrane integrity and helped to develop resistance against drought stress.

Chlorophyll content in plant is considered as key criteria to assess photosynthetic performance under drought stress. There was significant reduction in total chlorophyll content when the uninoculated plants were exposed to 5 days and 15 days of stress in both vegetative (23 and 27%, respectively) and reproductive stages (22 and 24%, respectively) as compared to their corresponding irrigated control (Table 2). In general inoculation led to an increase in total chlorophyll content at both stress and recovery during both stages. In vegetative stage, isolate MKS-6 inoculated plants showed significant increase in total chlorophyll content under both 5 (17%) and 15 DOS (28%) while isolate NAD-7 inoculated plants showed significant improvement only under 15 DOS (32%). In reproductive stage, inoculation led to significant improvement of total chlorophyll content under both 5 and 15 DOS. Following 5 days of rewatering period after termination of water deficit stress, complete recovery of total chlorophyll content was obtained comparable to corresponding irrigated control. Our results are in accordance with the finding of Syamsia *et al*. (2018), where endophytic fungi inoculated maize plants could maintain activity of photosynthetic pigments under water deficit stress, thereby imparting drought tolerance.

Overall, rhizobacterial inoculation resulted in better plant water status, stability of membrane and total chlorophyll content of mustard under water deficit stress. From the present investigation it was found that rhizobacteria modulates the plant physiological and biochemical attributes. This effect ultimately helps the plants to overcome deleterious effects of drought stress and maintaining yield stability.

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