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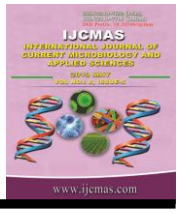

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Plant Growth Promotion of Rice as Influenced by *Ochrobactrum* sp. (MH685438) a Rhizospheric Bacteria Associated with *Oryzae sativa*

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A B S T R A C T

Plants subjected to different environmental stresses, of which drought is a major abiotic stress constraint for crop production. Current study, investigated the adverse effects of drought stress on growth and modulation of root system architecture of rice inoculated with drought-tolerant bacteria *Ochrobactrum* sp. (MH685438). Polyethylene glycol (PEG) solutions of different concentrations (5%, 10%, 15%, 20%, 25% & 30%) were used for drought stress induction artificially. It was observed that growth of bacteria significantly reduced under drought stress, while maximum reduction was caused by PEG (30%) applied. Drought-tolerant bacteria *Ochrobactrum* sp. (MH685438) identified based on 16S rDNA gene sequence was used to study the effect on drought stress tolerance of rice growth. Rice plants inoculated with *Ochrobactrum* sp. (MH685438) showed potential to withstand the drought upto 30 % of PEG, also increased the plant shoot and root length when compared to control. The bacterium also improved the germination percentage of rice seeds at different concentration of PEG 6000. Current investigation concluded that application of *Ochrobactrum* sp. (MH685438) may increase the tolerance capacity of crop plants especially rice against drought.

Introduction

Abiotic stress is a major constrain which can adversely affect the plant growth and productivity. Plants are exposed to number of potentially adverse environmental conditions such as water deficit, high salinity, extreme temperature, and submergence. In response, plants have evolved delicate mechanisms, from the molecular to the physiological level, to adapt to stressful environments. Drought is a situation where water potential and the turgor of plant leaf cells reduces to a level where normal functions are impaired. It causes stomatal closure and limits gas exchange, reduces water content, turgor, water potential and results in wilting of the plant (Shao *et al.*, 2008). Detrimental effects caused by different abiotic stresses are related to disruption of plant water status in one or the other way. The field drought condition can manifest physiological changes similar to other abiotic stresses like high temperature, disturbed ion intake and nutrient deficiencies in plants (Wang *et al.*, 2003). Verslues *et al.*, (2006) have comprehensively described the

physiological changes and stages of the drought response.

Plant growth promoting bacteria (PGPB) can mitigate the impact of abiotic stresses on plants through a process called induced systemic tolerance (IST), which includes bacterial production of cytokinins, production of antioxidants and degradation of the ethylene precursor 1- aminocyclopropane-1-carboxylate (ACC) by bacterial ACC deaminase. Rhizosphere colonizing bacteria were well studied for their role in stress tolerance (Sandhya *et al.,* 2011), but few studies were focused on phyllosphere bacterial amelioration of abiotic and biotic stress in plants.

Rice is the staple food for more than half of the world's population. Evolved in a semi-aquatic, low-radiation habitat, rice exhibits distinct tolerance and susceptibilities to abiotic stresses among domesticated cereal crops (Lafitte *et al.,* 2004). Substantial areas under rice cultivation in the tropics and subtropics are affected by drought. Plant productivity is considerably reduced due to improper nutrition of plants plus the osmotic and drought stress (Munn *et al.,* 1993).Rice is affected by drought stress at each developmental stage in all rice growing ecosystems and the crop responds differently to the drought stress in different life stages (Boonjung and Fukai, 1996).

Plants deploy drought avoidance mechanisms including leaf rolling, stomatal closure, reduced tillering and accumulation of osmoprotectants to prevent severe damage caused due to drought (Hadiarto and Tran, 2011). The effect of vegetative drought stress in upland rice results in reduced tiller number and reduced panicles, whereas drought stress during the reproductive growth phases cause heavy yield losses (Boonjung and Fukai, 1996).

The rhizobacteria assemblages of many agricultural crops have been studied, and the use of PGPR holds promise for plant growth promotion and alleviation of plant drought stress (Mayak *et al.,* 2004; Zahir *et al.,* 2008; Sandhya *et al.,* 2009). However, the drought-tolerant bacteria associated with crop species which are naturally adapted to drought, such as rice, have not been explored.

Ochrobactrum species have been described as free-living Alphaproteobacteria, and have been recovered from diverse habitats, including soil, plants and their rhizospheres, animals, and humans. In soil, Ochrobactrum strains were found to constitute 2% of the cultivable bacteria, and on the wheat rhizoplane this fraction was approximately 0.3% (Lebuhn *et al.,* 2000; Bathe *et al.,* 2004), indicating that Ochrobactrum is a substantial part of the currently cultivable soil and rhizosphere microbial communities. The diazotrophy of Ochrobactrum, strains with complete symbiotic ability in Acacia and Lupinus nodules (Ngom *et al.,* 2004; Trujillo *et al.,* 2005). In addition to its ability to establish symbiotic relationships with legumes, some species of the genus Ochrobactrum, as is the case of *O. antrhopi*, have been described as PGPR. Stenotrophomonas and Ochrobactrum strains have been isolated from various sources, mainly plant rhiospheres and aquatic habitat (Imran *et al.,* 2010; Hanssan *et al.,* 2010). Yousuf *et al.,* (2012) has reported the presence of strains of the Ochrobactrum genus in *A. hypogaea* rhizospheres. In present study, *Ochrobactrum* sp. (MH685438) have been isolated from rice rhizosphere and investigated for its ability to improve the plant growth and to mitigate drought stress on rice. The application of plant-growth-promoting bacteria (PGPB) is an alternative strategy for improving plant fitness under stressful conditions.

Materials and Methods

Bacterial strain and assessment of drought stress tolerance

The rice rhizosphere isolate *Ochrobactrum* sp. (MH685438) was selected for the study. The aforementioned isolate was previously isolated from the rhizosphere of rice and confirmed for its plant growth promoting characteristics.

The drought stress tolerance of selected isolate was tested using polyethylene glycol (PEG). The susceptibility of the selected bacteria in presence of PEG was relatively unknown. 1 mL of the bacterial culture was added to the test-tubes containing 70 mL of nutrient media amended with varying PEG concentrations (0%, 5%, 10%, 15%, 20%, 25% and 30%) for assessing the drought sustaining capacity of the isolate. All test-tubes were incubated on shaker at 28± 2 °C for 5 days. Bacteria growth viability under stress was monitored over the period of 5 days by measuring the optical density at 600nm.

In vitro assessment of plant growth of rice under induced drought stress

The drought tolerant *Ochrobactrum* sp. (MH685438) was used to evaluate the potential in alleviating drought-stress effects in host plant rice (*Oryzae sativa*) as described by Sandhya *et al.*, (2009). Seeds were surface sterilized and colonized with (10^8 cells/g) of drought tolerant Bacillus strain, shade dried and sown in germination sheet (sterilized). *In vitro* screening for drought tolerance was carried out using Polyethylene Glycol (PEG 6000 MW). In germination paper, a horizontal line was drawn at 3 cm from the top and was marked with 25 points at 1 cm intervals. Twenty five seeds were placed in the marked point on the moistened paper towel, ensuring that the seeds do not touch each other and a moistened second paper towel was carefully placed over the seeds. The paper towels along with a polythene sheet below it were then rolled loosely to form a tube and held with rubber band. The rolls were placed in the containers of different PEG concentration. Drought stress was stimulated using different concentrations viz., 5%, 10%, 15%, 20%, 25% and 30% of PEG 6000 respectively in 70 ml of Hoagland's nutrient solution. A control (0.0 bar) was maintained using sterile distilled water. The whole experimental set up was provided with light and dark at 12 h intervals. Water stressed seedlings and their corresponding unstressed controls were observed after 15 days of exposure to drought for germination percentage, root length, shoot length and root/shoot ratio at six different levels of treatment. The experiment was laid in completely randomized design with three replications.

Results and Discussion

Survival efficacy of *Ochrobactrum* sp. (MH685438) in response to induced drought by polyethylene glycol (PEG)

The selected bacterial isolate *Ochrobactrum* sp. (MH685438) was tested for the drought sustaining capacity in the presence of different PEG concentrations (0% to 30%). Based on the growth pattern it was shown that the bacterial isolate *Ochrobactrum* sp. (MH685438) was able to grow up to 30% PEG concentration (-1.32 bars of Osmotic Potential) (Table 1).

Ochrobactrum sp. (MH685438) isolate has higher drought tolerance compared to standard culture (MTCC 453- drought tolerant), which sustains up to the osmotic potential of -1.32 bars pressure (30 % PEG Conc.) and higher growth rate was recorded (0.51 ± 0.007 OD), whereas standard culture

has the potential of tolerating up to 15 % PEG concentrations (0.58 ± 0.007 OD) afterwards the growth was declined. *Ochrobactrum* sp. (MH685438) screened for drought tolerance could tolerate minimal water potential (-1.32 MPa). Similarly, Paulucci *et al.,* (2015), isolated and screened the osmotic stress-tolerant ACC deaminase-producing bacterial *Ochrobactrum pseudogrignonense* RJ12, and their PGP activities were evaluated in black gram and garden pea plants under water deficit conditions. Mishra *et al.,* (2017) reported that NBRISH6 (*Ochrobactrum* sp.) can withstand drought upto 60% and with maximum CFU *in vitro* under abiotic stress (drought) suggests that it may resist fluctuations under natural conditions too and therefore, presumably promote plant growth *in vivo*.

Effect of *Ochrobactrum* sp. (MH685438) on germination percentage, root and shoot length and vigor index of rice seedlings under water stress

Germination of rice seedlings was decreased with the increasing concentration of PEG *viz.*, 0 to 30 %. However, the effect of PEG was greatly reduced in rice seeds treated with bacterial cultures *viz*., *Ochrobactrum* sp. (MH685438) and standard culture (MTCC 453). Among the isolates tested, *Ochrobactrum* sp. (MH685438) greatly enhanced the germination percentage at -1.32 OP (58%) levels of PEG compared to uninoculated control (16%). According to the study of Lum *et al.,* (2014) drought-tolerant variety of paddy, Pulot Wangi tolerated PEG at the highest drought level (-8 bar) and showed no significantly difference relation to control. However, drought-sensitive variety, Kusam was markedly affected even at the lowest drought level used. In the current study we are using rice variety (CO 51) it was tolerated up to -1.32 bars of Osmotic Potential with the aid of *Ochrobactrum* sp. (MH685438) inoculation. Vardharajula *et al.*, (2011) studied the effect of seed inoculation of drought-tolerant *Bacillus* spp. strains on the growth and physiological and biochemical status of the maize seedlings exposed to drought stress. Drought stress drastically affected the growth of maize as reflected by stunted growth, less vigour, and wilting of leaves. However, inoculated plants survived up to 9 days after exposure to drought stress, respectively and started wilting thereafter (Fig. 1).

Effect of *Ochrobactrum* sp. (MH685438) on root length of rice seedlings under water stress

The *Ochrobactrum* sp. (MH685438) inoculated treatment showed significantly higher plant growth in terms of root and shoot length (Table 2 and 3), and vigour index (Table 4) as compared to standard and uninoculated control under both drought stress as well as non stress conditions.

Root length was decreased as the concentration of PEG increased, *viz.*, 0 to 30 %. However, culture treated seeds recorded better root growth than the uninoculated control at all concentrations of PEG. At higher concentration of PEG (30%), *Ochrobactrum* sp. (MH685438**)** treated seeds showed 7.05 cm root length followed by standard strain treated seeds (4.90 cm) whereas uninoculated control showed only 2.0 cm root length (Table 2). It is clear from the data recorded that our isolate *Ochrobactrum* sp. (MH685438) exhibited profound effect on paddy growth under water stress condition. Similarly, Mishra *et al.,* (2017), observed that, *Ochrobactrum* sp. NBRISH6 bacterial treatment to the plant showed 44% increase in Root length, fresh root (97%) and dry root (94%) weight in maize. Yasmin *et al.,* (2013) also reported that under drought stress, maize plants

inoculated with PGPR isolate enhanced root length by 43.3%. Naveed *et al.,* (2014) reported that maize plants inoculated with *Burkholderia phytofirmans* strain PsJN had significantly increased root biomass by 70 and 58 % in Mazurka and Kaleo cultivars respectively.

Effect of *Ochrobactrum* sp. (MH685438) on the shoot length of rice seedlings under water stress

One of the key responses to drought stress is the inhibition of shoot growth, which benefits plants by limiting the leaf area available for evaporative loss of limited water reserves (Skirycz and Inze, 2010). In addition, inhibiting shoot growth allows plants to divert essential solutes from growth requirements to stress-related house-keeping functions, such as osmotic adjustment. Therefore, inhibition of shoot growth is considered an adaptive response that helps plants to tolerate drought stress (Aachard *et al.,* 2006). Hence, inhibition of shoot growth could be a counter-productive response in the case of crop plants exposed to moderate drought stress (Neumann, 2008). However treatment of plants with PGPR typically increases shoot growth, under drought stress and plants inoculated with effective PGPR strains could maintain near-normal shoot growth rates, resulting in increased crop productivity.

Our results also have close relation with the above said hypothesis and the Ochrobactrum treated plants recorded higher shoot length compared to control non stressed plants. This result implies that Ochrobactrum inoculation might counteract or nullify the effect of moisture stress. In this study, under drought stress conditions, the plants inoculated with the tested *Ochrobactrum* sp. (MH685438) showed significantly increased shoot length of (11.05 cm) when compared to standard strain (6.90 cm) and control **(**2.0 cm**)**. Similarly, Mishra *et al.,* (2017) reported that, *Ochrobactrum* sp. NBRISH6 treatment to the maize plant showed 56% increase in shoot length, shoot fresh (144%) and dry weight (157%). Timmusk *et al.,* (2014) showed that under drought stress, wheat plants treated with PGPR had 78% higher biomass than non-treated plants, confirming the potential of PGPR to enhance plant performance under drought stress.

Effect of *Ochrobactrum* sp. (MH685438) on the vigor index of rice seedlings under water stress

Vigor index is the most one of the important traits pertaining to seed quality and seedling establishment in the field to be closely related to the vigor index assessed in lab scale experiments. Various studies proved that vigor index was improved by microbial inoculation. According to Sariah *et al.,* (2011) bacterization of rice seeds with *E. gergoviae* and *B. amyloliquefaciens* gave significantly high seed vigor index of 247.60 and 237.84, respectively.

Table.1 Growth rate of *Ochrobactrum* sp. (MH685438) with increasing concentrations of PEG 6000 (OD at 600 nm)

Bacterial strain	0%	5%	10%	15%	20%	25%	30%
***Ochrobactrum* sp. (MH685438)**	1.35 (0.028)	1.15 (0.012)	1.04 (0.008)	0.72 (0.004)	0.75 (0.014)	0.68 (0.004)	0.51 (0.007)
***Bacillus megaterium* (MTCC 453)**	1.10 (0.016)	0.96 (0.006)	0.69 (0.004)	0.58 (0.007)	0.08 (1.02)	0.15 (0.001)	0.01 (0.001)

Values in the parenthesis are mean ± standard error of seven replicates. PEG

Table.2 Effect of *Ochrobactrum* sp. (MH685438) on the root length of rice seedlings under induced drought

PEG Conc.	Root length (cm)		
	L1	L2	L3
T_1- 0 % PEG	15.50	13.90	9.50
T_2 - 5 % PEG	14.30	12.50	8.50
T_3 - 10 % PEG	11.85	9.10	7.80
T_4 - 15 % PEG	12.00	11.70	7.05
T_5 - 20 % PEG	11.95	8.20	5.75
T_6 - 25 % PEG	9.00	6.05	3.05
T_7 - 30% PEG	7.05	4.90	2.0
SEd	1.094	1.273	1.060
C.D (0.05)	0.019	0.018	0.190

L1 - *Ochrobactrum* sp. (MH685438)., L2 - Standard (MTCC 453), L3- Control

Table.3 Effect of *Ochrobactrum* sp. (MH685438) on the shoot length of rice seedlings under induced drought

PEG Conc.	Shoot length (cm)		
	L1	L2	L3
T_1- 0 % PEG	26.85	22.65	12.70
T_2 - 5 % PEG	26.00	18.95	11.40
T_3 - 10 % PEG	24.95	15.85	10.75
T_4 - 15 % PEG	22.85	13.85	9.30
T_5 - 20 % PEG	18.60	12.55	8.95
T_6 - 25 % PEG	14.05	10.00	6.05
T_7 - 30% PEG	11.05	6.90	2.0
SEd	2.342	2.013	1.379
C.D(0.05)	0.017	0.016	0.068

L1 - *Ochrobactrum* sp. (MH685438), L2 - Standard (MTCC 453), L3- Control

Fig.1 Effect of *Ochrobactrum* sp. (MH685438) on germination percentage of rice seedlings under water stress condition

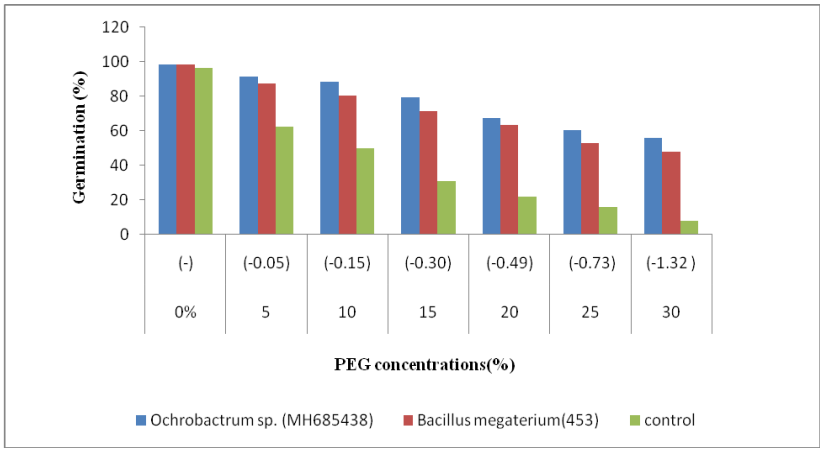
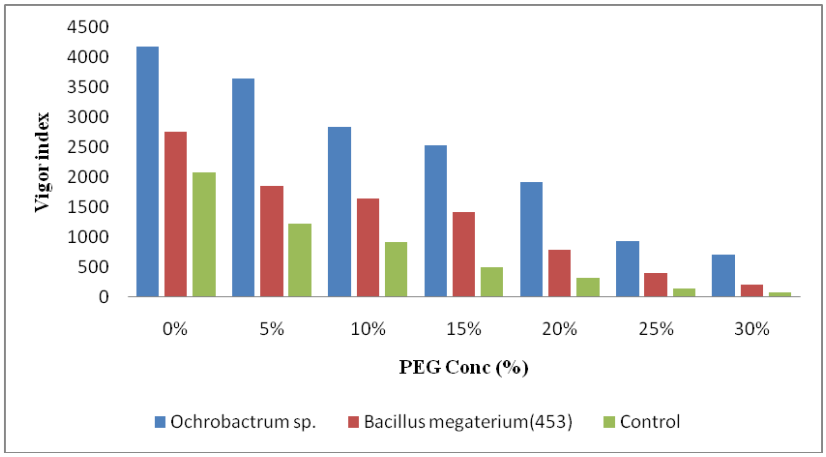


Fig.2 Effect of *Ochrobactrum* sp. (MH685438) on the vigor index of rice seedlings under induced drought



In the present investigation vigor index of rice under moisture stress condition was improved by *Ochrobactrum* sp. (MH685438) inoculation (4171 to 708 from 0 - 30% PEG) compared to standard strain (2755 to 204 from 0 – 30% PEG) as well as control (2086 to 85 from 0 - 30% PEG) (Fig. 2). Vigor index were declined with increasing PEG concentrations irrespective of the treatments, however *Ochrobactrum* sp. (MH685438) inoculation improves better compared to control. Batool *et al.*, (2014) compared the two varieties of maize in response to the water stress condition and the results indicated that seeds treated with diazotrophic bacteria, *Pseudomonas* spp.

Conclusion

PGPR may help plants tolerate drought stress via the enhancement and alteration of root parameters. From the present investigation, it was concluded that the isolate *Ochrobactrum* sp. (MH685438) has higher drought sustaining capacity and growth promoting activity compared to standard strain under induced moisture stress condition. Hence *Ochrobactrum* sp. (MH685438) proved to have a promising role in improving plant performance under drought condition.

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