

Assess the Role of Exopolysaccharide (EPS) Producing Bacteria in Soil Moisture Stress Alleviation

Devendra Singh¹, Rajendra Prasad Meena², Sukumar Taria³ and Geeta Singh*

¹Division of Microbiology, Indian Agricultural Research Institute

²National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi - 110012, India.

³Division of Plant Physiology, Indian Agricultural Research Institute *Principal Scientist, Division of Microbiology, Indian Agricultural Research Institute, New Delhi - 110012, India.

(Received: 06 August 2014; accepted: 01 October 2014)

A pot experiment was conducted to elucidate the effect of inoculating three exopolysaccharide (EPS) producing bacterial isolates namely *Bacillus cereus*, *Microbacterium resistens* and *Pseudomonas* sp. on the soil physical properties in the rhizosphere of *Triticum aestivum* L. Inoculation of wheat seeds with above three isolates caused a significant increase in the root-adhering soil (RAS) dry mass (dm) per root tissue (RT) dry mass (RAS/RT). The increase recorded due to *Microbacterium resistens* inoculation at -0.55 MPa hydric level after 20 DAS in RAS/RT was 157 % over the uninoculated control. Intense colonization of the wheat rhizosphere by these EPS producing bacteria was also associated with significant increase of mean weight diameter (MWD) and water stable aggregate (>250 μ m). Scanning electron microscopic studies showed the formation of biofilm of inoculated bacteria on the root surface and this, along with a better soil structure, might have protected the plants from the water stress. We demonstrate that EPS-producing bacteria were able to alleviate soils water stress and that EPS-producing bacterial populations play an important role in the rhizosphere through their contribution to soil aggregation.

Key words: Exopolysaccharide. RAS/RT. Mean Weight Diameter (MWD). Water Stable Aggregate (WSA). Scanning Electron Microscope, Biofilm.

Soil moisture stress affects nearly two-thirds area forming parts of the arid and semi arid eco systems. The moisture availability during the brief post emergence period of the crop plant is crucial for the productivity. A lack of rain during that period leads to significant losses in crop production. The role of rhizobacteria in plant growth promotion through direct and indirect mechanisms is well studied. These beneficial microorganisms colonize the rhizosphere/

endorhizosphere of plants. The rhizosphere contributes to soil organic matter directly through the root material itself and its decomposition, and indirectly through root exudation which stimulates microbial activity and biomass (Lynch, 1990; Lynch). Rhizosphere is a zone of intense microbial activity and it regulates the nutrient and moisture availability to crop plants. Microbial biomass, water-extractable polysaccharide and mean weight diameter of water-stable aggregates were significantly higher in root-adhering silty top soil than in its bulk soil counterpart (Haynes and Francis 1993). The water potential of rhizosphere soil is also a key physical parameter which determines (i) the availability of water, oxygen and substrates for plants and microorganisms, (ii) survival and activity of microorganisms (van Gestel

* To whom all correspondence should be addressed.
E-mail: geetasingh63@yahoo.co.in;
geetasinghkartik@gmail.com

et al., 1993). In turn, bacterial exopolysaccharides (EPS) can protect bacteria from hydric stresses (Roberson and Firestone, 1992). Soil structure plays a critical role in processes influencing crop yield. Soil aggregates are the basic units that determine the mechanical and physical properties of soil such as retention and movement of water, solid material and pores. The aggregates aeration, and temperature. Aggregate formation is depends on both the quality and the quantity of organic matter (Chaney and Swift 1984, Chenu, 1993). Experimental evidences clearly indicate the role of microbial EPS on soil aggregation. The amendment of soil with microbial EPS results in an increased soil aggregation (Lynch and Bragg, 1985). The influence of EPS-producing rhizobacteria, on the aggregation of root-adhering soil (RAS) especially under different levels of soil moisture is less studied (Watt et al., 1993). An understanding of the influence of EPS producing rhizobacteria on RAS aggregation is important because RAS forms the immediate environment where plants take up water and nutrients for their growth. Factors liable to change the physical properties of RAS can be expected to modify absorption of water and minerals by plants. Gouzou and coworkers, 1993 showed that inoculation of wheat with *Paenibacillus polymyxa* in a silty topsoil increased the RAS mass-to-root tissue (RT) mass ratio (RAS/RT ratio) by 57%. The EPS produced by *P. polymyxa* was implicated in the aggregation of RAS on wheat (Bezzate, 2000). The same effect on wheat was observed after inoculation with *Pantoea agglomerans*, indicating the importance of bacterial activity in the regulation of water content of the rhizosphere by improving the soil aggregation (Amellal, 1998). The purpose of this study was to determine the influence of inoculation with three different rhizobacteria selected for their EPS production on soil physical properties namely water stable aggregation, mean weight diameter of wheat RAS and its consequences on plant water status, under three different levels of soil water conditions.

MATERIALS AND METHODS

Wheat seed inoculation and sowing

The three bacterial cultures namely *Bacillus cereus*, *Micrococcus resistens* and

Pseudomonas sp. were grown in nutrient broth for 24 hr. Before inoculation of seeds, seeds were coated with carboxy methyl cellulose (CMC), 300 Seeds were taken in small poly bag and mixed with 1ml of 0.1% CMC solution. After 10 minute seeds were treated with bacterial culture separately. The control treatment was also coated with 0.1% CMC. Treated 15 seeds were sown 20 mm below the soil surface in plastic cylindrical pots of 12.5 cm diameter and 30 cm height, filled with 10 kg soil. After the field capacity of the soil was calculated, watering was done on the basis of moisture level of the soil to achieve three moisture levels viz. - 0.55 MPa, -0.20 MPa, -0.05 MPa. Gravimetric water content of the soil in each pot was adjusted after 5-6 days interval by spraying on the soil surface in each pot on the basis of moisture level (-0.55, -0.22 and -0.05 MPa).

Physical analysis RAS/RT ratio

The roots together with the adhering soil, which was subsequently called as the adhering soil: root tissue (RAS/RT) was taken from the pots 20 days and 40 days after sowing and 16 hrs after the last water addition. They were carefully separated from soil (plate 1,2,3). Adhering soil was separated from each root system by dipping in water and after it was filtered by filter paper no.1, before filtering, filter paper weights were taken. Soil with filter paper was oven dry at 105 degree centigrade temperature for 24 hrs. Roots were also oven dried at 105 degree centigrade temperature for 24 hrs to estimate the dry biomass.

Physical analysis of WSA and MWD

The aggregate size distributions was analysed by gently passing air-dried soil fraction through a set of sieves of sizes 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm and 0.1mm. The fractions of aggregates of mean diameter of 4 mm, 2 mm and 0.5 mm were weighed and their proportions to the whole sample of aggregates was calculated (Kemper and Koch, 1966). The results were expressed as Mean Weight Diameter (MWD) and water stable aggregate (WSA).

Colonization and Biofilm formation by *Pseudomonas* sp. Iso-13

After harvesting (40DAS), the root samples were fixed in 2.5% Gluteraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4°C. After the fixation, samples were dehydrated with acetone and mounted over the stubs with double-sided

conductivity tape. A thin layer of gold metal was applied over the samples using an automated sputter coater (JEOL JFC-1600). The samples were scanned under scanning electron microscope at various magnifications (Entomology Lab, IARI New Delhi, 2013).

RESULTS

Effect of inoculation on RAS/RT Ratio

There was a significant improvement in RAS/RT ratio due to bacterial inoculation at -0.55 MPa and -0.20 MP soil moisture level at 20 DAS interval. At -0.55 MPa moisture stress, *Microbacterium resistens* performed the best as it significantly improved (157.1%) RAS/RT over the control and other two inoculants tested namely *Bacillus cereus* and *Pseudomonas* sp. (table 1)

At -0.05 MPa normal moisture level (75% field capacity), inoculation with *Pseudomonas* sp. only significantly improved the RAS/RT ratio and rest all two bacterial cultures brought an insignificant increase the RAS/RT. At -0.55 MPa and -0.20 MPa moisture stress level the entire three bacterial cultures significantly increased RAS/RT ratio. Bacterial inoculation has a significant influence on RAS/RT as compared to uninoculated control in the wheat rhizosphere at -0.55MPa

moisture level. Similar trend was recorded at -0.20 MPa moisture level. However the performance of all the three inoculate was statistically equally at -0.22 MPa moisture stress level.

At -0.05 MPa or normal moisture level, *Bacillus cereus* and *Microbacterium resistens* inoculation resulted in insignificant improvement in the RAS/RT ratio in the wheat rhizosphere. *Pseudomonas* sp. was able to significantly improve (43.86%) the RAS/RT ratio even at -0.05 MPa water potential (normal moisture level). Interestingly this bacteria was isolated from the rhizosphere of wheat crop.

Inoculation with *Microbacterium resistens* was found to very effective under extreme moisture stress condition namely at -0.55 MPa and -0.20 MPa as it resulted in an increased 157.1% and 59.7% respectively. However as the soil moisture level approached normal (-0.05 MPa or 75% field capacity), the effectiveness of this culture in improving RAS/RT ratio was absent. This indicates that *Microbacterium resistens* is able to improve the RAS/RT ratio under moisture stress condition at early crop establishment stages.

At 40 days interval, inoculation with *Microbacterium resistens* maintained its superiority to improve RAS/RT over the other 2 bacterial inoculants namely *Bacillus cereus* and

Table 1. Effect of EPS positive bacterial inoculants on RAS/RT in wheat rhizosphere at different soil moisture levels at 20 DAS

Treatments	Dry mass of RAS (mg)	Dry mass of RT(mg)	RAS/RT (mg/mg)
Control (-0.55 Mpa)	249.75	15.18	16.47
<i>Bacillus cereus</i> (-0.55 MPa)	362.67	16.00	22.66
<i>Microbacterium resistens</i> (-0.55 Mpa)	604.84	14.27	42.35
<i>Pseudomonas</i> sp. (-0.55 Mpa)	430.63	15.13	28.46
CD(p=0.05)	91.95	0.72	5.99
SE(m)	27.76	0.22	1.81
Control (-0.20 Mpa)	719.00	20.31	35.4
<i>Bacillus cereus</i> (-0.20MPa)	1264.37	25.82	49.14
<i>Microbacterium resistens</i> (-0.20 Mpa)	1471.70	25.75	56.53
<i>Pseudomonas</i> sp.(-0.20 Mpa)	1310.79	24.21	54.3
CD (p=0.05)	425.89	N.S.	12.42
SE(m)	128.60	1.33	3.75
Control (-0.05 Mpa)	1477.26	25.17	58.73
<i>Bacillus cereus</i> (-0.05MPa)	1857.91	27.35	67.66
<i>Microbacterium resistens</i> (-0.05 Mpa)	1762.66	27.28	64.61
<i>Pseudomonas</i> sp. (-0.05Mpa)	2286.05	27.08	84.49
CD(P=0.05)	375.35	0.04	14.97
SE(m)	113.34	0.13	4.52

Pseudomonas sp. at maximum stress condition (-0.55MPa). But with a reduction in stress condition (-0.20 MPa and -0.05 MPa) its performance with respect to improving RAS/RT was relatively less than *Pseudomonas* sp. (table 2).

Inoculation with the three bacterial culture namely *Bacillus cereus*, *Microbacterium resistens* and *Pseudomonas* sp. RAS/RT ratio increased by 46.84%, 67.80% 62.77% respectively at maximum moisture stress (-0.55MPa). With a reduction in soil moisture stress from -0.55 to -0.20 MPa, the values of RAS/RT ratio decline to 29.19%, 32.09% and 43.49% in response to *Bacillus cereus*, *Microbacterium resistens* and *Pseudomonas* sp. Respectively. At normal soil moisture levels also

bacterial inoculation was found to significantly improve RAS/RT over the uninoculated control. The magnitude of increase recorded was 30.7%, 36.2% 53.3% over the uninoculated control.

As the magnitude of moisture stress declined, the response to bacterial inoculation also showed a decline in RAS/RT ratio as compared to corresponding control. Bacterial inoculants *Pseudomonas* sp. was significantly better than other two cultures in term of improving the RAS/RT at both 20 DAS and 40 DAS at normal soil moisture level.

Under maximum soil moisture stress (-0.55MPa) *Microbacterium resistens* was most effective at 20 DAS. With the establishment of crop

Table 2. Effect of EPS positive bacterial inoculants on RAS/RT in wheat rhizosphere at different soil moisture levels at 40 DAS

Treatments	Dry mass of RAS (mg)	Dry mass of RT(mg)	RAS/RT (mg/mg)
Control (-0.55 Mpa)	744	24.10	30.87
<i>Bacillus cereus</i> (-0.55MPa)	1210.72	26.47	45.33
<i>Microbacterium resistens</i> (-0.55 Mpa)	1577.81	30.42	51.80
<i>Pseudomonas</i> sp.(-0.55 Mpa)	1547.12	30.80	50.25
CD (P=0.05)	196.00	3.3	2.62
SE(m)	59.28	0.97	0.79
Control (-0.20 Mpa)	3433.68	62.33	55.11
<i>Bacillus cereus</i> (-0.20 MPa)	4962.82	69.69	71.2
<i>Microbacterium resistens</i> (-0.20 Mpa)	5412.71	74.32	72.80
<i>Pseudomonas</i> sp.(-0.20 Mpa)	6184.46	78.22	79.08
CD (P=0.05)	260.22	4.15	2.82
SE(m)	78.58	1.25	0.85
Control (-0.05 Mpa)	4063.39	67.08	60.57
<i>Bacillus cereus</i> (-0.05MPa)	5833.84	73.64	79.22
<i>Microbacterium resistens</i> (-0.05 Mpa)	6159.60	74.66	82.5
<i>Pseudomonas</i> sp.(-0.05 Mpa)	7730.44	83.40	92.83
CD (P=0.05)	362.84	3.68	6.49
SE(m)	109.56	1.11	1.96

Table 3. Effect of EPS positive bacterial inoculants on MWD at different moisture level at 20 DAS

Moisture levels Treatments	-0.55MPa MWD (mm)	-0.20MPa MWD (mm)	-0.05 MPa MWD (mm)	Mean
Control	0.545	0.758	0.73	0.678
<i>Bacillus cereus</i>	0.813	1.259	1.22	1.098
<i>Microbacterium resistens</i>	0.778	1.103	1.074	0.985
<i>Pseudomonas</i> sp.	0.865	1.281	1.237	1.127
Mean	0.75	1.1	1.065	

CD (P=0.05)culture: 0.1332

CD (P=0.05) moisture levels: 0.1153

CD (P=0.05) due to culture and moisture levels: N.S

Table 4. Effect of EPS positive bacterial inoculants on MWD at different moisture level at 20 DAS

Moisture levels Treatments	-0.55MPa MWD (mm)	-0.20MPa MWD (mm)	-0.05 MPa MWD (mm)	Mean
Control	0.55	0.84	0.917	0.769
<i>Bacillus cereus</i>	0.803	1.387	1.353	1.181
<i>Microbacterium resistens</i>	0.81	1.157	1.31	1.092
<i>Pseudomonas sp.</i>	0.87	1.277	1.36	1.069
Mean	0.758	1.65	1.235	

CD (P=0.05)culture: 0.1126

CD (P=0.05)moisture levels: 0.0975

CD (P=0.05)due to culture and moisture levels: N.S.

Table 6. Effect of EPS positive bacterial inoculants on WSA at different moisture level at 40 DAS

Moisture levels Treatments	-0.55MPa	-0.20MPa	-0.05 MPa	Mean
Control	31.753 (34.284)	45.5 (42.385)	63.23 (52.71)	46.83 (43.12)
<i>B. cereus</i>	40.72 (39.53)	71.4 (57.72)	65.93 (54.38)	59.35 (50.54)
<i>Microbacterium resistens</i>	44.78 (41.963)	64.84 (53.65)	62.79 (52.63)	57.46 (49.41)
<i>Pseudomonas sp.</i>	48.647 (44.20)	63.24 (52.71)	69.46 (56.45)	60.45 (51.12)
Mmean	41.47 (39.99)	61.24 (51.61)	65.35 (54.04)	

CD (P=0.05) culture: 4.69

CD (P=0.05) moisture levels: 4.05

CD (P=0.05) due to culture and moisture levels: N.S.

Table 5. Effect of EPS positive bacterial inoculants on WSA at different moisture level at 20 DAS

Moisture levels Treatments	-0.55MPa	-0.20MPa	-0.05 MPa	Mean
Control	31.44 (34.07)	43.26 (41.1)	46.76 (43.127)	40.49 (39.43)
<i>Bacillus cereus</i>	44.64 (41.88)	67.73 (55.43)	60.5 (51.07)	57.65 (49.46)
<i>Microbacterium resistens</i>	42.387 (40.543)	62.8 (52.4)	60.35 (50.97)	55.18 (47.97)
<i>Pseudomonas sp.</i>	44.65 (41.9)	67.04 (54.95)	61.97 (51.91)	57.89 (49.59)
mean	40.78 (39.6)	60.2 (50.97)	57.398 (49.27)	

CD (P=0.05) culture: 2.94

CD (P=0.05)moisture levels: 2.55

CD (P=0.05)due to culture and moisture levels: N.S.

at 40 DAS, *Microbacterium resistens* continued to be the best but *Pseudomonas sp* was able to possibly establish and was found statistically equivalent to *Microbacterium resistens*. At mild moisture stress (-0.20 MPa) and normal moisture level (-0.05 MPa) *Pseudomonas sp* was significantly better than *Bacillus cereus* and *Microbacterium resistens* in enhancing the RAS/RT over the control.

Effect of inoculation on root biomass

Inoculation exerted a significant a positive effect on root biomass. This effect was highly pronounced at 40 DAS. *Pseudomonas sp.* was the best and consistently improved the root biomass in respective of the soil moisture level. An increase

by 27.8%, 25.5%, 24.3% was recorded following inoculation *Pseudomonas sp.* at -0.55, -0.20 and -0.05 MPa moisture level respectively after 40 DAS.

Effect of inoculation on Mean Weight Diameter (MWD)

The soil MWD ranged from 0.545mm to 1.281mm at 20 DAS (table 3). While it increased in magnitude at 40 DAS ranging from 0.55mm- 1.39 mm. MWD was significantly higher following inoculation with the bacterial culture over their uninoculated control at both 20 DAS and 40 DAS at three different soil moisture levels. Initially, among the three cultures tested *Microbacterium resistens* was significantly less efficient than *Pseudomonas sp.* w.r.t. its capacity to effect the

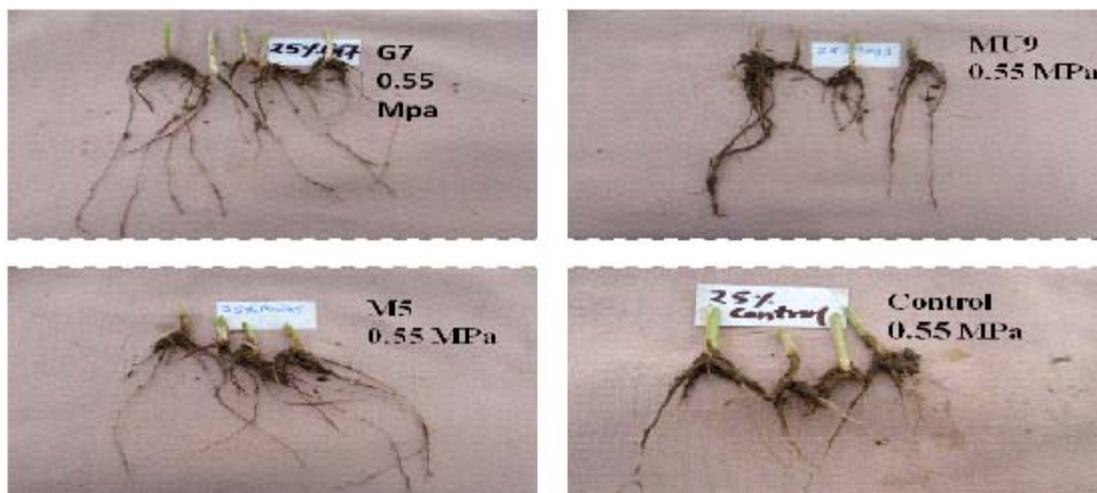


Plate 1. Root adhering soil in wheat at 0.55 Mpa by different treatments at 40 DAS



Plate 2. Root adhering soil in wheat at 0.20 Mpa by different treatments at 40 DAS

MWD, but with the progress of time at 40 DAS it was comparable to *Pseudomonas sp* and *Bacillus cereus*. Possibly because of relatively less compatibility of the legume rhizobacteria with that of a monocot. But after initial acclimatization even *Microbacterium resistens* was found to be comparable with the *Pseudomonas sp.* and *Bacillus cereus*. With an increase in soil moisture content from -0.55 MPa (25% field capacity) to -0.20 MPa (50% field capacity) bacterial inoculations significantly improved the MWD which was not the case with the uninoculated control at 20 DAS. At the 40 DAS even the uninoculated control showed a significant increase in MWD (table 4).

The bacterial inoculation was more effective at the moisture stress (-0.55 MPa and -0.20 MPa) condition to improve the MWD.

Effect of inoculation on Water Stable Aggregate (WSA)

WSA showed a significant increase from the soil moisture stress -0.55 MPa to -0.20 MPa. Further increase in soil moisture stress (-0.05 MPa.), failed to improve as it was statistical identical to -0.20 MPa. A significant and identical increase in WSA was recorded at -0.05 MPa and -0.20 MPa over the -0.55MPa soil moisture stress. All three cultures were found to be equally effective in increasing the WSA at 20 DAS. The observed



Plate 3. Root adhering soil in wheat at 0.05 Mpa by different treatments at 40 DAS

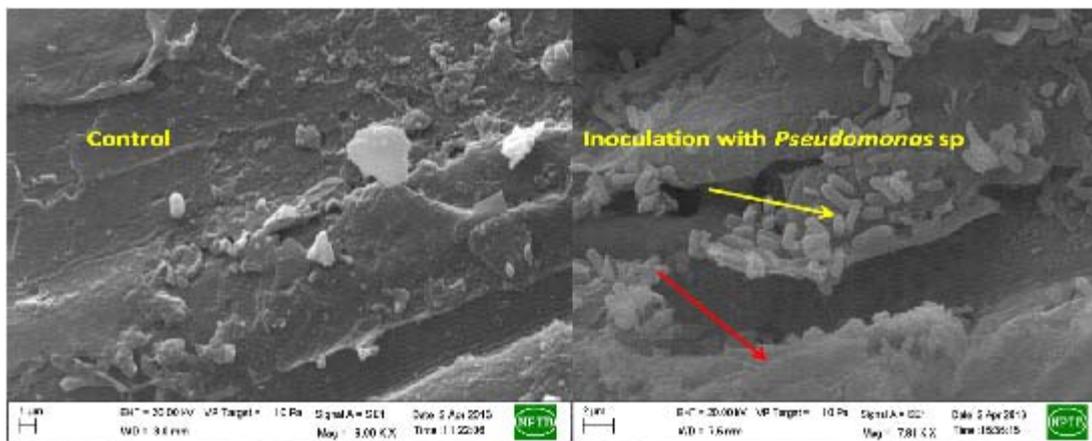


Plate 4. Scanning electron microscopic micrographs of plant roots colonized by *Pseudomonas sp.* And biofilm formation on wheat roots at -0.55 Mpa moisture stress level. Yellow arrow = Rods shaped *Pseudomonas sp.*, Red arrow= Biofilm

increase in WSA inoculation with *Bacillus cereus*, *Microbacterium* and *Pseudomonas sp* was 25.4%, 21.7% and 25.8% respectively over the uninoculated control (table 5).

At 40 DAS, the effect of inoculation with EPS producing bacteria significantly improved the WSA. The increase recorded following inoculation with *Bacillus cereus*, *Microbacterium resistens* and *Pseudomonas sp.* was 17.2%, 14.6% and 18.6% respectively over the un inoculated control (table 6). The soil moisture level also influences the WSA. The least level of WSA was under moisture deficient condition (-0.55MPa). The magnitude of

increase in WSA at -0.20 MPa and -0.05 MPa was 29.05% and 35.13% over the values recorded at -0.55 MPa moisture level. The interaction effect of bacterial inoculation and moisture level was found to be non significant at both interval 20 DAS and 40 DAS.

Biofilm formation and surface colonization

Scanning electron microscope confirmed the colonization of bacteria and biofilm formation on the surface of the roots (plate-4,5). There was found higher surface colonization and biofilm formation on inoculated plant roots in comparison to control.

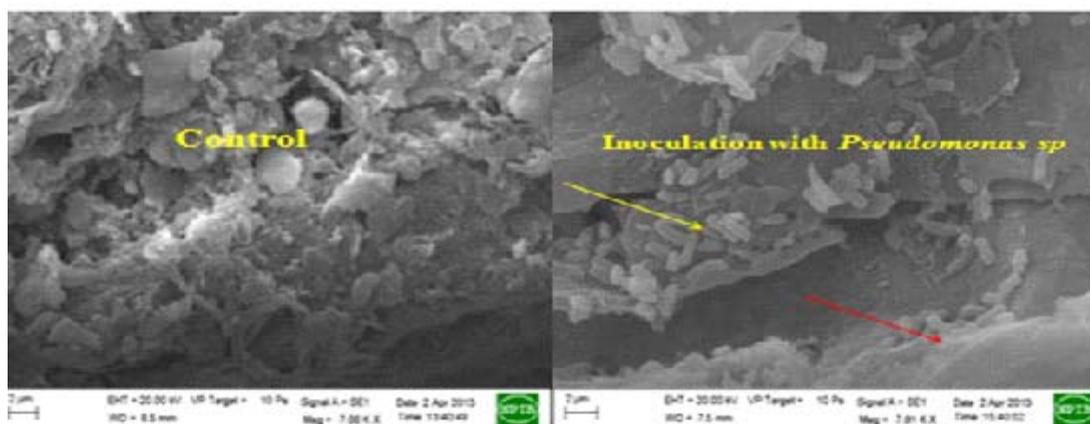


Plate 5. Scanning electron microscopic micrographs of plant colonized by *Pseudomonas sp.* Mpa moisture stress level. sp. And biofilm formation on wheat roots at roots -0.20

DISCUSSION

Soil moisture stress is responsible for low crop productivity in the arid and semi arid eco systems. Soil bacteria are known to secrete extra cellular polymers in the environment (Tisdall, 1994), called exopolysaccharides (EPS). EPS producing bacterial inoculants is an effective means to improve RAS/RT ratio in wheat under soil moisture stress as well normal conditions. *Microbacterium resistens* was the best inoculants for improving RAS/RT at -0.55 MPa at both 20 DAS and 40 DAS. while *Pseudomonas sp* is better option for mild moisture stress and normal soil condition as revealed by its ability to improve RAS/RT both at 20 DAS and 40 DAS. EPS are involved in adherence of the bacteria with the environmental surfaces, the association termed as biofilm (Mah and O'Toole, 2001). The underlying physical processes

could be aggregation of soil micro-aggregates due the adhesive effect of the bacterial EPS which are located in inter-micro aggregate pores and secondly a decrease of the capillary pressure due to the hydrophilic nature of bacterial EPS. These two processes may account for the observed increase of root-adhering soil. In addition, an increase in soil aggregation around roots of the inoculated plants would have aggravated the mechanical impedance (Chenu and Guerif 1991), the force applied per unit root area for growth of the roots. The inoculation with *Bacillus cereus*, *Microbacterium resistens* and *Pseudomonas sp* resulted in a significant improvement in a MWD, WSA at 20 DAS as well 40 DAS. Similar positive effect of microbial inoculation is reported by earlier workers (Gouzou *et al.*, 1993; Haynes and Francis 1993). Aggregate MWD decreased with decreasing water content in all treatments. This was highly

pronounced when the moisture stress increased from -0.20 MPa to -0.55 MPa. While an insignificant difference was observed in the MWD when moisture stress increased from -0.05 MPa to -0.20 MPa.

This observation may be explained to the clustering of the soil particle and micro-aggregate together under negative soil water potential. This may also be due to the breakdown the macro-aggregates at low water potential (Materchera *et al.*, 1994). There are ample evidences that microbial polysaccharide contribute to soil aggregation (Mah and O'Toole (2001), Alami *et al.*, (2000) and Lynch and Bragg, 1985). The high EPS producing *Pseudomonas sp* was more effective in increasing the mean weight diameter (MWD) of soil as well the water stable aggregate (WSA) at different intervals of observation. This trend was highly pronounced under high moisture stress condition (-0.55 MPa). There are records which indicate the role of EPS in soil aggregation (Bashan *et al.*, 2004 ; Watt *et al.* 1993) and concentration and composition of EPS which undergoes change under stress condition (Chenu *et al.*,1991) may account for the observed differential affect of the three bacterial culture on the water stable aggregate and mean weight diameter of soil subjected to moisture stress.

Bacterial polysaccharide are known to bind the soil particles forming micro-aggregates (<250 µm) and macro-aggregates (>250 µm) (Oades *et al.*, 1993). Further soil moisture availability play a important role in EPS production by the bacteria (Roberson and Firestone, 1992). Angers and Carter (1996) noted that the amount of WSA was often associated with soil organic carbon content and that particularly labile was positively related to macro-aggregate stability. EPS produced by the microorganisms possibly contributing to the soil organic content in the rhizosphere and a linear increase of aggregate stability and aggregate size with increasing levels of soil organic carbon is reported (Haynes, 2000).

Seed inoculation of wheat with *Bacillus cereus*, *Micrococcus resistens* and *Pseudomonas sp.* culture significantly improved the root biomass at all the soil moisture level. However this was most effective at high moisture stress (-0.55 MPa).

In uninoculated seedlings, due to the absence of EPS producing bacterial populations,

most of roots was devoid of RAS and thus was more susceptible to stress effect. Moreover, a higher population of EPS-producing bacteria on roots of inoculated plants may have stimulated root exudation (Wittenmayer and Merbach 2005), with stimulation of growth of inoculated bacteria with higher EPS production in the rhizosphere (Fischer *et al.* 2003, Sandhya *et al.*, 2009). Scanning electron microscope confirmed the colonization of bacteria and biofilm formation on the surface of the roots.

CONCLUSION

The results of the study suggest that inoculation of selected EPS-producing bacteria can serve as useful tool to improve the soil physical parameters and increase root weight thereby decrease the harmful effects of moisture stress on growth of crop plants and to improve crop productivity of the arid and semi arid soils. *Microbacterium resistens* was identified as a suitable candidate for extreme moisture stress condition at early crop establishment stages. While *Pseudomonas sp* was effective for medium stress and normal soil condition.

ACKNOWLEDGEMENTS

The authors thank the staff of microbiology for their help in data recording. Funding by the ICAR, India to carry out these studies is gratefully acknowledged.

REFERENCES

1. Alami Y, Heulin M, Milas R, de Baynast A, Heyraud, Villain A., Polysaccharide, microorganism and method for obtaining same, composition containing it and application. *European patent* 1998; **97**-1624. 970212.
2. Amellal N, Burtin G, Bartoli F, Heulin T., Colonization of wheat roots by EPS-producing *Pantoea agglomerans* and its effect on rhizosphere soil aggregation. *Appl Environ Microbiol* 1998; **64**:3740–3747.
3. Angers D A, Carter M R., Aggregation and organic matter storage in cool, humid agricultural soils. In 'Structure and organic matter storage in agricultural soils. (Eds M. R. Carter & B. A. Stewart) pp. 193-211. (CRC Press: Boca Raton.), 1996.

4. Bashan Y, Holguin G, De-Bashan LE., Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances. *Can J Microbiol.* 2004; **50**:521–577
5. Bezzate S, Aymerich S, Chambert R, Czarnes S, Berge O, Heulin T., Disruption of the *Paenibacillus polymyxa* levansucrase gene impairs ability to aggregate soil in the wheat rhizosphere. *Environ. Microbiol.* 2000; **2**:333–342.
6. Chaney K, Swift R S., The influence of organic matter on aggregate stability of some British soils. *J. Soil Sci.* 1984; **35**:223–230.
7. Chaney K, Swift RS., Studies on aggregate stability. I. Reformation of soil aggregates. *J. Soil Sci.* 1986; **37**:329–335.
8. Chenu C, EB Roberson., Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biol. Biochem.* 1996; **28**: 877–884.
9. Chenu C, Guérif J., Mechanical strength of clay minerals as influenced by an adsorbed polysaccharide. *Soil Sci. Soc. Am. J.* 1991; **55**:1076–1080.
10. Chenu C, J Guerif., Mechanical strength of clay minerals as influenced by an absorbed polysaccharide. *Soil Sci. Soc. Am. J.* 1991; **55**: 1076–1080.
11. Chenu C, Roberson EB., Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biol. Biochem.* 1996; **28**: 877–884.
12. Chenu C., Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. *Soil. Biol. Biochem.* 1989; **21**:299–305
13. Chenu C., Clay or sand polysaccharide associations as models for the interface between micro-organisms and soil: water-related properties and microstructure. *Geoderma.* 1993; **56**: 143–156.
14. Chenu C, Le Bissonnais, Y, Arrouays D., Organic matter influence on clay wettability and soil aggregate stability. *Soil Sci. Soc. Amer. J.*, 2000; **64**:1479–1486
15. Fischer SE, Miguel MJ, Mori GB., Effect of root exudates on the exopolysaccharide composition and the lipopolysaccharide profile of *Azospirillum brasilense* under saline stress. *FEMS Microbiol Lett.* 2003; **219**: 53–62
16. Gouzou L, Burtin G, Philipp R, Bartoli F, Heulin T., Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination. *Geoderma.* 1993; **56**:479–490.
17. Haynes R J., Interactions between soil organic matter status, cropping history, method of quantification and sample pretreatment and their effects on measured aggregate stability. *Biology & Fertility of Soils* 2000; **30**: 270–275.
18. Haynes RJ, Francis GS., Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *J. Soil Sci.* 1993; **44**:665–675
19. Kemper W D, Koch E J., Aggregate stability of soils from the western portions of the United States and Canada. *USDA Tech. Bull.* 1966; 1355.
20. Lynch J M, Bragg E., Microorganisms and soil aggregate stability. *Adv Soil Sci.* 1985; **2**:133–171.
21. Mah C, Thien-Fah, O Toole A George., Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology* 2001; **9**: 34–39.
22. Materechera SA, Kirby JM, Alston AM, Dexter AR., Modification of soil aggregation by watering and roots growing through beds of large aggregates. *Plant Soil* 1994; **160**:57–66.
23. Oades JM., The role of biology in the formation, stabilization and degradation of soil structure. *Geoderma* 1993; **56**:182–186
24. Roberson E B, Firestone M., Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl Environ Microbiol.* 1992; **58**:1284–1291.
25. Sandhya V, Ali SK, Minakshi Grover Z, Gopal Reddy, Venkateswarlu B., Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45, *Biol Fertil Soils* 2009; **46**:17–26
26. Tisdall J M, Oades J M., Stabilization of soil aggregates by the root systems of rye-grass. *Aust. J. Soil Research* 1979; **17**:429–441.
27. Van Gestel M V, Merckx R, Vlassak K. (1993) Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. *Soil Biol Biochem* **25**:109–123
28. Watt M, McCully M E, Jeffree C E., Plant & bacterial mucilages of the maize rhizosphere: comparison of their soil binding properties and histochemistry in a model system. *Plant Soil.* 1993; **151**:151–165.
29. Wittenmayer L, Merbach W., Plant responses to drought and phosphorous deficiency: contribution of phytohormones in root related processes. *J. Plant Nutr. Soil Sci.* 2005; **168**:531–540.