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Effect of inoculation of three rhizobial strains on maize hybrids

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Abstract

Maize (*Zea mays* L.) is an important cereal crop throughout the world. It is highly affected by biotic and abiotic stresses. Water deficiency is a major problem in semi-arid regions of Pakistan. It affects plant growth, therefore there is a huge loss of productivity and yield due to drought. Exopolysaccharides (EPSs) are of high importance for bacteria as a defense mechanism to prevent from desiccation and for adhesions by forming biofilms. EPSs producing bacterial strains were isolated from roots of desert plants of Thal, Pakistan and their potential was compared with pure culture strains *Bacillus subtilis* and *Mycobacterium Barkeri*. Effect of inoculation of EPSs producing strains on maize hybrids was evaluated at various moisture levels. The moisture treatments were comprised of 50%, 75% and 100% Field Capacity (FC). Various plant growth and soil parameters were determined. The results showed that the inoculation with *Bacillus subtilis* (KCTC-3135), *Mycobacterium Barkeri* (KCTC-3197) and strain (M1) isolated from roots of desert plants of Thal desert reduced the adverse effects of drought significantly. Moreover, inoculation with *Bacillus subtilis, Mycobacterium Barkeri* and M1 also improved the aggregate stability of soil, which improved water holding capacity of soil thus helping the plants to perform better in drought.

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Introduction

Potential of microbial strains isolated from water deficit condition is very effective to induce drought tolerance in plants. Microbes produce stress proteins, osmolytes and other bio stimulatory agents for their better survival under drought stress (Vanderlinde, 2010). Water deficit condition damage the bacterial cell by disruption of DNA structure and misfolding of RNA, in these condition bacteria produce certain types of proteins called chaperones which resolve RNA misfolding and facilitate biological functioning of bacteria via supporting transcription and translation of bacterial RNA under water shortage or drought. Some microbes can survive up to -3.5 MPa under water stress conditions and can improve drought tolerance of maize by decreasing stomatal conductance and transpiration losses under water stress condition through the production of lumichrome and abscisic acid in leaves. Catalases and oxidase play significant role in metabolism maintenance and avoid cell membrane damage under stress and can mitigate the impacts of drought stress by improving plant physiology (Yang, 2009). In stress conditions, like drought, bacteria can survive as microbial aggregates at solid-liquid interface and are covered by extracellular biopolymers which are highly hydrated called biofilm, these extracellular biopolymers/polysaccharides are macromolecules released by bacteria called as exopolysaccharides which are very helpful for normal bacterial activities under stresses like drought, which also play their role to increase plant growth (Flemming, 2010). (Characklis, 1990) stated that substrate (which forms biofilms) having rough surface released by bacteria increases the bacterial population and facilitates better attachment under water deficit conditions. Moreover, (Nichols, 2005) confirmed that production of biopolymers such as exopolysaccharides increases the potential of microbes in order to cope with water deficit conditions like drought, because these biopolymers facilitate attachment to the surfaces, give protection against antimicrobial agents released by plant or animal and avoid desiccation or drying of cell under stress environment like drought, hence these all features improve and increase the rhizobia ability to live under water shortage which is very beneficial for plant growth and development under drought condition. (Abbasi, 2011) stated that in stress environment, bacteria living in plant rhizosphere avoid the plant from pathogen attack by minimizing the adverse impacts of phytopathogenic organisms, like they release enzyme which causes degradation of fungal cell wall, ultimately enhance the plant growth indirectly. (Sheikh, 2006) evaluated that fungal attack on roots of okra plants significantly decreased when *Bacillus thuringiensis* (Bt10) and *Rhizobium meliloti* (R5) applied in the rhizosphere of okra, furthermore, root length, shoot length and seed germination also enhanced. (Hayat, 2010) discovered that microbes can produce siderophores under stress environment (drought, salinity, heavy metal, pH stress) which sequester the iron and making it available for the plants. Bacteria have certain set of environmental conditions, which are necessary for their normal growth. When bacteria exposed to abiotic stresses (drought, salinity, metal toxicity, high temperature and low temperature), adopt certain mechanisms to tolerate these stresses like they remain inactive under stress then become active when conditions become favorable. Bacterial adaptation to abiotic stresses is very complex and regulatory process due to involvement of different genes, so they adopt different mechanisms to tolerate stresses in soil (rhizosphere), like during salinity and drought bacteria tolerate the stress impacts by producing osmoprotectors such as glycine, proline, K^+, trehalose and glutamate, production of such compounds gives protection against severe conditions (Tobor, 2008). Some bacterial isolates having competitive and saprophytic ability are capable to survive and perform well under saline conditions in rhizosphere (Yap, 1983).

Microbes can be more beneficial for plant growth and development when applied as combination of different free living such as *Bascillus*, *Azospirrillum* and *Pseudomonos*. An experiment was conducted to investigate the efficacy of three rhizobacterium strains (*Serratia* sp. XY21, *Bacillus cereus* AR156 and *Bacillus subtilis* SM21) as consortium to mitigate drought stress in cucumber plant.

Results shown that inoculated plants have darker leaves and more chlorophyll contents than un-inoculated or control plants (Wang *et al*., 2012).

Material and methods

For this study, two maize hybrids, one drought tolerant and other drought sensitive with and without inoculum and three exopolysaccharides (EPSs) producing bacterial strains, one strain (M1) from Thal desert of Pakistan and two pure culture strains Bacillus *subtilis* (KCTC-3135) and *Mycobacterium barkeri* (KCTC-3197) were used.

Isolation of exopolysaccharides (EPSs) producing Bacterial Strains

Bacterial culture was being enriched with rhizosphere plants from Thal desert of Pakistan. After collecting samples from rhizosphere, they were introduced in nutrient broth medium at various incubation temperatures (30, 40 and 50°C). Optical density (OD) at 600nm was observed by using spectrophotometer at different time intervals for 24 hours, when maximum optical density was achieved cultures will be diluted 100 times in freshly prepared nutrient broth medium. This process was repeated thrice. Cultures were grown on nutrient agar medium in petri plates and were incubated again at 30, 40 and 50°C for 48 hours. Single colony was picked and purified in freshly prepared nutrient agar medium. This process of purification was repeated thrice.

Screening of exopolysaccharides (EPSs) producing bacterial strains

Bacterial strains were grown on RCV-glucose medium and were incubated at 30, 40 and 50°C. After 48 hours, the physical appearance of slimy and mucoid growth of bacterial colonies was observed. The strains producing slimy and mucoid growth were supposed to be EPSs producing strains. A ropy character was detected in cultures by forming long strands by extending with an inoculating loop and those strains were used in further study for crop inoculation.

Experimental Design Used and Parameters Recorded

Completely Randomized Design (CRD) under factorial arrangement was used in this experiment.

A weighed quantity of soil was filled into pots and its water retention capacity was determined by plotting a Soil Water Retention Curve (SWRC).

Two maize hybrids, one drought tolerant and another drought sensitive with and without inoculum were grown. When the crop was at four leaf stage stress was given at various moisture levels (100, 75 and 50% field capacity). Moisture levels were maintained gravimetrically. When crop was at 30 days of growth, no further irrigation was done and survival of plants in response to prolonged period of desiccation was estimated. Various plant growth parameters like leaf area, Chlorophyll contents, Relative water content (RWC), Shoot and root fresh and dry weights, Number of leaves per plant, Plant height and Root length were determined carefully.

Soil analyses including soil aggregate stability and organic matter were analyzed chemically. Soil as well as Plant NPK were analyzed using standard procedures. Data were collected and analyzed statistically following Completely Randomized Design (CRD) in three factors factorial settings using three replications by Analysis of Variance (ANOVA) and treatment means were compared using Tukey's Honestly Significant Difference (HSD) test (Steel, 1997).

Results and discussion

Chlorophyll content (SPAD value)

Under water deficit conditions, bacteria produce siderophores which make the iron available for plant required in chlorophyll synthesis, PGPR improve the chlorophyll contents via different mechanisms, such as enhancement of uptake and availability of other micro (Mg, Fe, Zn) and macro nutrients (NPK) also take part in strengthening of photosynthetic apparatus and chlorophyll contents. SPAD values indicated that inoculation of pure cultured bacterial strains enhanced chlorophyll contents in the leaves.

At 50% (FC) water deficit level, maximum increase in chlorophyll content was recorded by strain M1 inoculation (39.23%) following *Bacillus subtilis* (19.43%) and *Mycobacterium barkeri* (17.34%).

At 75% water deficit level, bacterial strains were more effective and statistically differed where increase in chlorophyll content was observed 34.30, 23.49 and 18.09% by strains M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective controls. **W**hile at 100% (FC) or normal water deficit, maximum increase was recorded by strain M1 (27.29%) following *Bacillus subtilis* (17.39%) and *Mycobacterium barkeri* (8.74%) over their respective non-inoculated control treatments (s).

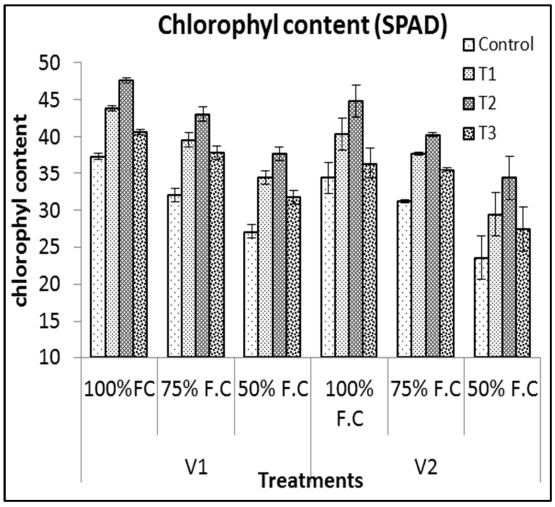

Fig. 1. Effect of inoculation of EPSs producing bacterial strains on chlorophyll content (SPAD) of maize hybrids at three different field capacity levels.

Relative water content (RWC)

Relative water content refers to the genetic capability of crop to mitigate the hazardous impacts of drought. Inoculation of M1 improved the relative water content by 39.08% at 50% drought level followed by *Bacillus subtilis* (21.83%) and *Mycobacterium barkeri* (12.97%) over control.

At 75% water deficit level application of M1 was observed most effective with 31.39% increase followed by *Bacillus subtilis* (18.07%) and *Mycobacterium barkeri* (9.42%) over control treatment. While maximum increase was found at 100% (FC) without stress, in which inoculation enhanced the relative water content up to 27.67% by M1, 15.41% by *Bacillus subtilis* and 9.32% by *Mycobacterium barkeri* as compared to non-inoculated control treatment(s).

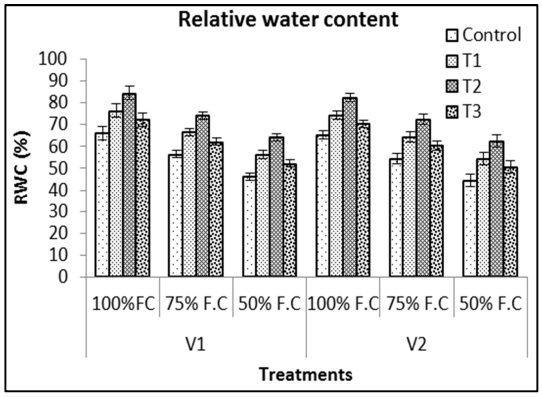

Fig. 2. Effect of inoculation of EPSs producing bacterial strains on relative water content (%) of maize hybrids at three different field capacity levels.

Shoot length

At 75% (FC) water deficit level, maximum increase in shoot length was recorded at strain M1 inoculation (16.26%) following Bacillus subtilis (9.53%) and *Mycobacterium barkeri* (8.26%) which was least effective at all water deficit levels, while at 50% water deficit minimum increase was recorded by *Mycobacterium barkeri* (7.3%), *Bacillus subtilis* (8.53%) then strain M1 (12.31%) over their respective non-inoculated control. At 100% (FC) level bacterial strains were also effective and statistically differed where increase in shoot length was observed 14.91, 9.23 and 7.69% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control.

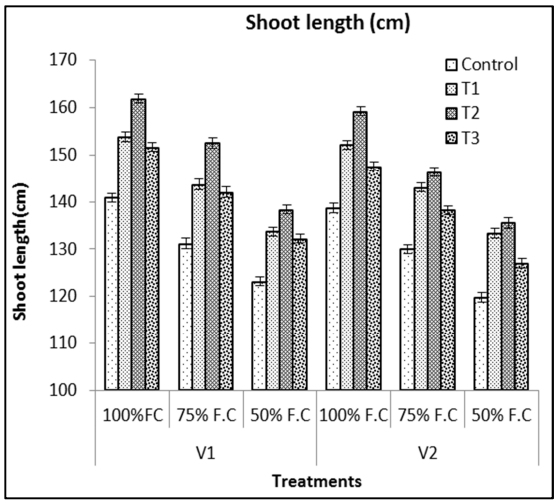

Fig. 3. Effect of inoculation of EPSs producing bacterial strains on shoot length of maize hybrids at three different field capacity levels.

Root length

At 50% (FC) water deficit level, maximum increase in root length was recorded at strain M1 inoculation (59.78%) following *Bacillus subtilis* (37.95%) then *Mycobacterium barkeri* (36.77%) which was least effective at all water deficit levels, while at 100% (FC) minimum increase was recorded by *Mycobacterium barkeri* (18.5%), *Bacillus subtilis* (28.46%) then strain M1 (39.75%) over their respective non-inoculated control. At 75% (FC) an increase in root length was observed 45.87, 33.91 and 23.03% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control.

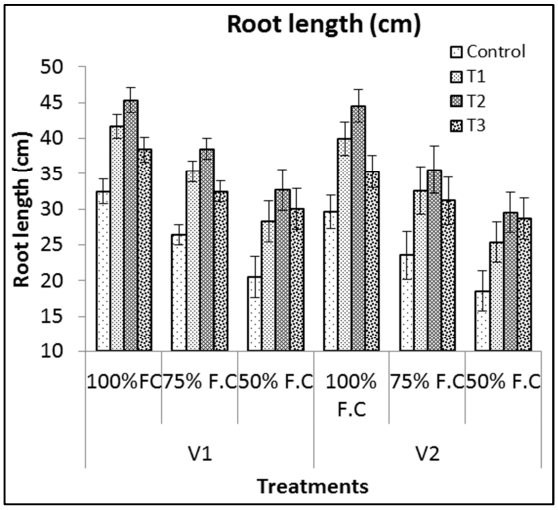

Fig. 4. Effect of inoculation of three EPSs producing bacterial strains on root length of maize hybrids at three field capacity levels.

Shoot Fresh Weight

Maximum increase in shoot fresh weight was recorded at 75% (FC) water deficit level by strain M1 inoculation (34.2%) following *Bacillus subtilis* (29.59%) then *Mycobacterium barkeri* (25.02%) which was least effective at all water deficit levels, while at 50% water deficit minimum increase was recorded by *Mycobacterium barkeri* (17.35%), *Bacillus subtilis* (18.66%) then strain M1 (27.10%) over their respective non-inoculated control. At 100% (FC) level bacterial strains were also effective and statistically differed where increase in shoot fresh weight was observed 30.30, 20.21 and 19.20% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control.

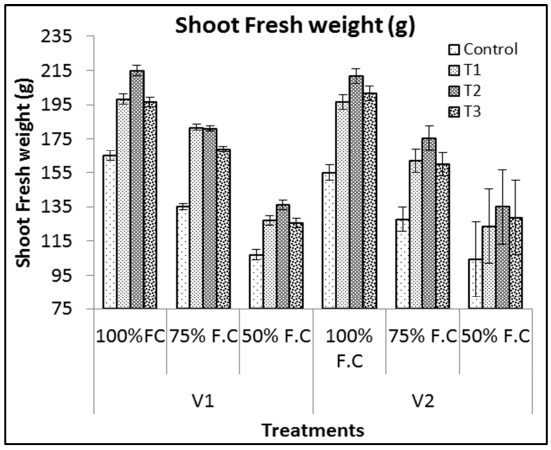

Fig. 5. Effect of inoculation of EPSs producing bacterial strains on shoot fresh weight (g) of maize hybrids at three different field capacity levels.

Root Fresh Weight

At 50% (FC) water deficit level, maximum increase in root fresh weight was recorded at strain M1 inoculation (52.99%) following *Mycobacterium barkeri* (44.93%) then *Bacillus subtilis* (43.4%) which was least effective at all water deficit levels, while at 100% (FC) minimum increase was recorded by *Mycobacterium barkeri* (16.03%), *Bacillus subtilis* (28.81%) then strain M1 (34.80%) over their respective non-inoculated control. At 75% (FC) level, an increase in fresh weight of roots was observed 40.044, 29.75 and 24.4% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control. Moreover, results were more statistically significant at inoculation with strain M1 at all water deficit levels.

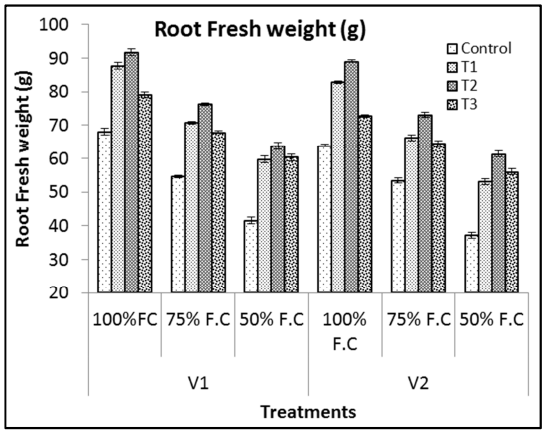

Fig. 6. Effect of inoculation of EPSs producing bacterial strains on root fresh weight of maize hybrids at three different field capacity levels.

Number of leaves

At 50% (FC) water deficit level, maximum increase in number of leaves was recorded at strain M1 inoculation (45.43%) following *Bacillus subtilis* (27.28%) and *Mycobacterium barkeri* (13.64%) which was least effective at all water deficit levels. At 75% (FC) level, an increase in number of leaves was observed 40.66, 25.89 and 14.78% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control. while at 100% (FC) minimum increase was recorded by *Mycobacterium barkeri* (8.51%) and *Bacillus subtilis* (14.25%) then strain M1 (22.86%) over their respective control.

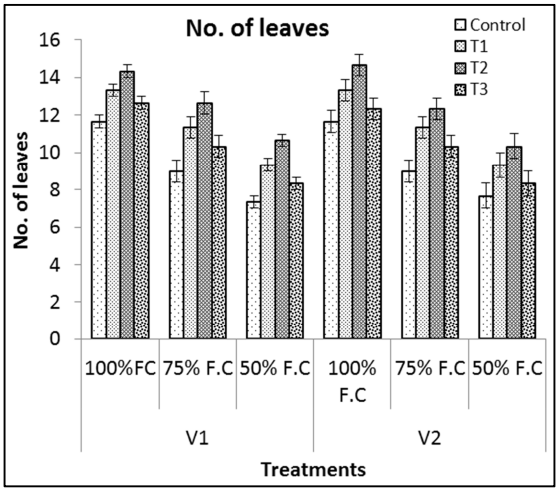

Fig. 7. Effect of inoculation of EPSs producing bacterial strains on number of leaves of maize hybrids at three different field capacity levels.

Soil aggregate stability

At 50% (FC) water deficit level, maximum increase in water stable aggregates was recorded by strain M1 (112.74%) following Bacillus subtilis (68.97%) and Mycobacterium barkeri (55.90%), while at 100% (FC) minimum increase was recorded by Mycobacterium barkeri (29.90%), Bacillus subtilis (38.25%) then strain M1 (66.44%) over their respective non-inoculated control.

At 75% (FC) level following increase in water stable aggregates was observed 70.58, 42.18 and 28.60% by strain M1, Bacillus subtilis and Mycobacterium barkeri respectively over respective control.

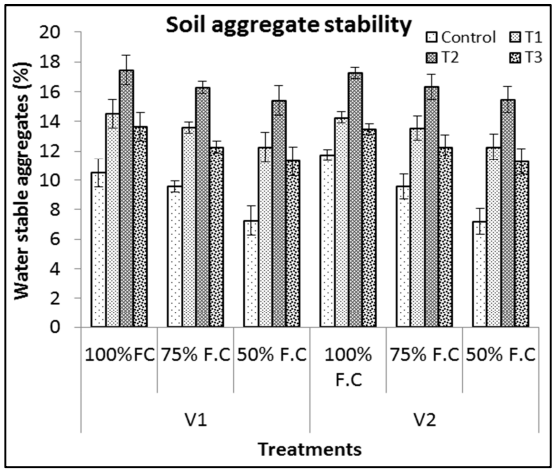

Fig. 8. Effect of inoculation of EPSs producing bacterial strains on soil aggregate stability at three different field capacity levels.

Soil Organic matter content

At 50% (FC) level following increase in soil organic matter content was observed 25.13, 10.69 and 5.88% by strain M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over their respective control treatment(s). Maximum increase in soil organic matter content was recorded by strain M1 (26.90%) following *Bacillus subtilis* (11.67%) and *Mycobacterium barkeri* (7.61%) at 75% water deficit level, while at 100% (FC) level minimum increase was recorded by *Mycobacterium barkeri* (4.21%), *Bacillus subtilis* (9.81%) then strain M1 (24.77%) over their respective non-inoculated control treatment (s).

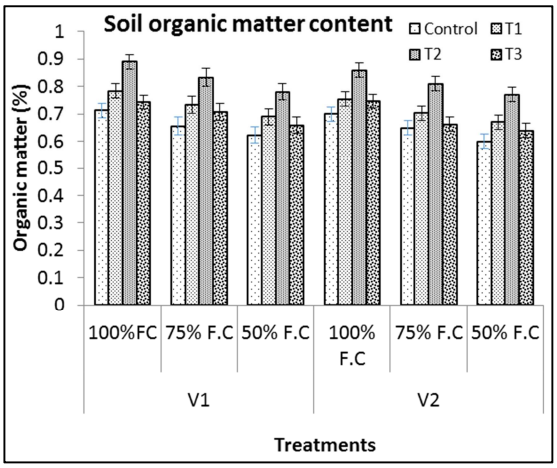

Fig. 9. Effect of inoculation of EPSs producing bacterial strains on soil organic matter content at three different field capacity levels.

Soil NPK Analysis

Nitrogen

Inoculation of bacterial strains significantly enhanced soil nitrogen in the pots. Inoculation with M1 improved the soil nitrogen 24.39% at 50% drought level followed by *Bacillus subtilis* (14.63%) and *Mycobacterium barkeri* (9.76%) strain over control treatment(s). At 75% water deficit level application of M1 was observed most effective with 24.56% increase following *Bacillus subtilis* (15.79%) and *Mycobacterium barkeri* (7.89%) over control treatments. While at 100% (FC) without stress, inoculation enhanced the soil nitrogen up to M1 (25.71%) following *Bacillus subtilis* (17.14%) and *Mycobacterium barkeri* 8.57% as compared to non-inoculated control treatment (s).

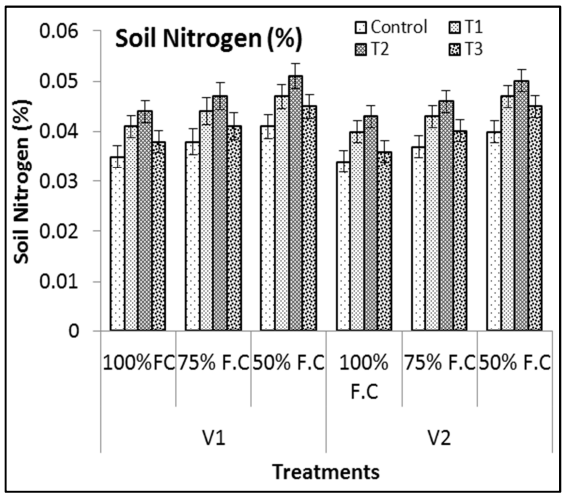

Fig. 10. Effect of inoculation of EPSs producing bacterial strains on soil nitrogen (%) at three different field capacity levels.

Phosphorus

Inoculation of bacterial strains significantly enhanced soil phosphorus in the pots. Inoculation with M1 improved the soil phosphorus by 32.19% at 50% drought level followed by *Bacillus subtilis* (14.83%) and *Mycobacterium barkeri* (23.57%) strain over control treatment(s). While at 100% (FC) without stress, inoculation with M1 enhanced soil phosphorus by 34.93% following *Bacillus subtilis* 14.10% and *Mycobacterium barkeri* 25.64% as compared to non-inoculated control treatments. At 75% water deficit level application of M1 was observed most effective with 30.35% followed by *Mycobacterium barkeri* (13.01%) and *Bacillus subtilis* (21.57%) over control treatment (s).

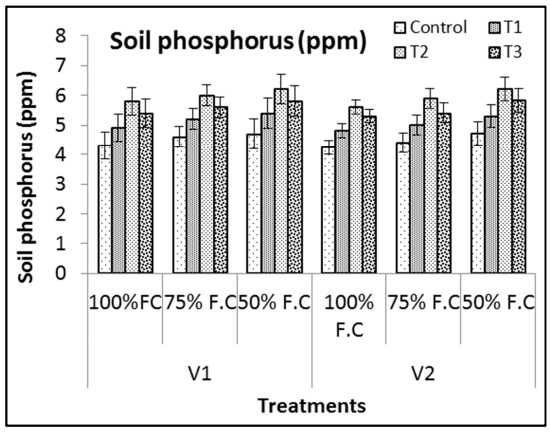

Fig. 11. Effect of inoculation of EPSs producing bacterial strains on soil phosphorus (ppm) at three different field capacity levels.

Potassium

Inoculation of M1 improved the soil potassium by 10.29% at 50% drought level followed by *Bacillus subtilis* (5.54%) and *Mycobacterium barkeri* (0.53%) over control treatment(s). While at 100% (FC) without stress, inoculation with M1 enhanced soil potassium up to M1 8.51% following *Bacillus subtilis* (4.06%) and *Mycobacterium barkeri* (0.49%) as compared to non-inoculated control treatments. At 75% water deficit level application of M1 was observed most effective with 8.89% increase in soil potassium followed by *Bacillus subtilis* (4.44%) and *Mycobacterium barkeri* (0.09%) over control treatment (s).

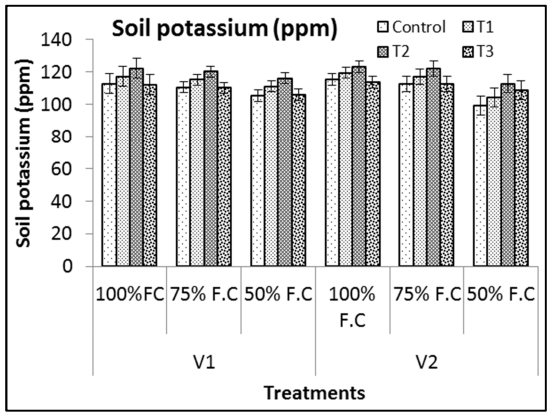

Fig. 12. Effect of inoculation of EPSs producing bacterial strains on potassium (ppm) at three different field capacity levels.

Plant NPK Analysis

Plant Nitrogen

At 50% (FC) level following plant nitrogen was observed 16.04, 8.64 and 3.7% by strains M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control treatment(s). At 75% (FC) water deficit level, results of all inoculations statistically differed significantly and maximum increase in plant nitrogen recorded was 26.04 % by strain M1 inoculation following *Bacillus subtilis* (18.75%) and *Mycobacterium barkeri* (11.46%), while at 100% (FC) maximum increase in plant nitrogen was recorded by strain M1 (35.31%) followed by *Bacillus subtilis* (10.34%) and *Mycobacterium barkeri* (4.31%) over their respective non-inoculated control treatment(s).

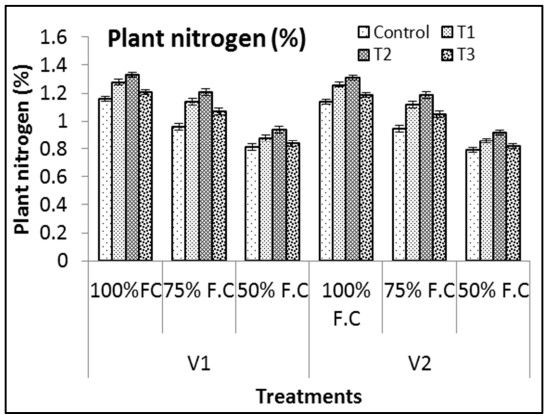

Fig. 13. Effect of inoculation of EPSs producing bacterial strains on plant nitrogen (%) at three different field capacity levels.

Plant potassium

At 50% (FC) water deficit level maximum increase in plant potassium was recorded by strain M1 inoculation (14.28%) following *Bacillus subtilis* (9.52%) then *Mycobacterium barkeri* (4.76%). At 75% (FC) level bacterial strains were effective and statistically differed where increase in potassium content in plant was observed 12.71, 8.47 and 4.24% by strains M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control treatment(s). While at 100% (FC) maximum increase was recorded by strain M1 (11.36%) followed by *Bacillus subtilis* (7.57%) and *Mycobacterium barkeri* (3.78%) over their respective non-inoculated control treatment(s).

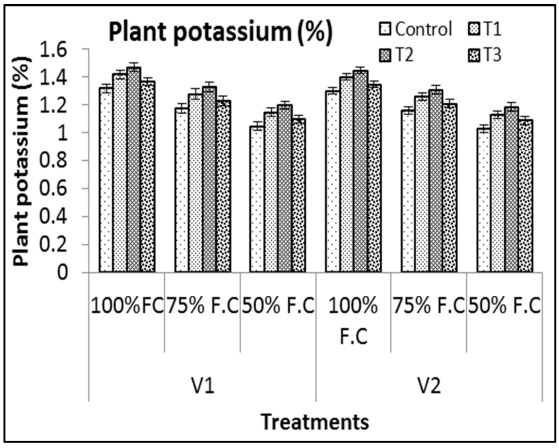

Fig. 14. Effect of inoculation of EPSs producing bacterial strains on plant potassium (%) at three different field capacity levels.

Plant phosphorus

At 50% (FC) water deficit level, maximum increase in phosphorus content in plant was recorded by strain M1 inoculation (42.85%) following *Bacillus subtilis* (28.57%) and *Mycobacterium barkeri* (9.52%). At 75% (FC) level bacterial strains were effective and statistically differed where increase in plant phosphorus was observed 33.31, 22.24 and 11.13% by strains M1, *Bacillus subtilis* and *Mycobacterium barkeri* respectively over respective control treatment(s). While at 100% (FC) maximum increase was recorded by strain M1 (28.57%) following *Bacillus subtilis* (19.04%) and *Mycobacterium barkeri* (9.52%) over their respective non-inoculated control treatment (s).

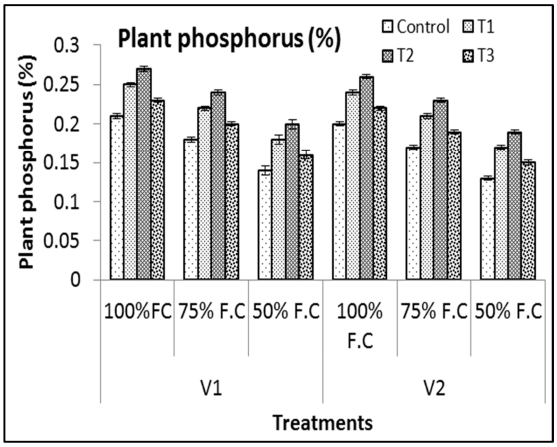

Fig. 15. Effect of inoculation of EPSs producing bacterial strains on plant phosphorus (%) at three different field capacity levels.

Conclusion

From this study it can be concluded that seed inoculation with EPSs producing bacterial strains enhanced the growth of maize crop by mitigating the adverse impacts of water stress via different direct and indirect mechanisms in pot conditions. Bacterial strains isolated from Thal desert of Pakistan were more efficacious at all water stress levels; moreover, their positive influence was more with increasing water deficit level. Results regarding comparison among three inoculations (KCTC-3135, KCTC-3197 and M1), their inoculation was most effective with stain M1 following *Bacillus subtilis* (KCTC-3135) and *Mycobacterium barkeri* (KCTC-3197) over respective non-inoculated control treatment(s).

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