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Performance evaluation of rhizobacteria on wheat crop under drought condition

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Abstract

Water shortage is a major issue to agribusiness in Pakistan and also other countries of world. Water deficiency hinders root advancement and effect the capacity of plants to take water. The microbes known as rhizobacteria have capacity to reduce stress and improve plant development through their drought tolerant mechanism. In this study two rhizobacterial strains (WK_1 and WK_2) were used in four different treatments (un-inoculated, inoculated with WK_1, WK_2 and Mixture), against three drought levels (65%, 40% and 25%) in a greenhouse pot trail. Drought levels were developed artificially after calculating field capacity of soil used in the experiment. Four kg autoclave soil per pot was used. All inoculated treatments showed increases in root length as compared to control at three drought levels. The highest increase in root length were as 59.09, 53.73 and 42.41% at 65, 40% and 25% drought level respectively followed by treatment inoculated with WK_1 (36.36, 38.73, 27.27%) at above said drought levels. The data collected regarding plant height showed the highest improvement from treatment inoculated with isolate WK_2 as 85.32, 41.42 and 37.6% at 25, 40 and 65% drought levels respectively followed by isolate WK_1 (6.68, 37.36, 24.75%) at three drought levels. Similarly trend of increase were found in case of seed weight per plant from treatment WK_2 and WK_1 at drought level of 25, 40 and 65%. Promising outcomes were acquired all through the research course. It was concluded that Bacterial strain WK_2 performed better at three different drought levels as compared to all other treatments.

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Introduction

Interaction between soil, microorganisms and plant can be neutral, harmful or beneficial (Adesemoye and Kloepper, 2009; Ahmad *et al.*, 2011). Types of microorganisms that colonize plant roots generally include algae, fungi, bacteria, actinomycetes and protozoa. Results of increase of plant growth due to the inoculation of microbes were reported by (Saharan and Nehra, 2011; Bhattacharyya and Jha, 2012). Bacteria are most important rhizospheric microbes among all of other microbial populations (Kaymak, 2010). Plant growth promoting rhizobacteria could be useful for promoting plant growth through the use of rhizobactera population, specifically under stress environment. The use of fungi and PGPR is a meaningful approach, it was revealed through various studies for sustainable agriculture (Denton, 2007; Ordookhani *et al.*, 2010; Najafi *et al.,* 2012), still gaps are present that need to be filled for maximizing the benefits of this natural population to get improved plant growth and development particularly in stress environment. Thus, present study discusses the knowledge on the active role of rhizobacteria.

PGPR have various mechanisms for promoting plant growth (Ahmad *et al.,* 2008) e.g siderophore production, suppression of pathogenic fungi, phosphate solubilization, nitrogen fixation, production of indole acetic acid (IAA), organic acids, NH_3, enzyme release (nitrogenase, phosphatase, soil dehydrogenase etc.) and mechanism of induced disease resistance Similarly, beneficial effects of bacteria to the plant include phytohormones production, nitrogen fixation and nitrate reduction due to this reason, PGPR are sometime isolated for such multiple traits for plant growth promotion.

Stress have harmful effect on plant e.g. nutritional and hormonal imbalance, and few physiological disorders e.g. abscission, senescence, epinasty and disease susceptibility (Ashraf, 1994, Niu *et al.*, 1995, El-Iklil *et al.*, 2000 and Nadeem *et al.*, 2010). Direct and indirect harmful impact of stresses on plant are ethylene concentration, drought stress increase ethylene level, chlorophyll contents changes, damage to photosynthetic apparatus and inhibit photosynthesis (Iturbe-Ormaetxe *et al.*, 1998).

Root growth got severely affected by drought stress. Most of the plant growth and yield parameters are affected under stress conditions Lucy *et al.*, 2004, Serraj, 2009, chitinase, Sandhya *et al.*, 2009). This research investigation was designed to evaluate performance of bacterial isolates under different drought condition.

Materials and method

This study was conducted to evaluate the performance of selected strains to alleviate the negative impact of drought stress on wheat (*Triticum astivum L.*) and enhance plant growth and biomass production. PGPR strains were characterized through morphological and bio-chemical characterization; research was carried out in green house of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. Soil samples were collected from PMAS-AAUR Research farm (Koont farm), from rhizosphere of wheat crop for this experiment. The collected sample was brought in environmental science laboratory. Soil samples were collected from the top soil (0-15cm depth) and then taken to the Environmental science laboratory of PMAS-AAUR in plastic bags.

Dilution plate technique was used for isolation. To isolate bacteria 90ml distilled water was taken in conical flask and 01gram soil was suspended into it. After settle down of solid particles, dilutions were carried out up to10^{-8}. Further 9mL water was taken to make 10^{-1} dilution and 1mL was taken out for making further dilutions. To isolate PGPR Mineral salt media (MSM) were used. The colonies were isolated and purified by four ways streaking. Two strains were selected and code names (WK_1 and WK_2) were given to them.

Morphological Characterization

These isolates were characterized on the basis of colony morphology (elevation, margin, colour and opacity) cell morphology and gram staining.

Phosphorus Solubilization

Purified colonies of bacterial strains were inoculated to Pikovskaya agar media (Pikovskaya, 1948) plates having tri-calcium phosphate as insoluble source of

phosphorus, under genotobiotic condition and then incubated at 28±2°C temperature for seven days. These plates were arranged with completely randomized design (CRD) with three replications. Colony diameter and halo zone diameter were recorded for seven days.

Solubilization index (SI)

Solubilization index (SI) of phosphate solubilizing rhizobacterial strains was determined by using formula of Edi-Premono *et al.*, 1996 as;

$$SI = \frac{\text{Halozone diameter + Colony diameter}}{\text{Colony diameter}}$$

Table 1. Physiological and morphological characterization of PGPR.

Bacterial isolates	Gram staining	Bacteria shape	Colony shape	Elevation	Margin	Opacity
WK_1	-ve	Rod	Circular	Punctiform	Entire	Undulate
WK_2	+ve	Rod	Circular	Punctiform	Raised	Undulate

Nitrogen Fixation

Petri plates having nitrogen free media were inoculated (NFM) (Okon *et al.*, 1977) with purified strains under aseptic condition in laminar flame hood. These plates were incubated at 28±2 C° for 3-4 days. Those bacterial colony exhibited growth in nitrogen free media were declared as nitrogen fixer.

Table 2. Biochemical Characterization of PGPR.

Sr. No.	Isolates	PSB (SI)	Nitrogen Fixer
1	WK_1	+	-
2	WK_2	+	+

Inocula Prepration

The chosen bacterial isolate were inoculated in 200 mL flask containing Luria- Bertini broth. Then these flasks were incubated in growth chamber having continuous shaking facility at 28±2C° for three to five days. The liquid cultures were transferred into autoclaved peat taken from Soil Biology and Biochemistry Laboratory, LRRI, NARC, Islamabad packed into half kg cellophane bags. Before coating with slurry hybrid seeds of Wheat (Chakwal 50) were surface sterilized with $HgCl_2$ (2%). A Pot study was conducted in green house of PMAS-AAUR. The autoclaved soil was used in all pots, each having 2 kg of soil. Wheat (Chakwal 50) was tested by inoculating rhizobacterial strains (WK_1, WK_2, Mix) under three drought level (60, 40, 25% of field capacity). Each treatment was replicated three times. Six seeds having inoculated PGPR were sown in pot. Strains used in this research are mentioned below.

Following treatments were applied

1. T_1= un-inoculated
2. T_2= WK_1
3. T_3= WK_2
4. T_4= MIX (WK_1 + WK_2)

Root length, plant height, weight of seeds per plant from all treatments was recorded.

Statistical Analysis

Software statistic 8.1 was used for the data collected on plant growth and yield parameters in all pots.

Results and discussion

A pot study was carried out in glass house of PMAS-AAUR at four drought levels 65%, 40%, 25%. Two PGPR isolates (WK_1, WK_2) were tested. The data collected on root length indicated continuous decrease with increasing drought level in control i.e. 26.67cm at 40% drought and decreased to 22cm at 25%. While those treatments in which seed were inoculated with bacterial strains increased root length at all drought levels compared to their control. At drought level 65% bacterial strains WK_1, WK_2 and Mix enhanced root length 36.36, 59.09 and 18.18%, while at 40% increase in root length was 38.73, 53.73 and 7.5% respectively over control. Whereas at 25% bacterial strains WK_1, WK_2 and Mix increased root length 27.27, 42.41 and 19.68% respectively over control. The inoculation with bacterial isolate WK_2 showed the highest increase of 59.09, 53.73 and 42.41% at 65, 40% and 25% respectively followed by isolate WK_1 (36.36, 38.73,27.27%) at same drought level (Fig. 1).

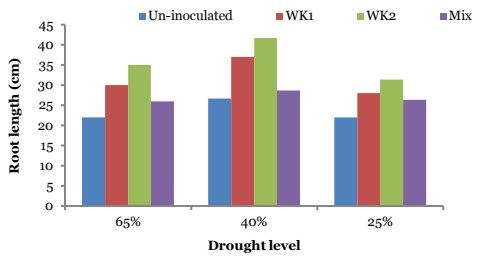

Fig. 1. Outcome of Bacterial inoculation on root length of wheat at different drought levels.

The shoot length data also showed continuous reduction with increasing drought in control i.e. 44.33 cm at 65%, 33cm at 40% and reduced up to 25 cm at 25%. Whereas seeds inoculated with bacterial strains high in PGPR activity increased shoot length at all drought levels as compared to their respective control.

The inoculation with bacterial isolate WK_1, WK_2 and Mix increased plant height 24.75, 37.6 and 24.81% respectively at drought level 65%, whereas an increase of 37.36, 41.42 and 35.36% respectively was observed over control at 40% While isolate WK_1, WK_2 and Mix increased plant height 6.68, 85.32 and 4% at 25%. The highest improvement of 85.32, 41.42, and 37.6% was noted at 25%, 40 and 65% respectively because of inoculation with isolate WK_2, followed by isolate WK_1 (6.68, 37.36, 24.75%) at same drought level (Fig. 2).

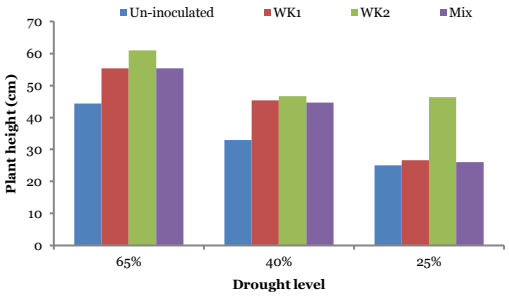

Fig. 2. Outcome of bacterial inoculation on plant height of wheat at different drought levels.

Data of seed weight is graphically presented (Fig. 3). All inoculated treatments WK_1, WK_2 and Mix have shown significant increase (18.18, 31.81 and 9.09%) as compared to un-inoculated at 65% drought. Whereas plants inoculated with bacterial strains at 40% drought level also indicated improvement (57.14, 128.57 and 42.85%) in number of over un-inoculated but this increase was 100% at 25% drought level. However highest improvement in seed weight per head was observed in WK_2.

Under field condition plants face different types of stresses like salt stress, drought stress nutritional stress, injury stress etc. The microbial population present in rhizospheric region help plant to reduce the negative impact of stress. Glick *et al.*, (1998) presented a model that describe how bacteria having ACC-deaminase enzyme break down 1-amionocyclopropane-1-carboxylate (ACC) and maintained normal ethylene level under stress condition. In this research trail two bacterial strains (WK_1 and WK_2) were evaluated for their stress tolerance ability against three different drought levels. The isolate WK_2 performed better as compared to other treatments at 40 percent drought level. Whereas all inoculated treatments showed improvement as compared to un-inoculated treatments.

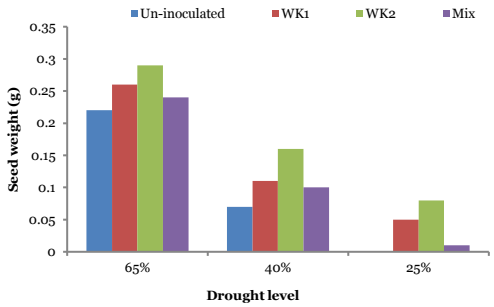

Fig. 3. Outcome of bacterial inoculation on seeds weight (g) at different drought levels.

The behaviour of mixture treatment was opposite to those reported by Saleemi, (2011) in case of wheat crop, where co-inoculation treatment of PGPR gave better results as compared to single inoculation. It can be due to non-compatible combination of isolates. There are large number of researchers who have reported that bacterial inoculation significantly improves plant growth under stress condition (Kiani *et al.*, 2016, Nadeem *et al.*, 2013. It was inferred that this research will create dry spell resilience against the dry season for rain fed zone where the water requirement of the crop is absolutely dependent on rain.

Conclusions

All inoculated treatments showed an increase at all drought levels over control, but more significantly at 40 percent drought. The treatmentsWK_2 and WK_1 showed maximum benefit as compared to control. Results of rhizosphere interactions revealed that WK_2 can be used as benchmark strain for the development of drought tolerant for the rain fed region of our country.

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