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# Stimulation of Plant Growth and Drought Tolerance on Wheat by Endophytic Bacteria from Dry Environment

**Disheeta L Akbari[1]*, Akbari LF[2] and Golakiya BA[1]**

## Abstract

The four drought tolerant endophytic bacteria isolated from the grasses of Kutch were examined for their ability to increase drought tolerance in wheat growing under water stress condition. The two combination cultures were inoculated on wheat seeds and effect of these bacteria seem to have a developed mechanisms to cope with drought stress fewer than 55% water holding capacity. Their inoculation increased wheat growth, shoot and root biomass, root and shoot length as compared with the control. The potent bacteria from harsh environment are a promising, novel way to improve plant water use efficiency and drought tolerance.

**Keywords**

Drought tolerant endophytic bacteria; Inoculation; Water stress; Wheat

## Introduction

The world population is increasing day by day inferring major challenges for agricultural to secure food availability. A major challenge for plant growth is shortage of water and it limiting the crop productivity. Drought plays a major role in destabilizing the productivity in wheat plant, wheat are often exposed to drought, which adversely affects both yield and seed quality worldwide. Several beneficial plant-microbe interactions that could enhance plant yield and health have been studied and utilized for the benefit of agricultural productivity over the last few decades. Endophytic bacteria can be defined as those bacteria that colonize the internal tissues of the plants showing no external sign of infection or negative effect on their host [1].

As a relatively simple and low cost alternative strategy, the use of plant growth promoting bacteria has been highlighted as a promising broad-spectrum means to improve plant growth [2]. Although the exact mechanisms of plant drought stress tolerance enhancement by endophytic bacteria remain possible explanations include:

(1) associative $N_2$ fixation (2) solubilization of immobilized mineral nutrients such as phosphorus and zinc or mineralization of organic phosphorus compounds (3) oxidation of sulfur (4) sequestration of iron by siderophores (5) production of different types of phytohormones like auxins, cytokinins, and gibberellins (6) production of amino-cyclopropane-1-carboxylic acid (ACC) deaminase (7) production of volatile growth stimulants such as acetoin and 2,3-butanediol (8) antibiosis (9) induced systemic resistance (ISR) (10) hydrogen cyanide (HCN) production and (11) production of a wide range of cell wall degrading enzymes and (12) formation of bacterial biofilm like exopolysaccharides [3,4]. The aim of the research work was to compare bacterial isolates from stressed and controlled environments in their capacity to enhance drought stress tolerance of wheat (*Triticum aestivum*).

## Materials and Methods

### Bacterial strains

In this study four drought tolerant endophytic bacteria were used which were isolated from grasses of banni region of Kutch (Table 1). The bacteria were identified by the partial DNA sequencing of 16s rRNA gene in Deptt. of Biotechnology, J.A.U., Junagadh.

**A pot experiment on wheat with water stress:** A pot experiment was carried out in a natural(uncontrolled) condition employing completely randomized design with four replications and six treatments T1-Control, T2-Control+endophytic bacteria (A+B), T3-Control+endophytic bacteria (C+D), T4-control+water stress, T5-control+endophytic bacteria+water stress(A+B) and T6-control+endophytic bacteria+water stress(C+D).

**Pots, soil preparation and irrigation:** All treatments were conducted in large pots and small pots containing 12 kg and 1 kg of unfertilized, unsterile loamy garden soil (pH 7-8) respectively. Experiment was conducted in large and small pots to give the water stress at two different stages of wheat growth. First stress was given after 20 days of plant emergence and second stress after 40 days. Garden soil was amended by adding farm yard manure (5000 kg/ha), recommended doses of N, P and K fertilizers (160-100-60 $kgha^{-1}$) were applied to each pot. A tap water applied to large pots was 2 l and 200 ml for small pots with 100% water holding capacity. Water stress was applied by stopping irrigation for 14 days after 20 days and 40 days of wheat plant emergence. In the stress days control (T1 pot) plants irrigated with water at alternative days while T2 and T3 pot treated with endophytic bacterial culture and irrigated with water at alternative days. In T4 water and endophytic culture was not applied while in T5 and T6 pot bacterial culture was once added at the initial day of water stress [5].

**A field capacity was calculated after 14 days of water stress by this formula:**

Water at Fc = (soil mass at dry field capacity – oven mass / oven dry mass) × 100

### Seed treatment and planting

Certified seeds of wheat GW-366 were obtained from Wheat Research Station, Junagadh Agriculture University, Junagadh were surface sterilized with 0.1% $HgCl_2$ for 5 min. followed by three times washing with sterilled distilled water and also washed with 70% ethanol for 3 min followed by two times washing with sterilled distill water. Sterilized wheat seeds were incubated in two combinations

***Corresponding author:** Disheeta L Akbari, Department of Biotechnology, College of Agriculture, Junagadh Agriculture University, Junagadh, Gujarat, India, Tel: +91 285 267 2080; E-mail: disheetaakbari@gmail.com

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of bacterial strain for 1 h. Growth of individual bacteria in nutrient broth was mixed at the time of incubation in another sterile flask. Seeds were then sown (2 cm depth with equal distance) in each pot (10 seeds/ pot) during November 2014-2015 season. Same process was done with the seeds dipped in uninoculated broth for control setup. The experiments were conducted at average daily temperatures ranging 10 to 20°C [6].

### Plant growth analysis

Plant survival was watched daily after stress application using stress plants. After stopping irrigation, plants were observed for signs of wilting and when shrinkage of leaves and stem were clearly visible wheat plants were harvested and determined a root length, stem and leaf length also taken a fresh and dry weight. A soil moisture content of both normal and reduced irrigated pots was determined. The statistical data was generated by one way ANOVA [7].

## Results and Discussion

### Pot experiment

The pot trial experiment monitored the plant growth promotion of drought tolerant endophytic bacteria in well-watered and water stress conditions. The response to an efficient colonization of wheat plant with the A+B and C+D combination of drought tolerant endophytic bacteria recorded in (Tables 2 and 3) (Figure 1). The Table 2 and 3 showed the comparison between inoculated and uninoculated controls in well water and water stress conditions, where inoculated wheat plant enhanced root, stem and leaves length. Whereas Tables 4 and 5 showed general increased in dry matter and germination of seed. This is favorable feature because an increase in crop canopy enhances photosynthesis and productivity [8].

**The wheat plant after 20 days of plant emergence with 14 days of water stress:** The present results on effect of drought tolerant endophytic bacteria on growth of wheat plant after 20 days of plant emergence showed in Table 2 and depicted in Figure 1 indicate that both the combinations i.e. A+B and C+D were found effective in enhancing growth of the plant (root, stem and leaves) under 100% (well-watered) and 55% (water stress) water holding capacity. The combination of C+D was found significant in increasing growth of the wheat plant (16.63cm root, 27.00 cm stem, 18 cm leaves) under water stress condition as compared to combination A+B (12.75 cm root, 25.00 cm stem, 15.88 cm leaves) and control (7.63cm root, 13.13cm stem, 7.63 cm leaves). Again this combination also found significant in promoting plant growth as compared to well-watered condition (12.63 cm roots, 24 cm stem, 13.38 cm leaves).

**Table 1:** Drought tolerant endophytic bacteria and source of isolation.

| Name | Tissue associated | Plant | Max Identity (%) |
|---|---|---|---|
| *Enterobacter cloacae* strain DSM | Root | *Cenchrus biflorus* | 97 |
| *Pseudomonas synxantha* strain NBRC103159 | Stem | *Eleusine indica* | 92 |
| *Pseudomonas cedrina* strain CFML96-198 | Stem | *Eleusine indica* | 96 |
| *Pantoea ananatis* AJ13355 | Root | *Cenchrus biflorus* | 97 |

**Table 2:** Effect of drought tolerant endophytic bacteria on growth of wheat plant after 20 days of plant emergence (small pot).

| Treatment | Water holding capacity (%) | Height (cm) | | |
|---|---|---|---|---|
| | | Root | Stem | Leaves |
| Control | 100 | 10.13 | 13.25 | 8.75 |
| Control+Endo. (A+B) | 100 | 12.50 | 21.75 | 14.63 |
| Control+Endo. (C+D) | 100 | 12.63 | 24.00 | 13.38 |
| Control+Water stress | 55 | 7.63 | 13.13 | 7.63 |
| Control+Water stress+Endo. (A+B) | 55 | 12.75 | 25.00 | 15.88 |
| Control+Water stress+Endo. (C+D) | 55 | 16.63 | 27.00 | 18.00 |
| S.Em. ± | | 0.44 | 0.78 | 0.63 |
| C.D. at 5% | | 1.31 | 2.34 | 1.88 |
| C.V. % | | 7.32 | 7.62 | 9.73 |
| A=*Enterobacter cloacae* strain DSM; B=*Pseudomonas synxantha* strain NBRC103159<br>C=*Pseudomonas cedrina* strain CFML96-198; D=*Pantoea ananatis* AJ13355 | | | | |

**Table 3:** Effect of drought tolerant endophytic bacteria on growth of wheat plant after 40 days of plant emergence (big pot).

| Treatment | Water holding capacity (%) | Height (cm) | | |
|---|---|---|---|---|
| | | Root | Stem | Leaves |
| Control | 100 | 15.88 | 17.13 | 8.68 |
| Control+Endo. (A+B) | 100 | 24.88 | 25.72 | 17.13 |
| Control+Endo. (C+D) | 100 | 26.93 | 29.60 | 18.63 |
| Control+Water stress | 55 | 10.13 | 15.15 | 7.13 |
| Control+Water stress+Endo. (A+B) | 55 | 25.80 | 30.82 | 19.05 |
| Control+Water stress+Endo. (C+D) | 55 | 27.50 | 32.42 | 19.13 |
| S.Em.± | | 0.63 | 0.88 | 0.74 |
| C.D. at 5% | | 1.89 | 2.63 | 2.20 |
| C.V. % | | 5.83 | 7.06 | 9.94 |
| A=*Enterobacter cloacae* strain DSM; B=*Pseudomonas synxantha* strain NBRC103159<br>C=*Pseudomonas cedrina* strain CFML96-198; D=*Pantoea ananatis* AJ13355 | | | | |

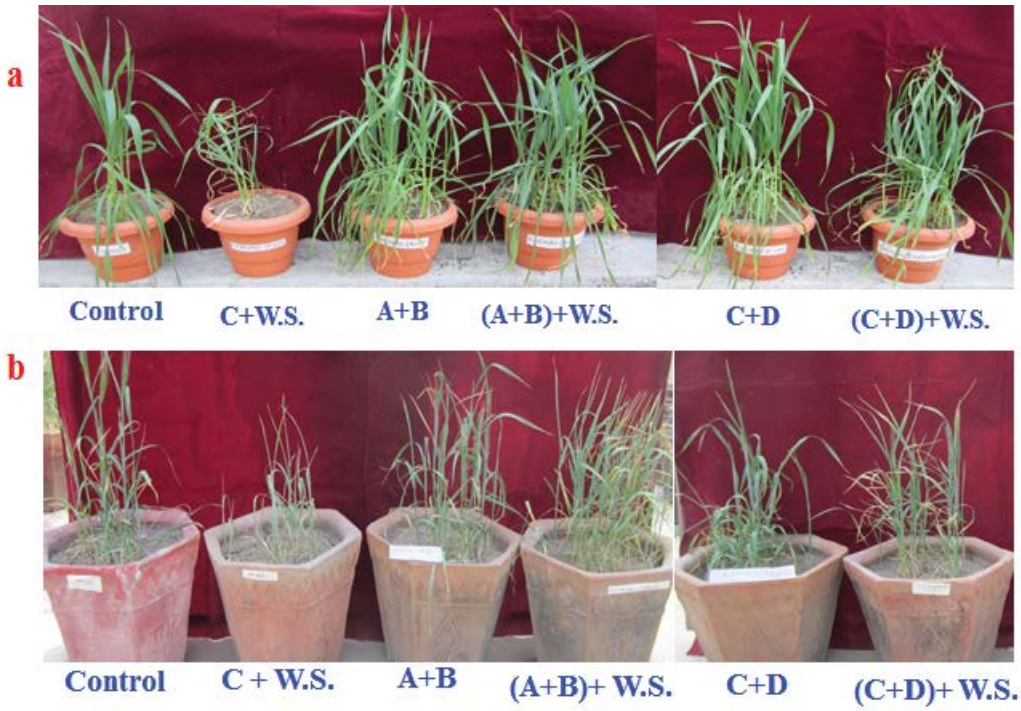


**Figure 1:** Plant growth promotion under 14 days of water stress (55% of water field capacity)after 20 (a) and 40 (b) days of plant growth. a=A+B combination b=combination, A=Enterobacter cloacae strain DSM; B=*Pseudomonas synxantha* strain NBRC103159; C=*Pseudomonas cedrina* strain CFML96-198; D=*Pantoea onanatis* AJ13355.

**Table 4:** Effect of drought tolerant endophytic bacteria on germination rate, weight and water use efficiency of 20 days wheat plant (small pot).

| Parameters | Well-watered | | | Water stress | | |
|---|---|---|---|---|---|---|
| | Control | Control+Endo. (A+B) | Control+Endo. (C+D) | Control+Water stress | C+Water stress+Endo. (A+B) | C+Water stress+Endo. (C+D) |
| *Root fresh weight (g) | 1.0 | 1.48 | 1.68 | 0.30 | 1.50 | 1.76 |
| Root dry weight (g) | 0.44 | 0.50 | 0.52 | 0.17 | 0.71 | 0.75 |
| Stem fresh weight (g) | 4.56 | 6.04 | 6.23 | 2.55 | 9.42 | 9.73 |
| Stem dry weight (g) | 1.22 | 1.47 | 2.14 | 0.74 | 2.69 | 2.78 |
| **Germination (%) | 60 | 100 | 100 | 70 | 100 | 100 |
| ***Water use efficiency (g $g^{-1}$) | 0.0095 | 0.0113 | 0.0150 | 0.0053 | 0.0192 | 0.0201 |

*Average of five plant
**Measured under well water condition
*** Water use efficiency is defined as the ratio of total plant dry mass per total water used
A=*Enterobacter cloacae* strain DSM; B=*Pseudomonas synxantha* strain NBRC103159
C=*Pseudomonas cedrina* strain CFML96-198; D=*Pantoea ananatis* AJ13355

The fresh & dry weight (g) of roots and stems were measured under well-watered and water stress conditions 20 days after plant emergence showed in Table 4. The results indicates that fresh and dry root and stem weight were 1.68 g & 0.52 g and 6.23 g & 2.14 g, respectively, recorded in C+D combination under well-watered condition were higher as compared to A+B combination and control. Whereas under water stress conditions, the C+D combination was found better than well-watered condition in increasing fresh & dry root and stem weight, where 1.76 g & 0.75 g and 9.73 g & 2.78 g, respectively recorded followed by water stressed A+B combination. However, fresh & dry root and stem weight in water stress control was lower than well-watered control because of plant can acquired the water easily under well-watered condition. The water use efficiency of water stress plant inoculated with endophytic bacteria was higher than the well-watered plant. The maximum water use efficiency recorded in the water stress C+D combination(0.0201 g $g^{-1}$) followed by A+B combination(0.0192 g $g^{-1}$).

**The wheat plant after 40 days of plant emergence with 14 days of water stress :**The data presented in Table 4 indicates that both the combinations i.e. A+B and C+D where found effective in enhancing growth of the plant(root, stem and leaves) under 100% (Well-watered) and 55% (water stress) water holding capacity. The combination of C+D was found significant in increasing growth of the wheat plant (27.50 cm root, 32.42 cm stem, 19.13 cm leaves) under water stress condition as compared to combination A+B (25.80 cm root, 30.82 cm stem, 19.13 cm leaves) and control (10.13 cm root, 15.15 cm stem, 7.13 cm leaves). This combination also found significant in promoting plant growth as compared to well-watered condition (26.93 cm root, 29.60 cm stem, 18.63 cm leaves).

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**Table 5:** Effect of drought tolerant endophytic bacteria on germination rate, weight and water use efficiency of wheat plant (big pot).

| Parameters | Well-watered | | | Water stress | | |
|---|---|---|---|---|---|---|
| | Control | Control+Endo. (A+B) | Control+Endo. (C+D) | Control+Water stress | C+Water stress+Endo. (A+B) | C+Water stress+Endo. (C+D) |
| *Root fresh weight (g) | 2.8 | 3.01 | 3.04 | 1.22 | 3.80 | 4 |
| Root dry weight (g) | 1.08 | 1.05 | 1.07 | 0.78 | 1.76 | 1.93 |
| Stem fresh weight (g) | 6.24 | 8.57 | 10.50 | 4.84 | 13.23 | 13.34 |
| Stem dry weight (g) | 2.0 | 3.53 | 4.39 | 1.78 | 5.68 | 5.70 |
| **Germination (%) | 68.33 | 100 | 100 | 60 | 100 | 100 |
| ***Water use efficiency (g $g^{-1}$) | 0.0021 | 0.0027 | 0.0031 | 0.0014 | 0.0042 | 0.0045 |

*Average of five plant
**Measured under well water condition
***Water use efficiency is defined as the ratio of total plant dry mass per total water used
A=*Enterobacter cloacae* strain DSM; B=*Pseudomonas synxantha* strain NBRC103159
C=*Pseudomonas cedrina* strain CFML96-198; D=*Pantoea ananatis* AJ13355

A fresh & dry root and stem weight were 4 g & 1.93 g and 13.34 g & 5.70 g, respectively, in C+ D combination under water stress condition (Table 5). These were higher as compared to A+B combination in term of fresh & dry weight of roots and stems were 3.80 g & 13.23 g and 1.76 g & 5.68 g, respectively while in water stressed control dry weight of root and stem was 0.78 g and 1.78 g. Whereas the fresh & dry root and stem weight, under well water condition of the C+D combination was found 3.04 g, 10.50 g and 1.07 g & 4.39 g, respectively, followed by well water A+B combination and control. The wheat seed treated with endophytic isolates showed 100% germination of seed as compared to control of well water and water stressed (68.33% and 60%, respectively).

These results interpreted that both the combinations works effective as compared to control in well water and water stress conditions because of the endophytic bacteria harbor drought tolerant activities gave much promising results in water stress condition , gave vigor and freshness to the leaves (Figure 1) and a strength to the plant for survival. The endophytic bacteria gave its effect throughout the life of plant and it was proved in intervals of 20 and 40 days of wheat plant and significantly increasing in root, stem and leaves height.

Marulanda [9] suggested that co-inoculation of microorganisms such as *Bacillus thuringiensis* and *G. intraradices* (a drought-tolerant fungal specie) reduced by 42% the water required to produce 1 mg of shoot biomass compared with an uninoculated control in maize plant. These results were the first evidence of the ability of a rhizosphere bacterium to increase plant water use efficiency and also proved that *B. megaterium* acted as an endophyte bacterium. The water content of plants was enhanced by bacterial inoculation which represents a positive bacterial effect on plant development under drought conditions. This bacterial activity is very important for preventing damage and enhancing plant survival in semiarid and arid areas. The underlying mechanism of enhancing plant tolerance to drought (in terms of growth) by bacterial inoculation could be the ability of these plants to increase their water content, because plants with a well-developed root system have the greatest ability to take up water. The enhancement of root growth by bacterial inoculation could be due to IAA produced by bacteria. The advantages of a well-developed and persistent bacterial community include better survival and effectiveness in plant development in osmotically stressed environments because the activity of such microbial communities may be essential in the establishment and nutrition of plants in such environments. Whereas plant root and shoot biomass were limited by osmotic stress, the microbial inoculation attenuated the negative effect of such detrimental factors. The PGPR are associated with plant roots (inside and/or outside) and either directly or indirectly stimulates plant growth, but there is a gradient of root proximity and intimacy depending on the niche. There are bacteria living in the soil near the roots, bacteria colonizing the rhizoplane (root surface), and bacteria residing in root tissue (inside cortical cells Gray and Smith) [10]. These aspects are important for intimacy with the associated plant, from almost casual to extremely regulated and housed in specialized structures. In general, for an effective growth stimulation a close interaction between microorganisms and host plants is a prerequisite for utilization of plant assimilates and microbial metabolites, respectively, by the partners. Particular and specific interactions between plants and microbial groups need to be compatible at a physiologic level.

According to Kavamura [7] the inoculation of *Bacillus* spp. and *Pantoea* sp. in *Z. mays* L. seedlings showed significant increases in leaf area, stalk length and shoot dry biomass under water stress, however these effects are not clearly correlated to the production of IAA, phosphate solubilisation and other mechanisms. Araújo-Garrido [11] observed that bacteria that promoted maize growth were not necessarily those that produced more IAA. Increase in growth, yield and nutrient absorption by plants may occur through the expression of one or more plant growth promoting characteristics. In this way, the *in vitro* selection of PGPR with multiple traits and their greenhouse evaluation under controlled conditions is important [12].

Timmusk [6] worked on increase of wheat drought stress tolerance by *B. thuringiensis* AZP2. They observed five times greater survival and 78% higher biomass in inoculated plants under drought stress condition and confirming the potential of bacterial priming in enhancing plant performance under drought. About 43% of *B. thuringiensis* AZP2 treated wheat plants survived the severe 10 day drought stress exposure followed by 4 days recovery after rewatering. Following germination, the capacity of roots to extract moisture and nutrients from the soil become the key traits determining plant survival. Root hair length and density are critical when it comes to water and nutrient acquisition from surrounding environment. Although root hair formation can be massively enhanced when it is expose to bacterial inoculation. Another important root trait in plant protection against drought stress is the creation of bacterial biofilm with attached soil mulch. The AZP2-induced denser and longer root hair framework forms an excellent matrix for the bacterially excreted biofilm comprised of cells and extracellular matrix producing a thick sticky layer around root hair. Hence, induction of long and dense root hair should be considered as an important drought stress tolerance

enhancement strategy. The dense biofilm matrix also limits diffusion of biologically active compounds secreted by bacteria and these are therefore concentrated on the root surface, facilitating water uptake. In addition, biofilm formation on root hair substantially improves root-to-soil contact, enhancing plant nutrient acquisition from soil and suggesting that biofilm formation importantly contributes to improving plant nutrition as well. Alginate, a hygroscopic bacterial polysaccharide, can play an important role in determining the biofilm capacity to enhance water status of seedlings [13]. Bacterial alginate water holding capacity is very high and it loses water slowly, thereby keeping root cells hydrated long enough to allow for cellular metabolic adjustments necessary to enhance drought stress tolerance. The drought tolerance enhancement of alginate might be due to its hygroscopic properties, but can also result from its role in biofilm architecture that contributes to reduced evaporation loss [14]. Despite several mechanisms suggested, the mode of how endophytic bacteria enhance plant drought stress tolerance is largely unknown. In particular, comparative genomic analysis of the sequence reveals gene clusters for alginate, ACC deaminase and auxin (IAA) production and regulation. Any of the traits alone and in combination could have been responsible for the bacterial drought tolerance enhancement [15,16].

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## Author Affiliations

Top

[1]*Department of Biotechnology, College of Agriculture, Junagadh Agriculture University, Junagadh, Gujarat, India*

[2]*Department of Plant Pathology, College of Agriculture, Junagadh Agriculture University, Junagadh, Gujarat, India*