J. Plant Production, Mansoura Univ., Vol. 3 (7): 2091 - 2106, 2012

IMPROVMENT OF WHEAT PLANTS GROWTH AND PRODUCTIVITY UNDER DROUGHT STRESS CONDITIONS BY USING SOME ENDOPHYTIC BARCTERIAL STRAINS

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ABSTRACT

This study was carried out at laboratory and greenhouse of Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University during two winter seasons of 2009 and 2010. The main objective of this study is: Effect of two treatment of bacterial endophytes strains *Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2) individually whether as grains soaking and foliar application on growth and yield parameters of two wheat plants (*Triticum aestivum* L.) cultivars (Sakha 93 and Gmiza 9) grown under three levels of irrigation water deficit stress 75, 50 and 25 % field capacity. Growth parameters that were take into account were plant height, number of leaf/plant, flag leaf area, fresh and dry weight/plant. In addition, wheat yield parameters included spike length and dry weight, number of spikelets/spike and grains number/spike. Generally, irrigation water deficit have an undesirable effect on growth and yield parameters of the tested wheat cultivars. Application of *Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2) strains individually were carried out by spray foliar and grains soaking treatments increased the tested vegetative and yield growth parameters for two cultivars, after 40, 70 and 130 days from planting compared with untreated plant. Entophytic bacterial strains treatments play an important role in protection of wheat plants against the adverse effects of drought stress, improve water productivity and will lead to significant water savings for irrigation sector.

Keyword: *Triticum aestivum,* endophytes, vegetative growth parameters, yield growth parameters, *Azotobacter chrocooccum*, *Pseudomonas* sp., and water deficit.

INTRODUCTION

Plants have evolved complex systems of defense against, as well as adaptation to, the variable and often potentially damaging environmental conditions to which they are exposed during their growth and development. Among environmental factors, water availability is probably the most limiting for crop quality and productivity, comprising economical output and human food supply (Roche *et al*., 2009). Water deficit is a multidimensional stress affecting plants at various levels of their organization (Yordanov *et al*., 2000). Thus, the effects of stress are often manifested at morpho-physiological, biochemical and molecular level, such as inhibition of growth (Bahrani *et al*., 2010), accumulation of compatible organic solutes (DaCosta and Huang 2009), changes in phytohormones endogenous contents (Huang, 2008;

Dobra *et al*., 2010), modifications in expression of stress responsive-genes (Xiong and Yang 2003; Yamaguchi-Shinozaki and Shinozaki, 2005; Huang *et al*., 2008).

Plant growth-promoting bacteria include both free living and symbiotic bacteria, typically found in the soil, that facilitate the growth and development of plants (Glick *et al*., 1999). This can occur directly promote plant growth either by providing the plant with a compound that is synthesized by the bacterium or by facilitating the uptake of nutrients from the soil. Thus, plant growth-promoting bacteria can directly facilitate the proliferation of plants by fixing atmospheric nitrogen; producing siderophores which can mineral solubilize and provide it to plants; synthesizing phytohormones, such as auxin, cytokinin and gibberelin, which can enhance various stages of plant growth; solubilizing minerals such as phosphorus; and synthesizing enzymes that can modulate plant growth and development (Glick, 2007).

They are also known as the plant growth promoting rhizobacteria (PGPR) because they colonize the plant roots and promote growth to the plants. There are the two levels of complexity in relationship between plant growth promoting rhizobacteria and host plant. These levels are rhizopheric and endophytic (Hayat *et al*., 2010). Among different strategies to cope with drought issues seed priming (pre-sowing seed treatment) is an easy, low cost and low risk technique and this approach has recently been used to overcome the drought problem in agriculture land (Iqbal and Ashraf, 2006). Although priming induced-drought tolerance has been reported in some crops, knowledge about physiological, biochemical and anatomical basis of priming induced-beneficial effects under stressful environment is still in frequent.

Azotobacter sp besides fixing nitrogen it is also secrete certain growth hormones such as IAA, GA and Cytokinins (Coppola, 1971) which promote vegetative growth and root development.

More recently, the function of seed-borne endophytes that improve seedling development have been demonstrated in a study in which seed-borne *Pseudomonas* sp. SENDO 2, *Acinetobacter* sp. SENDO 1, and Bacillus sp. SENDO 6 improved cardon cactus growth by solubilising rock minerals (Puente *et al*., 2009).

Wheat (*Triticum aestivum* L.) belongs to the Poaceae (Gramineae) family and it is one of the most important cereal crops and stands the top among the cereal crops of Egypt. Wheat is the world's most widely adapted crop, supplying one-third of the world population with more than half of their calories and nearly half of their protein (Rajaram, 2001). Increasing wheat production to meet higher demands by growing population is still a challenge in many countries. Higher production is only possible via higher yielding, better quality and drought tolerance varieties (Hamam, 2008).

Wheat production is an essential national target to fill the gap between production and consumption. Production could be increased through cultivation of high yielding cultivars and appropriate agronomic practices (Tawfik *et al*., 2006). It clearing that, there is found an enormous pressure on irrigation water in Egypt due to drought and competing water demands. Increasing wheat production under irrigation water deficit (drought) has

become important during recent years worldwide. Therefore, the present work was preformed to study the effect of different water deficient irrigation levels on growth, crop yield features of wheat plants treated with two different endophytic bacterial strains [*Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2)].

MATERIALS AND METHODS

The present investigation was conducted under greenhouse conditions at the Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, during the two growing winter successive seasons of 2009 and / 2010 and 2010 / 2011 to study the effects of irrigation water deficit and two endophytic bacterial strains and their interaction on growth and yield parameters of two wheat cultivars (*Triticum aestivum* L.).

Source of wheat cultivars:

Wheat grains (***Triticum aestivum*** L.) cultivars Sakha 93 and Gmiza 9 were obtained from wheat research Dept. Sakha of Agricultural research station, Kafr El-sheaikh, Egypt.

Source of microorganisms:

Two bacterial strains {*Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2)} were obtained from Dr. Elsayed Belal, Associate professor of Agricultural microbiology, Dep. of Agric. Botany, Fac. of Agriculture, Kafrelsheikh University and these bacterial strains were isolated in previous study as entophytic bacteria from wheat plants (unpublished data).

Cultivation of microorganisms:

Azotobacter chrocooccum (E1) and *Pseudomonas* sp. (E2) was cultivated in nutrient liquid medium**.** 200 ml nutrient liquid medium were inoculated with 2 ml of a cell suspension of (*Azotobacter chrocooccum* (E1) or *Pseudomonas* sp. (E2) (nutrient broth medium, 10^8 cfu / ml) was incubated at 30 ^oC and 150 rpm for 3 days. The cultures were incubated at 30^o C and 150 rpm for 5 days. Thereafter**,** two bacterial strains were applied on wheat as follow:

Seed treatments:

Two bacterial strains were applied at the time of planting as seed treatment. Grains were immersed in each bacterial suspension (10^8 cfu / ml) for 30 min. Grains were witted with 10 % sugar syrup, and were thoroughly mixed with an amount of bacterial suspension (10^8 cfu / ml) for 30 min. enough to obtain 10^8 cfu / per gram of seeds and then dried. Grains were then sown in each pot (10 Grains / pot). On the other hand, grains wheat were immersed in the manner in 10 % sugar syrup and were thoroughly mixed with an amount of nutrient broth medium (without bacterial growth).

Wheat plant spraying:

Wheat plants (20 days from planting) were sprayed weekly intervals with inculated bacterial suspension (10^8 cfu / ml) from each bacterial strains.

Pots, soil preparation and grains wheat planting:

Each pot (30 cm in diameter) contained 8 Kg of air dried clay soil. The chemical analysis was determined by conventional methods, twelve

grains / pot were sown at equal distances and depth. After two weeks from sowing, the seedlings were thinned to ten seedlings / pot, three of them were kept for the morphological character throughout the experimental period. The soil used in this experiment was fertilized with nitrogen at rate a 360 kg/h of urea fertilizer (contain 46 % nitrogen). Super phosphate fertilizer (phosphorus 15 %) was added at a rate of 240 kg/ha before planting. Potassium was not added because the Egyptian soil is rich in this element. Chemical analysis of the soil samples were taken before planting in the two seasons, mechanical and chemical analysis of the experimental soil were determined (Metwaly, 2012) according to Page, (1982).

Plant growth parameters:

Growth features were determined on three samples were taken throughout the experimental period 40, 70 and 130 days after sowing. Samples were taken at 40, 70 and 130 days from sowing to estimate plant height (cm), fresh and dry weights of wheat plants cultivars (dried in an electric oven at 70 ^{o}C for 72 h till constant weight) g/plant. Flag leaf area (cm2/plant) as affected by the treatments was estimated as the average of area of leaves of randomly taken five wheat plants using LI-3100 area meter.

Yield and its components:-

At harvest time, the following data were recorded: 1- Number of spikes per plant: determined by counting number of fertile spikes per plant. 2- Number of kernels per spike: computed by counting number of grains of the main spike. 3- 100-kernel weight, (gm): determined as the mean weight of 100 kernel random sample.

Experimental design and statistical analysis:

The pots were arranged in a randomized complete block design with three replicates in each treatment and ten plants in each pot. Data of growth features and all characters as well as chemical and physiological studies of investigated wheat plant were tested by analysis of variance. Duncan's multiple range tests were used for comparisons among treatments mean (Duncan, 1955).

RESULTS AND DISCUSSION

Results obtained in 2009/2010 showed almost the same trend as those of 2010/2011 season, so data of the first season were found enough to be presented. Generally, irrigation water deficit have an undesirable effect on growth and yield parameters of tested wheat cultivars.

Growth parameters:

Data presented in Table (1, 2 and 3) show that, the irrigation water deficit levels (75, 50 and 25 % FC) decreased significantly all tested vegetative growth parameters (plant height, flag leaf area, number of leaves/plant, and fresh and dry weight/plant) of wheat cultivars under the study at all sampling dates. The highest reduction were recorded under the sever irrigation water deficit (25 % FC) treatment. The obtained results were compared to well-irrigated plants (control 100 % FC).

1

Table 2 : Leaf area and leaves number of wheat cultivars as affected by different levels of water stress and two endophytic bacteria and their interactions.

Treatments	Gmiza 9		Sakha 93	
	Flag Leaf area	Leaves number	Flag Leaf area	Leaves number
Control	23.97^{D}	6.00^{A}	26.84^{CDE}	5.00^{A}
E 1 Gs	36.94^{A}	6.33^{A}	41.56^{A}	6.00^{A}
E 1 Sp	28.92^{BC}	6.33^{A}	34.83^{B}	5.67^{A}
E 2 Gs	30.43^{B}	7.33^{A}	39.69^{A}	6.00^{A}
E 2 Sp	27.56^{C}	7.00^{A}	37.46^{AB}	5.67^{A}
W 1	14.85^{GH}	6.33^{A}	20.55^{FGH}	5.00^{A}
W 1 + E 1 Gs	23.29^{D}	7.00^{A}	29.03^{C}	5.33^{A}
W 1 + E 1 Sp	22.63^{DE}	6.33^{A}	25.11^{C-F}	6.00^{A}
W 1 + E2 Gs	24.95^{D}	7.00^{A}	24.79^{C-F}	5.67^{A}
W 1 + E 2 Sp	22.47^{DE}	7.00^{A}	24.41^{DEF}	5.33^{A}
W 2	13.09^{H}	6.67^{A}	15.78^{I}	5.33^{A}
W 2 + E1 Gs	20.10^{EF}	7.00^{A}	24.37^{DEF}	5.33^{A}
W 2 + E1 Sp	19.51^{F}	6.00^{A}	22.59^{EFG}	5.33^{A}
W 2 + E 2 Gs	18.86^{F}	6.67^{A}	27.36^{CD}	5.67^{A}
W 2 + E 2 Sp	17.35^{FG}	7.00^{A}	24.19^{DEF}	5.00^{A}
W 3	9.92^{I}	6.00^{A}	11.69^{J}	5.33^{A}
W 3 + E 1 Gs	15.26^{GH}	6.67^{A}	18.89^{GHI}	5.00^{A}
W 3 + E 1 Sp	14.79^{GH}	6.33^{A}	19.14^{GHI}	5.67^{A}
W 3 + E 2 Gs	12.89^{H}	7.00^{A}	18.28^{GHI}	5.67^{A}
W 3 + E 2 Sp	13.54^{H}	6.00^{A}	17.66^{HI}	5.00^{A}

E1 = *Azotobacter chrocooccum* E2 = *Pseudomonas sp* Gs = Grains soaking Sp = foliar application control = 100 % field capacity W1 = 75% field capacity W2 = 50 % field capacity W3 = 25 % field capacity

Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test

The reduction in vegetative growth parameters of two different cultivars of wheat grown under irrigation water deficit may be attributed to the decrease in net photosynthetic rates (photoinhibition) in plants due to the reduction in photosynthetic pigments (Metwaly, 2012). Moreover, this effect may be attributed to the results of massive and irreversible expansion of small daughter cells produced by meristematic divisions and growth inhibition is therefore related to the inhibition of cell expansions as well as reduced rates of new cell production may make additional contribution to the inhibition of growth (Bhatt and Srinivasa, 2005). Moreover, this effect may be attributed to losses in tissue water content, which reduce turgor pressure in the cell, thereby inhibiting enlargement and division of cells causing a reduction in plant growth (Amon, 1972). The reduction in growth parameters may be due to the reduction in water absorption, nutrient uptake under drought stress conditions (Mahgoub, 1996). The adverse effect of drought stress on vegetative growth parameters may be attributed to the decrease in net photosynthetic rates (photoinhibition) in plants due to stomatal closure, which decreases or prevents water loss but reduces CO_2 availability for chloroplast (Flexas, *et al*., 2004 and Lawlor and Cornic, 2002). These results related to the microscopic anatomical, relative water content, water deficit, chlorophyll

pigments and essential nutritional elements uptacke (Metwaly, 2012). Similar results were in agreement with (Saleem, 2003; Ghamarnia and Gowing, 2005 and Selim and El-Nady, 2011).

Table 3: Fresh and dry weight of wheat plants Gmiza 9 and Sakha 93 cultivars as affected by different levels of irrigation water deficit and two bacterial endophytes and their interactions at different stages during 2009 / 2010 season.

Treatments	Gmiza 9						Sakha 93					
	Fresh weight (g) after			Dry weight (g) after			Fresh weight (g) after			Fresh weight (g) after		
	40 days	70 days	Mean	40 days	70 days	Mean	40 days	70 days	Mean	40 days	70 days	Mean
Control	4.37^{FG}	9.81^{CD}	7.09	1.12^{EF}	3.36^{BC}	2.24	3.53^{IJ}	7.33^{F}	5.43	0.89^{DE}	`2.92^{E}	1.91
E 1 Gs	5.56^{A}	11.71^{A}	8.64	1.37^{A}	3.93^{A}	3.33	5.31^{A}	10.05^{A}	7.68	1.47^{A}	3.79^{ABC}	2.63
E 1 Sp	4.77^{CD}	10.95^{AB}	7.86	1.27^{BC}	3.78^{AB}	2.53	4.14^{DE}	8.96^{BC}	6.55	0.97^{D}	3.96^{AB}	2.47
E 2 Gs	5.28^{B}	10.74^{B}	8.01	1.26^{BCD}	3.85^{A}	2.56	5.03^{B}	9.11^{BC}	7.07	1.45^{A}	3.89^{AB}	2.67
E 2 Sp	4.64^{DE}	10.47^{BC}	7.56	1.23^{CD}	3.98^{A}	2.61	4.32^{D}	8.44^{CDE}	6.38	1.32^{B}	3.87^{AB}	3.00
W 1	3.33^{JK}	5.28^{FG}	4.31	0.83^{I}	1.56^{FG}	1.19	3.31^{J}	6.44^{G}	4.88	0.86^{EF}	2.23^{F}	1.55
W1 + E 1 Gs	5.34^{B}	9.72^{CD}	7.52	1.31^{B}	3.63^{AB}	2.47	5.07^{B}	9.19^{BC}	7.13	1.49^{A}	3.85^{AB}	2.67
W1 + E 1 Sp	4.36^{FG}	9.23^{D}	6.78	1.12^{EF}	3.13^{CD}	2.13	4.09^{DEF}	7.74^{EF}	5.92	0.89^{DE}	2.73^{E}	1.81
W 1 + E2 Gs	4.14^{B}	10.49^{CD}	7.32	1.21^{D}	3.52^{ABC}	2.37	4.77^{C}	8.57^{BCD}	6.67	1.35^{B}	4.14^{A}	2.75
W1 + E 2 Sp	4.48^{EF}	9.30^{D}	6.89	1.11^{EF}	3.54^{ABC}	2.33	4.23^{D}	9.32^{AB}	6.78	1.12^{C}	3.41^{D}	2.27
W 2	3.16^{KL}	4.48^{G}	3.82	0.71^{J}	1.17^{GH}	0.94	2.78^{K}	3.67^{J}	3.23	0.64^{G}	1.46^{H}	1.05
W 2 + E1 Gs	4.88^{C}	7.59^{E}	6.24	1.13^{E}	2.78^{DE}	1.96	3.98^{EFG}	5.33^{H}	4.66	1.29^{B}	2.75^{E}	2.02
W 2 + E1 Sp	4.06^{H}	7.21^{E}	5.64	0.98^{G}	2.75^{DE}	2.36	3.39^{J}	4.84^{HI}	4.12	0.83^{EF}	2.67^{E}	1.75
W2 + E 2 Gs	4.71^{CD}	7.53^{E}	6.12	1.08^{F}	2.61^{E}	1.85	3.94^{EFG}	7.75^{EF}	5.85	0.97^{D}	3.48^{CD}	2.23
W2 + E 2 Sp	4.25^{GH}	7.19^{E}	5.72	0.93^{H}	2.66^{E}	1.80	3.77^{GH}	7.82^{DEF}	5.79	0.89^{DE}	3.65^{BCD}	2.72
W 3	3.05^{L}	3.24^{H}	3.15	0.59^{L}	0.91^{H}	1.05	2.47^{L}	2.22^{K}	2.35	0.57^{G}	0.62^{I}	0.60
W3 + E 1 Gs	4.13^{H}	5.34^{FG}	4.74	0.87^{I}	1.74^{F}	1.31	3.69^{HI}	4.65^{I}	4.17	0.96^{D}	1.70^{GH}	1.33
W3 + E 1 Sp	3.66^{I}	5.25^{FG}	4.46	0.71^{J}	1.32^{FGH}	1.02	3.35^{J}	4.49^{I}	3.92	0.79^{F}	1.55^{H}	1.17
W3+ Et 2 Gs	4.14^{H}	4.68^{FG}	4.41	0.84^{I}	1.60^{FG}	1.22	3.88^{FGH}	4.68^{HI}	4.28	0.90^{DE}	1.94^{FG}	1.42
W3 + E 2 Sp	3.52^{IJ}	5.60^{F}	4.56	0.66^{K}	1.35^{FGH}	1.01	3.40^{J}	4.17^{IJ}	3.79	0.83^{EF}	2.62^{E}	1.7

E1 = *Azotobacter chrocooccum* E2 = *Pseudomonas sp* Gs = Grains soaking Sp = foliar application control = 100 % field apacity W1 = 75% field capacity W2 = 50 % field capacity W3 = 25 % field capacity

Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test

Application of *Azotobacter chrocooccum* (E1) and *Pseudomonas* sp. (E2) strains individually were carried out by spray foliar and grains soaking treatments increased the tested vegetative growth parameters for two cultivars, after 40, 70 and 130 days from planting. Burd *et al.,* (2000) reported that plant growth promoting rhizobacteria (PGPR) might enhance plant growth parameters by producing phytohormones that increase the local availability of nutrients and facilitating the uptake of nutrients by the plants. The increasing percentage of plant height (cm) for Gmiza 9 cultivar by application of bacterial endophytes was about 16.59 % under normal irrigation water (100 % FC). On the other hand, for Sakha 93 cultivar the percentage was 16.71 % compared with control treatment. In addition, the increasing percentage of flag leaf area (cm^2) for Gmiza 9 cultivar by

application of bacterial endophytes was about 37.35 % under normal irrigation water (100 % FC). On the other hand, for Sakha 93 cultivar the percentage was about 45.69 % compared with control treatment.

The increasing percentage of plant height (cm) for Gmiza 9 cultivar by application of bacterial endophytes was 17.85 % under irrigation water deficit level (75 % FC). On the other hand, for Sakha 93 cultivar the percentage was 8.89 % compared with the same irrigation water deficit level without endophytes treatments.

The increasing percentage of fresh weight (g / plant) for Gmiza 9 cultivar by application of bacterial endophytes was about 55.93 % under irrigation water deficit level (50 % FC). For Sakha 93 cultivar the percentage was 54.66 % compared with the same irrigation water deficit level without endophytes treatments. On the other hand, concerning the dry weight the increasing percentage of Gmiza 9 cultivar was about 53.21 % under irrigation water deficit level (50 % FC). For Sakha 93 cultivar the percentage was about 39.89 % compared with the same irrigation water deficit level without endophytes treatments.

The increasing percentage of plant height (cm) for Gmiza 9 cultivar by application of bacterial endophytes was about 29.34 % under irrigation water deficit level (25 % FC). On the other hand, for Sakha 93 cultivar the percentage was 20.07 % compared with the same irrigation water deficit level without endophytes treatments.

Plants are sessile and prone to multiple stresses in changing environmental conditions. Of the several strategies adopted by plants to counteract the adverse effects abiotic stress e.g. drought stress, phytohormones provide signals to allow plants to survive under stress conditions. They are one of the key systems integrating metabolic and developmental events in the whole plant and the response of plants to external factors, and are essential for many processes throughout the life of a plant, influencing the yield and quality of crops.

Azotobacter sp besides fixing nitrogen it is also secrete certain growth hormones such as IAA, GA and Cytokinins (Coppola, 1971) which promote vegetative growth and root development. Auxin mediate numerous aspects of plant growth and development, including vascular tissues differentiation and root formation. Relatively little information is available on the changes in auxin content induced by water stress. Osmotic stress (150–300mM NaCl) decreased IAA content in tomato roots, but the leaf IAA content remained relatively unchanged (Dunlap and Binzel, 1996). Although root drying can decrease root auxin concentration by up to 70% (Masia, *et al*., 1994), it has not been investigated whether this changes xylem auxin concentration. Dehydration of detached leaves did not alter xylem auxin concentration (Hartung, *et al*., 1992). Auxin is considered to be the primary hormone involved in the initiation and growth of lateral and adventitious roots, ABA may play a role in regulating lateral root growth under stress conditions (De Smet *et al*. 2006). During drought conditions, *Arabidopsis* produces specialized, short lateral roots that remain in a dormant or no growing condition while the plant is under stress. These roots replace dehydrated lateral roots once drought conditions are relieved. Therefore, for *Arabidopsis*,

the additional ABA produced in response to drought stress inhibits the outgrowth of lateral roots from existing meristems (De Smet *et al*. 2006). This activity would stimulate root growth and adventitious root production; thus, increasing the vegetative growth parameters associated with drought conditions and with increased drought tolerance.

Many plants contain a mixture of different GAs, and at least 70 GAs have been isolated from natural sources. Cleavage of the ring system results in loss of activity. They are easily transported in both xylem and phloem. The little that is known about changes in endogenous GA content under water stress has been previously reviewed (Pospı and ilova, 2003), with either no change or decreases in GA content reported. The effects of foliar application of gibberellic acid (GA3) are variable (Pospı and ilova, 2003) although retardation of stomatal closure in water-stressed lettuce leaves following GA3 treatment has been observed (Aharoni, *et al*., 1977). This is consistent with a report that GA3 could reverse triazole-induced stomatal closure in isolated epidermal strips of Commelina benghalensis (Santakumari and Fletcher, 1987). Some nonstomatal effects of GA3 application (increased ribulose-1,5-bisphosphate carboxylase activity (Yuan and Xu, 2001) have been reported, thus any report of GA3 effects on photosynthesis should carefully analyze whether stomatal or nonstomatal effects are important.

Cytokinins are produced in plant meristematic regions including the roots (Chen, et. al., 1985) and transported in both the xylem and the phloem. Cytokinin is considered to be a negative regulator of root growth and branching, and root-specific degradation of cytokinin may also contribute to the primary root growth and branching induced by drought stress (Werner *et al*. 2010). Using *Arabidopsis* plants that display increased expression of cytokinin oxidase/dehydrogenase (CKX) genes under the control of a root-specific reporter, Werner *et al*. (2010) demonstrated that an increase in cytokinin degradation in the roots results in an increase in both primary root length and lateral root formation during drought conditions. This activity would stimulate root growth and lateral root production; thus, increasing the rootto-shoot is associated with drought conditions and with increased drought tolerance (Werner *et al*. 2010). Under osmotic stress, gibberellin and cytokinin are able to increase germination percentage and seedling growth in chickpea (Kaur *et al*. 1998, 2000). GAs or cytokinins were able to increase growth of plants under osmotic stress (Kaur *et al*. 1998). Yet, knowledge about the variation of cytokinin and gibberellin contents in plants under water stress is scarce (Yang *et al*. 2001; Xie *et al*. 2003).

Cytokinin was also shown to be a positive regulator of auxin biosynthesis, and it was postulated that a homeostatic feedback regulatory loop involving both CK and IAA signaling acts to maintain appropriate cytokinin and IAA concentrations in developing root and shoot tissues (Jones, *et al*., 2010). Yet, to date, little is known about the relationship of endogenous hormone contents and cell damage under water stress in maize plants.

Microbial inoculants that can promote plant growth and productivity is internationally accepted as an alternative source of N-fertilizer. It is environmental friendly and can be used to ensure a sustainable wheat production. In this bio-fertilizer technology new systems are being developed

to increase the biological N_2 fixation (BNF) with cereals and other non-legumes by establishing N_2-fixing bacteria within the roots (Cocking, 2000). Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective bio-fertilizer. Inoculation of associative and free living N_2-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed plant growth promoting rhizobacteria (PGPR) (Kloepper *et al*., 1980; Bashan and Holguin, 1998). Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998). They have been widely reported to fix atmospheric nitrogen with grasses and cereals (Dobereiner, 1997) and enhance nutrient uptake (Lin *et al*., 1983; Murty and Ladha, 1988). Increasing in essential nutritional elements and water absorption led to enhancement of vegetative growth parameters of the tested wheat cultivars in combination with irrigation water deficit under the study.

Yield parameters:

Regarding yield parameters e.g. spike dry weight, spike length, spikelets number/spike and grains number/spike were reduced in the tested wheat cultivars grown under the different irrigation water deficit during the both seasons (Tables, 4 and 5).

Table 4: Spike dry weight (gm) and spike length (cm) of wheat cultivars Gmiza 9 and Sakha 93 wheat plants as affected by different levels of irrigation water deficit and two different bacterial endophytes and their interactions during 2009 / 2010 season.

Treatment	Spike dry weight and length			
	Gmiza 9		Sakha 93	
	Spike dry weight	Spike length	Spike dry weight	Spike length
Control	1.69^{D}	12.83^{DE}	1.81^{D}	7.23^{DE}
E 1 Gs	2.61^{A}	14.93^{A}	2.74^{A}	8.77^{A}
E 1 Sp	2.47^{AB}	14.10^{B}	2.61^{B}	8.13^{B}
E 2 Gs	2.32^{B}	14.07^{BC}	2.75^{A}	8.30^{B}
E 2 Sp	1.89^{C}	13.37^{CD}	2.07^{C}	7.67^{CD}
W 1	1.11^{EF}	10.53^{HIJ}	1.48^{I}	6.27^{HI}
W 1 + E 1 Gs	1.62^{D}	13.27^{D}	1.71^{EF}	7.17^{EF}
W 1 + E 1 Sp	1.56^{D}	12.17^{EF}	1.58^{GH}	7.23^{DE}
W 1 + E2 Gs	1.63^{D}	13.50^{BCD}	1.76^{DE}	7.83^{BC}
W 1 + E 2 Sp	1.49^{D}	12.00^{F}	1.58^{GH}	7.33^{DE}
W 2	0.70^{HI}	9.40^{L}	1.28^{J}	5.30^{JKL}
W 2 + E1 Gs	1.15^{E}	11.70^{FG}	1.68^{EF}	6.73^{FGH}
W 2 + E1 Sp	1.13^{E}	10.83^{HI}	1.51^{HI}	6.47^{GHI}
W 2 + E 2 Gs	1.04^{EF}	11.63^{FG}	1.64^{FG}	6.87^{EFG}
W 2 + E 2 Sp	1.06^{EF}	11.20^{GH}	1.46^{I}	6.20^{I}
W 3	0.51^{I}	7.33^{M}	0.85^{L}	4.50^{M}
W 3 + E 1 Gs	0.83^{GH}	10.43^{IJ}	1.21^{JK}	5.73^{J}
W 3 + E 1 Sp	0.92^{FG}	9.90^{JKL}	1.14^{K}	5.17^{KL}
W 3 + E 2 Gs	0.69^{HI}	10.33^{IJK}	1.27^{J}	5.57^{JK}
W 3 + E 2 Sp	0.65^{HI}	9.67^{KL}	1.14^{K}	4.97^{L}

E1 = *Azotobacter chrocooccum* E2 = *Pseudomonas sp* Gs = Grains soaking Sp = foliar application control = 100 % field capacity W1 = 75% field capacity W2 = 50 % field capacity W3 = 25 % field capacity

Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test

The reduction in wheat yield under irrigation water deficit attributed with the reduction in vegetative growth parameters. Metwaly, (2012) indicated that, the adverse effect of irrigation water deficit on the same wheat cultivars related to the decrease in chlorophyll pigments contents, which led to the decreasing in net photosynthetic rate.

Table 5: Spikelets number and grains number of wheat cultivars Gmiza 9 and Sakha 93 wheat plants as affected by different levels of irrigation water deficit and two different bacterial endophytes and their interactions during 2009 / 2010 season.

Treatment	2009/2010 season			
	Gmiza 9		Sakha 93	
	Spikelet numbers	Grains number	Spikelet numbers	Grains numbers
Control	18.00^{CD}	26.67^{BCD}	11.33^{C}	26.67^{D}
E 1 Gs	19.67^{A}	34.00^{A}	14.33^{A}	32.00^{B}
E 1 sp	18.33^{BC}	29.33^{B}	13.33^{AB}	29.33^{C}
E 2 Gs	19.33^{AB}	29.33^{B}	13.67^{AB}	35.33^{A}
E 2 sp	18.33^{BC}	33.33^{A}	12.67^{B}	30.33^{C}
W 1	13.33^{HI}	24.00^{DEF}	8.33^{FG}	18.33^{H}
W 1 + E 1 Gs	16.33^{EF}	27.33^{BC}	11.33^{C}	24.67^{E}
W 1 + E 1 sp	15.67^{F}	25.33^{CDE}	10.33^{CD}	22.67^{FG}
W 1 + E2 Gs	17.00^{DE}	28.00^{BC}	11.33^{C}	26.33^{D}
W 1 + E 2 sp	15.33^{FG}	24.33^{DEF}	9.67^{DE}	21.33^{G}
W 2	11.00^{KL}	19.33^{G}	6.33^{HI}	15.67^{JK}
W 2 + E1 Gs	14.33^{GH}	25.33^{CDE}	10.33^{CD}	23.67^{EF}
W 2 + E1 sp	13.33^{HI}	23.33^{EF}	9.33^{DEF}	21.33^{G}
W 2 + E 2 Gs	15.33^{FG}	26.67^{BCD}	9.67^{DE}	23.00^{F}
W 2 + E 2 sp	12.67^{IJ}	22.33^{F}	8.67^{EF}	18.67^{H}
W 3	8.33^{M}	12.67^{J}	5.33^{I}	12.33^{L}
W 3 + E 1 Gs	11.67^{JK}	18.33^{GH}	9.33^{DEF}	18.33^{H}
W 3 + E 1 sp	11.33^{KL}	16.33^{HI}	7.33^{GH}	16.33^{IJ}
W 3 + E 2 Gs	11.00^{KL}	17.67^{GH}	9.00^{EF}	17.67^{HI}
W 3 + E 2 sp	10.33^{L}	14.67^{IJ}	6.67^{H}	14.67^{K}

E1 = *Azotobacter chrocooccum* E2 = *Pseudomonas sp* Gs = Grains soaking Sp = foliar application control = 100 % field capacity W1 = 75% field capacity W2 = 50 % field capacity W3 = 25 % field capacity

Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test.

In wheat, yield is greatly reduced mostly when drought stress occurs during the heading or flowering and soft dough stages. Drought stress during maturity resulted in about 10% decrease in yield, while moderate stress during the early vegetative period had essentially no effect on yield (Bauder, 2001). Gupta *et al*., (2001) studied physiological and yield attributes of two wheat genotypes with stress at boot and anthesis. They reported that number of grains, grain yield, biological yield and harvest index decreased to a greater extent when water stress was imposed at anthesis stage.

The increasing percentage of spike dry weight (g) for Gmiza 9 cultivar by application of bacterial endophytes was about 36.59, 38.44 and

50.00 % under irrigation water deficit level (75, 50 and 25 % FC) respectively. On the other hand, for Sakha 93 cultivar the percentage was about 11.92, 24.05 and 35.00 % under irrigation water deficit levels (75, 50 and 25 % FC) respectively. These results were compared with the each level of irrigation water deficit without endophytes treatments. The increasing percentage of spike length (cm) for Gmiza 9 cultivar by application of bacterial endophytes was about 8.93 % under normal irrigation water (100 % FC). On the other hand, for Sakha 93 cultivar the percentage was about 18.49 % compared with control treatment. Moreover, the increasing percentage of spikelets number for Gmiza 9 cultivar by application of bacterial endophytes was about 34.08 % under irrigation water deficit level (25 % FC). On the other hand, for Sakha 93 cultivar the percentage was about 43.48 % compared with the same irrigation water deficit level without endophytes treatments. In addition, the increasing percentage of 100 kernel weight (g) for Gmiza 9 cultivar by application of bacterial endophytes strains was 19.32, 21.08 and 31.69 % under irrigation water deficit level (75, 50 and 25 % FC) respectively. On the other hand, for Sakha 93 cultivar the percentage was 10.83, 11.36 and 14.13 % under irrigation water deficit levels (75, 50 and 25 % FC) respectively. These results were compared with the each level of irrigation water deficit without endophytes treatments.

Overcome or reduction of the bad effects of different irrigation water deficit by wheat grains socking or foliar application of endophytic bacterial strains due to synthesizing phytohormones such as auxin, cytokinin and gibberelin, which increase various growth and yield parameters. Moreover, increasing in essential mineral elements uptake enhanced the growth and yield parameters.

Finally, irrigation water deficit have an undesirable effect on growth and yield parameters of tested wheat cultivars. The grains soaking and foliar application of endophytic bacteria strains overcome the adverse effect of irrigation water deficit levels on all growth and yield parameters of wheat cultivars under this study. Moreover, endophytic bacteria strains application play an important role in protection of wheat plants against the adverse effects of drought stress, improve water productivity and will lead to significant water savings for irrigation sector. However, the study has raised an important question: Will the endophytic bacteria strains application of tested wheat cultivars have significant benefits under field conditions?

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تحسين نمو وانتاجية نباتات القمح تحت ظروف الإجهاد الجفافي باستخدام بعض البكتريا التي تنمو داخل النبات

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****فرع النبات الزراعي - *فرع ميكروبيولوجي زراعية**

أجريت هذه الدراسة في معمل وصوبة قسم النبات الزراعي – كلية الزراعة – جامعة كفرالشيخ خلال موسمي 2010/2009 و 2011/2010م لدراسة تأثير سلالتين من بكتريا تنمو داخل النبات وهما: *Azotobacter chrocooccum* (E1) و *Pseudomonas* sp. (E2) بنقع الحبوب ، أو الرش علي المجموع الخضري لصنفين من القمح هما سخا 93 وجميزة 9 علي صفات النمو الخضري والمحصولي. اشتملت صفات النمو الخضري علي ارتفاع النبات والوزن الطازج والجاف للنبات، وكذلك المساحة الورقية لورقة العلم وعدد الأوراق علي النبات. كما تضمنت صفات النمو المحصولي علي طول السنبلة ووزنها الجاف ، وعدد السنيبلات في السنبلة وعدد الحبوب في السنبلة ووزن 100 حبة. وقد تأثرت جميع الصفات بدون استثناء تحت الدراسة سلبيا تحت ظروف نقص ماء الري ، بينما أدت المعامله بالبكتريا سالفة الذكر الي الي زياده في مقاييس النمو الخضري والمحصولي. كما أدت المعاملة بكل من السلالتين البكتريتين إلي التغلب أو التقليل من الأثر السيئ لنقص ماء الري (الجفاف) والتحسين من النمو والانتاجية المائية والتي تعمل علي توفير الماء لقطاع الري.

قام بتحكيم البحث

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Table 1: Plant height of wheat cultivars Gmiza 9 and Sakha 93 as affected by different levels of irrigation water deficit and two bacterial endophytes and their interactions at different stages during 2009 / 2010 season.

Treatments	Gmiza 9				Sakha 93				Mean of means
	Plant height (cm) after				Plant height (cm) after				
	40 days	70 days	130 days	means	40 days	70 days	130 days	means	
Control	14.97^{CD}	40.67^{E}	49.33^{D}	34.99	17.50^{D}	45.67^{D}	49.67^{D}	37.61	36.3
E 1 Gs	18.33^{A}	47.33^{B}	59.00^{A}	41.55	20.83^{A}	56.33^{A}	59.33^{A}	45.49	43.52
E 1 Sp	17.27^{B}	45.67^{C}	56.33^{B}	39.76	18.57^{BC}	54.00^{B}	55.33^{BC}	42.63	41.19
E 2 Gs	15.27^{CD}	48.67^{A}	60.00^{A}	41.31	19.00^{B}	57.33^{A}	57.00^{B}	44.44	42.88
E 2 Sp	14.60^{D}	43.33^{D}	54.33^{C}	37.42	18.40^{BCD}	52.50^{C}	53.67^{C}	41.52	39.47
W 1	10.80^{H}	34.33^{GH}	44.00^{GH}	29.71	14.33^{GH}	40.67^{G}	39.67^{JK}	31.56	30.64
W 1 + E 1 Gs	17.27^{B}	40.33^{E}	48.33^{DE}	35.31	17.97^{CD}	44.67^{DE}	49.33^{D}	37.32	36.34
W 1 + E 1 Sp	15.33^{CD}	38.33^{F}	46.33^{F}	33.33	16.53^{E}	43.33^{EF}	45.67^{EFG}	35.18	34.26
W 1 + E2 Gs	16.83^{B}	40.00^{E}	49.00^{D}	35.28	15.93^{EF}	45.33^{D}	47.33^{E}	36.19	35.76
W 1 + E 2 Sp	14.50^{D}	35.67^{G}	47.00^{EF}	32.39	15.23^{FG}	42.33^{F}	42.67^{HI}	33.41	32.90
W 2	6.67^{J}	30.33^{IJ}	40.00^{J}	25.67	12.50^{J}	35.00^{J}	38.67^{K}	28.72	27.19
W 2 + E1 Gs	15.13^{CD}	37.33^{F}	45.67^{FG}	32.71	14.50^{GH}	42.33^{F}	46.67^{EF}	34.50	33.61
W 2 + E1 Sp	14.33^{DE}	35.33^{GH}	44.33^{GH}	31.33	14.50^{GH}	40.33^{GH}	44.33^{GH}	33.05	32.19
W 2 + E 2 Gs	15.67^{C}	37.33^{F}	45.33^{FG}	32.78	13.83^{HI}	44.67^{DE}	46.67^{EF}	35.06	33.92
W 2 + E 2 Sp	13.33^{FG}	34.00^{H}	43.33^{HI}	30.22	14.90^{G}	39.33^{GH}	41.33^{IJ}	31.85	31.04
W 3	9.83^{I}	25.00^{K}	35.33^{L}	23.39	12.97^{IJ}	32.33^{K}	35.00^{L}	26.77	25.08
W 3 + E 1 Gs	14.33^{DE}	34.67^{GH}	44.33^{GH}	31.11	14.53^{GH}	40.00^{GH}	45.33^{FG}	33.29	32.20
W 3 + E 1 Sp	12.83^{FG}	31.33^{I}	40.33^{J}	28.16	13.17^{IJ}	36.67^{I}	42.33^{I}	30.72	29.44
W 3 + Et 2 Gs	13.50^{EF}	35.33^{GH}	42.23^{I}	30.35	14.47^{GH}	39.00^{H}	44.67^{G}	32.71	31.53
W 3 + E 2 Sp	12.47^{G}	29.67^{J}	38.00^{K}	26.71	13.17^{IJ}	35.33^{J}	40.33^{JK}	29.61	28.16

E1 = *Azotobacter chrocooccum* E2 = *Pseudomonas sp* Gs = Grains soaking
Sp = foliar application control = 100 % field capacity W1 = 75% field capacity
W2 = 50 % field capacity W3 = 25 % field capacity
Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test