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ORIGINAL ARTICLES

Water Stress Mitigation on Growth, Yield and Quality Traits of Wheat (*Triticum aestivum* L.) Using Biofertilizer Inoculation

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ABSTRACT

The aims of this research were to investigate the effects of biofertilization with a mixture of different microbial and fungi strains on wheat growth, water use efficiency and yield under water stress conditions plant. Microbial strains were *Azospirillum brasilense, Bacillus megaterium var phosphaticum*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas fluorescence* as well as the plant growth-promoting fungus as arbuscular mycorrhizal fungi (AMF). The biofertilizer was mixed with wheat seeds (verity Sakha-93) immediately before cultivation. Chemical and mechanical analyses of soil and irrigation water were done before sowing. Generally, the results showed significant increases and improvements in irrigation water use efficiency, growth, yield, yield attributes, protein and carbohydrate contents of wheat due to biofertilizer treatments under water stress. Biofertilizer inoculation increased grain, straw and biological yield/faddan by 26, 18 and 20 %, respectively. Meanwhile, biofertilizer inoculation reported about 30% increase in water use efficiency and irrigation water use efficiency increased from 1.12 to 1.46 for without biofertilizer and with biofertilizer treatments, respectively. It may be concluded from this study that biofertilizer inoculation to newly reclaimed soil is effective in improving crop productivity. It is unlikely that a single strain in the mixture was responsible for this but is more likely to be due to the mixture of several strains which save and ease nutrient supply to wheat. These increases represent applicable practice which may help to obtain a greater sustainability of the agro ecosystems.

Key words: Wheat, Biofertilizer, Water stress, Growth, Yield

Introduction

Wheat (*Triticum aestivum* L.) is the world's most important crop. Greater importance of bread wheat can be expected as a main source of food for solving the increasing population of the world. In arid and semiarid regions with Mediterranean climate, wheat crop usually encounter drought during the grain filling period. Wheat quality is controlled not only by genetic factors, but also by environmental conditions, especially the supply of water and fertility in soil that can change wheat quality under normal cropping condition (Triboi *et al*., 2003).

Drought is a worldwide problem, constraining global crop production and quality seriously and recent global climate change has made this situation more serious (Dobbelaere and Okon, 2003; Frommel *et al*., 1993). In many regions of the world, drought stress is one of the most important factors that decrease agricultural crop production (Zahedi *et al*., 2009). In this sense, the ability of a given plant cell to tolerate a restricted water supply depends on three known physiological mechanisms of adaptation: (*i*) active or passive solute accumulation in vacuoles, (*ii*) changes in cell wall elasticity, and (*iii*) changes in the relative partitioning of water into apoplastic and symplastic fractions (Girma and Krieg, 1992). The percentage of drought affected land areas doubled from the 1970s to the early 2000s in the world (Isendahl and Schmidt, 2006). About 33% of wheat fields in the world and about 55% in the developing countries are suffering from drought stress. In these regions, water deficit influences all developmental stages of wheat from germination to seed formation and finally yield (Trethowan et al., 2001).

Drought stress affects wheat growth from germination till physiological maturity. The stress response in wheat and other crops depends upon the severity of the stress, rate, duration of exposure and the plant developmental stage (Bartel and Bartel, 2003). In wheat, germination, tillering and reproductive stages are considered the most sensitive stages to drought stress (Casati and Walbot, 2004). Drought is a complex physical chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements (Creus, *et al*., 2004 and Cakmakc *et al*., 2006). Katerji *et al*., (2009) reported that drought reduced the grain (37%) and straw (18%) yield. Johari-pireuvatlou (2010) reported that wheat yield decreased from 25 to 85% under drought stress. Maralian *et al*., (2010) reported that seed yield reduced with water stress as compared with the control. If water

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stress occurred at tillering or heading stages, the seed yield decreased more than 37%. Tatar and Gevrek (2008) showed that wheat dry mater production decreased and proline content increased under drought stress. Ashraf *et al*., (1994) reported that drought stress will reduce concentration of chlorophyll b more than chlorophyll a.

The extensive use of chemical fertilizers has disturbed the delicate ecological balance of the soil, contaminated groundwater, developed resistant races of pathogens and increased human health risks (Tawfik *et al*., 2006). During the last two decades, because of negative environmental impact of chemical fertilizers and their increasing costs, application of soil microorganisms in various parts of the world has increased. Plant growth-promoting bacteria (PGPB) are a diverse group of soil microbes capable of increasing crop yields. Most bacterial strains investigated belong to the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Bacillus* however; some are members of the *Enterobacteriaceae* (Chandanie-Kubota and Hyakumachi, 2006). When present in plants in proper amounts, they stimulate the density and length of root hairs, the rate of appearance of lateral roots, root surface area, improve plant growth under water stress conditions, produce growth regulators, fix nitrogen and solubilize inorganic phosphates (Babana *et al.*, 2012). On this regard, some plant growth-promoting fungi (PGPF) e.g. arbuscular mycorrhizal fungi (AMF) that form mutual endosymbiosis with plant roots are very important to benefit crop plants overcome their biotic and abiotic stresses. Arbuscular mycorrhizal fungi (AMF) symbiosis is known to alter the microbial population composition quantitatively and qualitatively in the mycorrhizosphere (mycorrhizosphere effect) as a result of altered host physiology and root exudates. These effects cause roots to take up more water and mineral nutrients resulting in faster plant growth. Therefore, under appropriate agronomic conditions, these processes would increase crop yield (Alejandra-Pereyra *et al*., 2009). This investigation was carried out to study water stress and biofertilizer inoculation effects on growth, yield, and quality traits of wheat under water stress conditions.

Materials and Methods

Two split plot experiments with three replications were conducted at the Agricultural Production and Research Station, National Research Centre (NRC), El Nubaria Province, Egypt, during the two successive winter seasons 2010/2011 and 2011/2012, to study the effect of biofertilizer inoculation (with biofertilizer or without biofertilizer) and reducing irrigation requirements (100% IR, 80% IR, 60% IR and 40% IR) on growth, yield, and quality traits of wheat under water stress conditions in the newly reclaimed sandy soil. Biofertilizer inoculation and water requirement treatments were allocated in the main and sub plot, respectively. Soil sample was taken at different depths from 0 to 60 cm for mechanical and chemical analyses as described by Chapaman and Pratt (1978) (Table 1 and 2).

Table 1: Chemical and mechanical analyses of soil.

Character Depth (cm)	Chemical analysis				Mechanical analysis			Texture
	OM (%)	pH (1:2.5)	EC (dSm^{-1})	$CaCO_3$ %	Course sand	Fine sand	Silt clay	
0-20	0.65	8.70	0.35	7.02	47.76	49.75	2.49	Sandy
20-40	0.40	8.80	0.32	2.34	56.72	39.56	3.72	
40-60	0.25	9.30	0.44	4.68	36.76	59.40	3.84	

Table 2: Characteristics of soil.

Character Depth (cm)	Saturation Point (%)	Field Capacity (%)	Wilting Point (%)	Available Water (%)	Hydraulic conductivity(cm/hr)
0-20	21.00	10.10	4.70	5.40	22.50
20-40	19.00	13.50	5.60	7.90	19.00
40-60	22.00	12.50	4.60	7.90	21.00

The soil was ploughed twice and divided into plots, during seed preparation, 150 kg calcium super phosphate/fed (15.5% P_2O_5) and 50 kg potassium sulfate (48 % K_2O) were added. The experimental unit consisted of 15 rows each of 3.5 meter length and 20 cm between rows where, the size of each plot was 10.5 m^2 (1/400 faddan).

Wheat grains of cultivar Sakha-93 sown by drilling seed manually in the rows at 20-cm apart at the rate of 60 kg/fad (faddan=4200 m^2). Sowing date was on 17th and 20th November in 1st and 2nd season, respectively. 120 kg N/fed as ammonium sulphate (20.6% N) was added in six equal doses after complete germination and every two weeks till beginning spike emergences stage. Sprinkler irrigation was applied as plants needed (3/4" diameter and discharge was 1.2 m^3/h at 2.5 bar operating pressure and 12 m service radius).

Microbiological biofertilizer used:

The biofertilizer under our study was consisted of two parts, the first was bacterial mixture of *Azospirillum brasilense, Azotobacter chrococcum, Bacillus megaterium var phosphaticum* and *Pseudomonas sp.* as soil plant growth-promoting bacteria (PGPB) and the second part was arbuscular mycorrhizal fungi (AMF) as plant growth-promoting fungus (PGPF). The microorganisms strains were obtained from Agricultural Microbiology Department, NRC (Hoballah *et al.*, 2012). Each bacterium strain was grown in the appropriate liquid medium up to reaching about 10^5 to 10^6 CFU/ ml. Arbuscular mycorrhizal conidia were extracted from soil by wet sieving and sucrose density gradient centrifugation according to Syliva et al., (1993) then carried on vermiculite (commercial clay mineral) for giving mycorrhizal inoculum. Mycorrhizal conidia counted were 100-150 mycorrhizal spores/gram vermiculite in the mycorrhizal inoculums and was determined by binocular stereo microscope (Olympus SZ). At sowing, the mixture of 10 ml of each liquid medium were added to 1 kg of vermiculite as a carrier then mixed with 1kg of mycorrhizal inoculum and 200 ml of 1% Arabic gum solution as an adhesive agent for producing the microbiological biofertilizer mixture used. Wheat grains (verity Sakha-93) were mixed with the inoculants immediately just before cultivation.

Total water irrigation (m^3/faddan/season) for the district was estimated according to the meteorological data of the Central Laboratory for Agricultural Climate (CLAC) depending on Penman-Monteith equation was shown in Fig. (1), the seasonal water irrigation applied was found 2060 m^3/faddan (100 % IR), 1648 m^3/faddan (80 % IR), 1236 m^3/faddan (60 % IR) and 824 m^3/faddan (40 % IR). Normal cultural practices of growing wheat conducted in the usual manner followed by the farmers of this district.

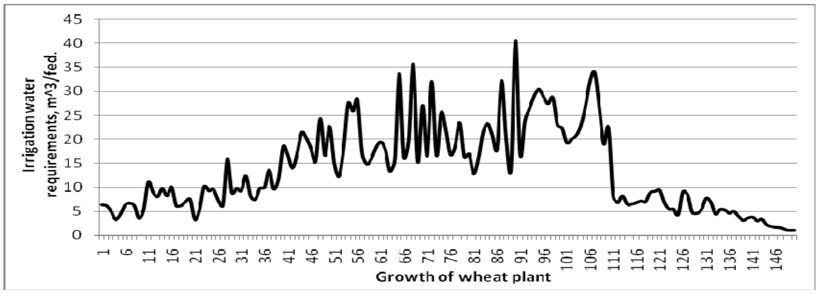

Fig. 1: The relation between growth of wheat plant and irrigation water requirements.

Data recorded:

Growth characteristics:

During both growing seasons, wheat plants from each plot - in quarter square meter- were cut from ground surface at heading time. Plant height (cm), number of leaves /main stem, flag leaf area (cm^2) and Chlorophyll content were recorded. Flag leaf area (cm^2) was estimated according to the method described by Bremner and Taha, (1966). Total chlorophyll was estimated according to Richards and Thompson (1952). Leaf area = leaf length x maximum leaf width x 0.75 according to (Stickler et al., 1961).

Yield and yield attributes:

At harvest, a random sample of 50 cm length X 50 cm width was taken from each plot to determine, spike length (cm), number of spikes/m^2, number of spikelets /spike, seed index. In addition, grain, straw and biological yields “ton/faddan” were determined from the whole area of experimental unit and then converted to yield per faddan.

Irrigation water use efficiency (IWUE):

IWUE was calculated according to James (1988) as follows: IWUE (kg/m^3) =Total yield, (kg/fad.) / Total applied irrigation water, (m^3/fad./season).

Grain protein content:

Total N content in grains determined and protein% was calculated by multiplying N-content by 6.25 according to Chapman and Pratt (1978)

Total carbohydrate content of wheat, %:

Total carbohydrates, were determined (as glucose) after acid hydrolysis and spectro photometrically determined using phenol sulfuric acid regent according to Dubbois *et al*. (1956).

Statistical analysis:

The analysis of variance of split plot experiment was carried out using MSTAT-C Computer Software Program (MSTAT, 1988). Since the trend was similar in both seasons, Bartlett's test and the combined analysis of the two growing seasons were done. Least significant difference test was applied at 0.05 probability level to compare mean treatments.

Results and Discussions

1. Growth characteristics:

1.1. Effect of drought and biofertilizer inoculation on some growth characteristics of wheat:

Data presented in Table (3) show that effect of biofertilizer inoculation and reduction of water requirements on plant height, number of leaves/main stem, flag leaf area and total chlorophyll. Biofertilizer inoculation affected significantly on plant height and total chlorophyll while, number of leaves /main stem and flag leaf area did not show any significant differences. These results are in coincidence with these obtained by (Christiansen *et al*., 1991) who reported that, significant increase in plant dry matter by wheat when inoculated with various soil microorganisms such as *Azospirillum brasilense, Azotobacter chrococcum, Bacillus megaterium var phosphaticum* or *Pseudomonas* sp.. Also, such microorganisms stimulate the density and length of root hairs, the rate of appearance of lateral roots, root surface area, improve plant growth under water stress conditions and plant dry weight (Andres –Naiman *et al*., 2009 and Babana *et al*., 2012).

Table 3: Effect of drought and biofertilizer inoculation on some growth characteristics of wheat.

Character Treatment		Plant height (cm)	Leaves number/main stem	Total chlorophyll (mg/g fresh weight)	Flag leaf area (cm^2)
Biofertilizer	With	117.34	4.83	34.48	23.92
	Without	114.17	4.25	29.95	22
LSD 0.05		0.95	NS	0.55	NS
Irrigation requirements	100% IR	119.5	5.16	41.21	28.54
	80% IR	117.5	4.5	35.18	24.67
	60% IR	115.83	4.33	28.85	19.34
	40% IR	110.17	4.16	23.68	19.34
LSD 0.05		3.23	NS	7.57	2.92

IR: Irrigation Requirements

Decreasing the irrigation requirements from 100% to 40% significantly decreased most of studied characters where, decreasing the irrigation requirements (from 2304 m^3 to 992 m^3) decrease plant height from 119.5 to 110.17cm, flag leaf area from 28.54 to 19.34 cm^2 and total chlorophyll from 41.21 to 23.68, respectively. Meanwhile, decreasing irrigation requirements from 100% to 80% did not show significant differences in plant height and total chlorophyll, while decreasing irrigation requirements from 100% to 60 % significantly decreased most of studied characters except number of leaves/main stem. Decreasing irrigation requirements from 80% to 60% did not show significant differences in the studied characters, except flag leaf area, while decreasing irrigation requirements from 60% to 40% did not show significant differences in most of studied characters except, plant height. These differences may be due to changes in photosynthesis process, which is the most significant process influence crop production, which is also inhibited by drought stress. Some photosynthesis process studies have shown that the photosynthetic rate of leaves decreases as relative water content and water potential decrease (Cornic and Massacci, 1996). Limitation of net photosynthetic rate in low moisture stressed plant is mainly through stomatal closure (Cronic, 2000) and/or by metabolic impairment (Flexas and Medrano, 2002). These results are in harmony with those obtained by Tatar and Gevrek (2008),they showed that wheat dry mater production decreased under drought stress. Ashraf *et al.,* (1994) reported that drought stress will reduce concentration of chlorophyll b more than chlorophyll a.

1.2. Effect of interaction between drought stress and biofertilizer inoculation on some growth characteristics of wheat:

Data presented in Table (4) show that effect of interaction between biofertilizer inoculation and reducing of water requirements (from 100% to 40% irrigation water requirements) on plant height, leaves number/main stem, flag leaf area and total chlorophyll where, most studied characters significantly affected except, number of leaves /main stem. However, biofertilizer inoculation + 100% irrigation requirements significantly surpassed the other treatments except, biofertilizer inoculation+ 80% irrigation requirements. The data presented show that the use of biofertilizer inoculation could save better circumstances for nutrient release especially under low irrigation levels, consequently greater water use efficiency to produce better biomass so the reduction of irrigation requirements from 100 % to 80 % offset the impact of the shortage in irrigation requirements in the studied characters. Water availability mostly affects accumulation of some organic compatible solutes such as sugars, betaines and proline which adjust the intercellular osmotic potential is also early reaction of plants to water stress. Zlatev and Stoyanov (2005) suggested that proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. Vendruscolo *et al.*, (2007) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. Also, PGPF when present in plants in proper amounts, they stimulate the density and length of root hairs, the rate of appearance of lateral roots, root surface area, improve plant growth under water stress conditions, produce growth regulators, fix nitrogen and solubilize inorganic phosphates (Andres–Naiman *et al*., 2009 and Babana *et al*., 2012). Moreover, some plant growth-promoting fungi (PGPF) e.g. arbuscular mycorrhizal fungi (AMF) that form mutual endosymbiosis with plant roots are very important to benefit crop plants overcome their biotic and abiotic stresses. These effects cause roots to take up more water and mineral nutrients resulting in faster plant growth. Therefore, under appropriate agronomic conditions, these processes would increase crop yield (Alejandra-Pereyra *et al.,* 2009).

Table 4: Effect of interaction between drought stress and biofertilizer inoculation on some growth characters of wheat.

Character Treatment		Plant height (cm)	Leaves number/main stem	Total chlorophyll (mg/g fresh weight)	Flag leaf area (cm^2)
With biofertilizer	100% IR	121.00	5.67	42.85	28.67
	80% IR	119.67	5.00	39.62	28.33
	60% IR	117.00	4.33	37.64	27.33
	40% IR	116.67	4.33	32.75	22.00
Without biofertilizer	100% IR	115.34	4.66	31.03	20.67
	80% IR	115.00	4.00	26.57	19.67
	60% IR	111.00	4.00	26.43	19.00
	40% IR	109.34	4.33	20.93	18.00
LSD $_{0.50}$		1.53	NS	3.67	2.69

IR: Irrigation Requirements

2. Yield and yield attributes:

2.1. Effect of drought stress and biofertilizer inoculation on yield and yield attributes of wheat:

Data presented in Table (5) illustrated that biofertilizer inoculation of wheat significantly increased number of spikelets/spike, 100-seed weight, grain, straw and biological yield/faddan while, spike length and number of spike/m^2 did not significantly affected by biofertilizer inoculation. Biofertilizer inoculation tended to increase in the grain, straw and biological yield/faddan by 26, 18 and 20 %, respectively. These results are in harmony with Puente *et al*., (2005) observed number of spikelets/spike when wheat seeds where inoculated with several *Azospirillum brasilense* strains. Kloepper *et al*., (1992) has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Some investigators indicated that grain yield can be improved by *Azospirillum* inoculation (Okon and Labandera, 1994). Root association with *Azospirillum* spp. has been shown to promote vegetative growth in several plant species (Baldani *et al*., 1987). Also it was found that apart from fixing atmospheric nitrogen under limited conditions (Boddey and Döbereiner, 1988). *Azospirillum*, in general, stimulated both rates of root elongation and appearance of lateral and adventitious roots (Okon *et al*., 1988).

With regard to irrigation requirements, reducing of irrigation water duty from 100 % to 40 % significantly decreased most studied characters except, spike length and biological yield/faddan, where spike length decreased from 11 to 9.5 cm, number of spikelets/spike from 17.34 to 14.16, seed yield /plant from 2.62 to 1.8 g, seed index from 4.80 to 4.31 g , number of spike / m^2 from 491 to 457, grain yield /faddan from 2.43 to 0.92

ton, straw yield /faddan from 6.27 to 5.36 ton and biological yield/faddan from 7.85 to 7.18 ton, respectively. Decreasing irrigation water quantities from 100% to 80% did not show significant differences in most of studied character except seed yield/plant, seed index and grain yield/faddan, while decreasing irrigation water requirements from 100% to 60 % significantly decreased most of studied characters except, spike length, straw and biological yield /faddan. Decreasing irrigation water requirements from 80% to 60% did not show significant differences, except, number of spike/ m^2, while decreasing irrigation requirements from 60% to 40% did not show significant differences in most of studied characters except, number of spike/m^2 and grain yield/faddan. Water stress interferes with both the production of photo assimilate and the import of assimilated material into the developing grains. Yield potential in cereals is apparently limited by the capacities of both the sink (Fischer *et al*., 1977) and the assimilatory source (Fischer and HilleRisLambers, 1978). Several workers have reported that kernel growth increased in response to both artificial reductions in sink capacity or artificially increased assimilatory capacity (Blade and Baker, 1991). Conversely, kernel growth has been shown to be retarded by reductions in assimilation near anthesis (Jedel and Hunt, 1990) indicating that assimilate supply controls expression of kernel weight. Theoretically, increasing the number of kernels that comprise the sink even at the expense of kernel weight can increase grain yield. Blade and Baker (1991) expressed the view that sink-related limitations to spring wheat yield are not important in a semi-arid environment where drought stress typically limits photoassimilation. These results are agreement with those obtained by Katerji *et al*., (2009), Johari-pireuvatlou (2010) and Maralian *et al*., (2010). Studies have shown that drought stress reduced all yield components so that numbers of fertile spikes as well as number of grain per spike decreased by 60% and 48%, respectively (Giunta *et al*., 1993).

Table 5: Effect of drought stress and biofertilizers on yield and yield attributes of wheat.

Character Treatment		Spike length (cm)	Spikelets number/ spike	100-seed weight (g)	Spikes number/ m^2	Yield (ton fed^{-1})		
						Biological	Straw	Grain
Biofertilizer	With	10.56	16.42	4.78	497.00	5.31	3.23	2.08
	Without	9.67	14.08	4.23	475.25	3.91	2.27	1.64
LSD 0.05		NS	1.56	0.32	NS	0.66	0.64	0.27
Irrigation requirements	100% IR	11.00	17.34	4.80	491.00	4.80	2.37	2.43
	80% IR	10.00	15.33	4.51	487.67	4.85	2.68	2.17
	60% IR	9.83	14.17	4.43	475.00	4.60	2.68	1.92
	40% IR	9.50	14.16	4.31	457.00	4.18	3.27	0.92
LSD 0.05		NS	2.05	0.18	6.71	NS	NS	0.07

IR: Irrigation Requirements

2.2. Effect of interaction between drought stress and biofertilizer inoculation on yield and yield attributes of wheat:

Data presented in Table (6) showed that the interaction between biofertilizer inoculation and reducing irrigation water requirements on yield and yield attributes, where the different treatments show significant differences on the studied characters except, spike length and seed index. However, biofertilizer inoculation + 100% irrigation requirements surpassed other treatments in the studied characters with significant differences except biofertilizer inoculation+ 80% irrigation requirements without significant differences between the two treatments except, number of spikelets/spike. Biofertilizer inoculation + 100% irrigation requirements recorded the highest values of different studied characters followed by biofertilizer inoculation+ 80% irrigation requirements while 40% irrigation requirements without biofertilizer inoculation recorded the lowest values of different studied characters. Decreasing of irrigation water requirements from 80 to 40 % with biofertilizer inoculation showed significant differences in some studied traits i.e., number of spikes/m^2, grain and biological yield/faddan. Okon, (1985) reported that as a consequence of deeper plant rooting, inoculated plants showed enhanced mineral and water uptake, which in turn could benefit crops growing in water-deficient soils**.**

3. Water Use Efficiency:

3.1. Effect of drought stress and biofertilizer inoculation on irrigation water use efficiency of wheat:

Data presented in Table (7) illustrated that biofertilizer inoculation significantly increased irrigation water use efficiency of wheat compared to non-treated one, however, it achieved about 30% increase in water use efficiency where, irrigation water use efficiency increased from 1.12 to 1.46 for without biofertilizer and with biofertilizer treatments, respectively. Ghazi, (1998) reported that the inoculated plants used less water to produce one unit of shoot dry matter, in other words higher water-use efficiency than non-inoculated plants. Higher WUE in inoculated plants than non-inoculated plants may be due to that inoculation increased the ability of roots to absorb soil moisture, thus maintaining opened stomata in leaves and enhancing dry matter production. Enhanced water conductivity has been attributed to increase the area for water uptake provided by inoculation in soil (Bethlenfalvay *et al*., 1988).

Table 6: Effect of the interaction between drought stress and bio-fertilizers on yield and yield attributes of wheat.

Character Treatment		Spike length (cm)	Spikelets number/ spike	100-seed weight (g)	Spikes number/ m^2	Yield (ton fed^{-1})		
						Biological	Straw	Grain
With biofertilizer	100% IR	11.33	19.00	5.20	497.00	5.40	2.80	2.60
	80% IR	10.34	15.67	4.80	495.34	5.53	3.20	2.33
	60% IR	10.35	15.66	4.67	473.00	5.73	3.43	2.30
	40% IR	10.00	15.34	4.46	452.00	4.57	3.50	1.07
Without biofertilizer	100% IR	10.67	15.67	4.40	485.00	4.20	1.93	2.27
	80% IR	9.67	15.00	4.20	480.00	4.17	2.17	2.00
	60% IR	9.34	12.67	4.21	474.00	3.47	1.93	1.53
	40% IR	9.00	13.00	4.13	462.00	3.80	3.03	0.77
LSD at 5% level		NS	2.24	NS	4.33	NS	NS	NS

IR: Irrigation Requirements

Regarding irrigation requirements, reducing of water requirements from 100 % to 40 % significantly affected water use efficiency of wheat plants where, 60% irrigation requirements achieved the highest water use efficiency (1.55), with significant differences with other water requirement treatments followed by 80% irrigation requirements, 100% irrigation requirements and 40% irrigation requirements, respectively where no significant differences between the three treatments.

Table 7: Effect of drought stress and biofertilizers on irrigation water use efficiency of wheat.

Treatment Character	Biofertilizers		LSD $_{0.05}$	Irrigation requirements				LSD $_{0.05}$
	With	Without		100% IR	80% IR	60% IR	40% IR	
Irrigation Water Use Efficiency	1.46	1.12	0.12	1.18	1.31	1.55	1.12	0.20

IR: Irrigation Requirements

3.2. Effect of interaction between drought stress and biofertilizer inoculation on water use efficiency of wheat:

Fig. (3) illustrate the effect of the interaction between biofertilizer and irrigation requirements on irrigation water use efficiency where no significant differences among treatments was observed. The treatment biofertilizer + 60 % irrigation requirements recorded the highest water use efficiency followed by the treatments biofertilizer+80 % irrigation requirements, biofertilizer+ 100 % irrigation requirements and biofertilizer + 40 % irrigation requirements, respectively. The absence of biofertilizer in the different irrigation combinations recorded the lowest irrigation water use efficiency values**.** Inoculation enhanced plant dry matter in wheat genotypes tested under both well-watered and water stressed conditions. Enhanced growth effects on inoculated plants have been attributed to improve water relations resulting from enhanced P nutrition (Ruiz-Lozano *et al*., 1995).

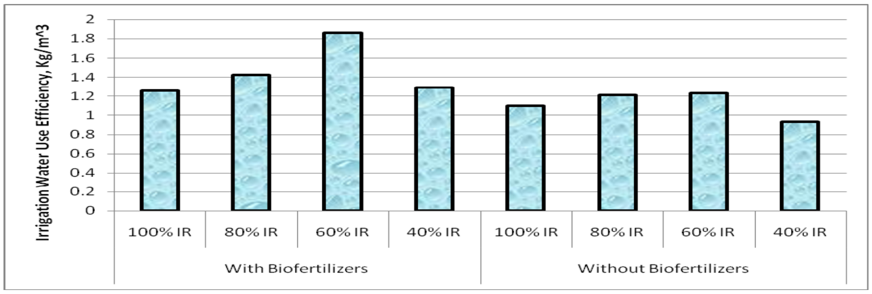

Fig. 3: Effect of interaction between drought stress and biofertilizers on irrigation water use efficiency of wheat.

4. Grains quality:

4.1. Effect of drought stress and biofertilizer inoculation on protein and carbohydrate contents of wheat:

Data presented in Table (8) show that biofertilizer inoculation significantly increased protein and carbohydrate contents of wheat compared to the non-inoculated treatments, inoculation resulted in 12% and 14% increase in protein and carbohydrate contents, respectively. The increased wheat grain protein content could be attributed to an increase in root uptake of inorganic N (Saubidet *et al*., 2002).

Regarding irrigation requirements, reducing of water requirements from 100 % to 40 % significantly affected on protein and carbohydrate contents of wheat plants where, 100% irrigation requirements achieved the highest protein (12.48 %) and carbohydrate contents (76.67%), with significant differences with other water requirement treatments flowed by 80% irrigation requirements, 60% irrigation requirements and 40% irrigation requirements, respectively. Hasanpour *et al*., (2012) reported that light stress treatment caused a non-significant increase for protein percentage but this was significant under severe stress treatment.

Table 8: Effect of drought stress and bio-fertilizers on protein and carbohydrate contents of wheat.

Treatment Character	Biofertilizers		$LSD_{0.05}$	Irrigation requirements				$LSD_{0.05}$
	With	Without		100% IR	80% IR	60% IR	40% IR	
Protein content (%)	11.68	10.42	0.13	12.48	11.68	10.53	9.48	0.44
Carbohydrate content (%)	77.02	67.28	2.46	76.67	73.50	70.90	67.52	2.14

IR: Irrigation Requirements

4.2. Effect of the interaction between drought stress and biofertilizer inoculation on protein and carbohydrate contents of wheat:

Data presented in Figs (4 and 5) show that effect of the interaction of bifertilizer and water irrigation requirements on protein and carbohydrate contents in wheat. Insignificant differences were observed among the treatments. Biofertilizer + 100% irrigation requirements treatment recorded the highest protein and carbohydrate contents followed by biofertilizer + 80% irrigation requirements while, the lowest recorded values was reported without biofertilizer + 40% irrigation requirements for both characters protein and carbohydrate contents. Creus *et al*., (2004) concluded that inoculated wheat plants under water stress during anthesis exhibited better growth due to an ''elastic adjustment'' that enhanced grain yield and quality. Hasanpour *et al*., (2012) reported that the highest amount for protein percentage was obtained from the dual inoculation treatment. The dual inoculation treatment was the best treatment in terms of yield, grain protein in drought conditions.

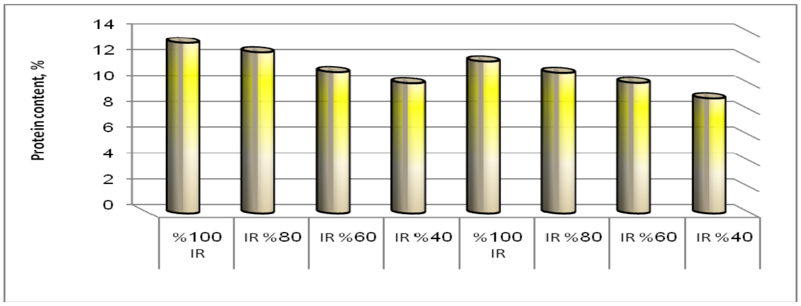

Fig. 4: Effect of the interaction between drought and biofertilizer inoculation on protein percentage of wheat.

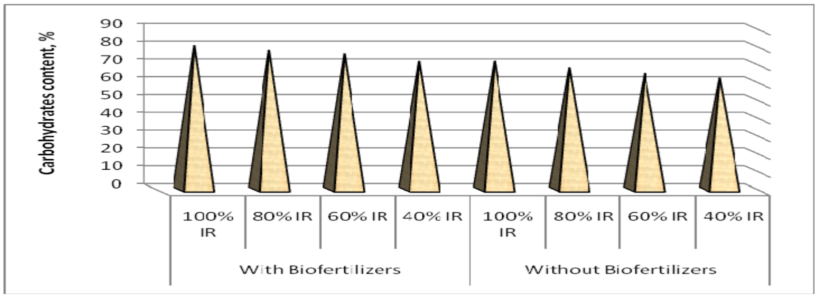

Fig. 5: Effect of the interaction between drought stress and biofertilizer inoculation on carbohydrates content of wheat.

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