

Using two plant growth promoting bacteria to sustainably the drought-induced loss in *Triticum aestivum* yield

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Abstract

In a greenhouse experiment, the inoculated and uninoculated grains with *Azospirillum brasilense* NO40 or *Stenotrophomonas maltophilia* were sown in unsterilized sandy soil and watered normally till the 8th day. Thereafter, the drought stress was initiated by watering pots once every 10 days while the unstressed pots were irrigated normally once every 5 days. Samples of spikes and dry grains were collected after 120 days from sowing. The results indicated that the inoculated-drought-stressed plants maintained significantly higher values of all of the measured yield parameters, where the yielded grains had higher amounts of the direct reducing sugars, sucrose, starch; lower contents of total soluble proteins and the total free amino acids, and altered protein patterns compared to those of the uninoculated-drought-stressed plants. SDS-PAGE of the yielded grains showed that drought led to the appearance of some newly synthesized stress protein bands and disappearance of others. Inoculation with PGPB resulted in the re-appearance of some of the disappearing bands and the synthesis of new others. Meanwhile, wheat inoculation under normal conditions resulted in significantly promoted grain yields with higher contents of carbohydrates, total soluble proteins and total free amino acids than that of the uninoculated control. It has been proved that treating wheat plants with the PEG-tolerant *Azospirillum brasilense* NO40 or *Stenotrophomonas maltophilia* that were compatible with the systems into which they were introduced and possess multiple plant growth promoting traits, can be an efficient strategy to enhance wheat growth and productivity, not only under normal conditions, but also under drought stress.

Keywords: *Azospirillum brasilense* NO40; carbohydrate; protein patterns; soluble proteins; *Stenotrophomonas maltophilia*; *Triticum aestivum*; wheat; yield criteria

Introduction

Wheat (*Triticum aestivum* L.) is the most important grain crop in Egypt, representing almost 10% of the total agricultural production, 20% of all agricultural imports, and more than 60% of the imported grains (FAO and EBRD, 2015). Furthermore, Egypt is the world's largest importer of wheat (FAO and EBRD, 2015;

FAS/USDA, 2018). One of the key strategic targets is to sustainably maximize the economic return per unit of land and water in general, and for wheat in particular, in order to cope with the food demands of the rapidly growing population, that on average, over a quarter of it lives under the poverty line, with hopefully no or with minimal expansion in imports, especially with the significant depreciation in the Egyptian Pound against the abrupt rise in the world wheat prices (FAO and EBRD, 2015; WFP/EB, 2018). However, achieving this target has become more challenging due to the current and aggravating fresh water shortage (FAO, 2015; OED/FAO, 2018).

Egypt is a water-poor country where the fresh water resources are limited: fixed quota of Nile water (the main source), shallow groundwater reservoirs and small amounts of rainfall (FAO-Aquastat, 2018), with projections suggesting that by 2025, Egypt will reach the absolute water scarcity ($500 \text{ m}^3/\text{capita}/\text{year}$) (MIWR, 2014), and by 2050, Egypt will be at severe water stress (Damania *et al.*, 2017). In addition to being limited, the water resources are deteriorating: in quality due to the alarming levels of water pollution by the untreated sewage, industrial effluents, and agricultural runoff laden with fertilizers (WB, 2017), and in quantity due to the heavy consumption by the ever-increasing population, and by the presence of much irrigated agriculture (Mekonnen and Hoekstra, 2016), and due to the complicated relationships with the Nile Basin states (OED/FAO, 2018). Moreover, the ongoing climate change is predicted to increase the variability and uncertainty about the timing and intensity of rainfall, and to elevate temperatures which in turn will lead to higher evapotranspiration rates and more crop water requirements (MSEA, 2010; FAO, 2015; WB, 2017). Thus, to secure enough wheat for the fast-growing population of Egypt, high yields of wheat must be sustained, but with less amounts of water.

However, the lack of adequate moisture, i.e. drought stress, permanent or temporary, can cause significant damage for the plant at any developmental stage, hence, drought has been known as the most important limiting factor for crop growth and productivity worldwide (Daryanto *et al.*, 2017). The drought-induced yield loss varies depending on the plant species, genotype, age and developmental stage; organ or cell identity; the stress severity, duration and progression rate; and on the surrounding conditions (De Leonardis *et al.*, 2012; Claeys *et al.*, 2014; Ngara and Ndimba, 2014).

The beneficial soil microbes such as the plant growth promoting bacteria (PGPB) have been welcomed as a relatively simple, low-cost, environmentally sustainable solution for improving the crops' tolerance to drought stress, and therefore reducing the drought-mediated yield losses (Singh and Singh, 2013; Ngumbi and Kloepper, 2016). PGPB can induce physical and chemical changes in plants that result in enhanced tolerance to abiotic stress; these changes have been termed induced systemic tolerance (IST) by Yang *et al.* (2009).

In our previous study (Omar *et al.*, 2017) two PGPB (*Azospirillum brasilense* NO40 and *Stenotrophomonas maltophilia*) were selected as promising candidates for enhancing the drought tolerance of wheat plants, because both of them were able to fix atmospheric nitrogen and to maintain high growth at different PEG6000 concentrations (up to 30%). In our subsequent study, they enhanced the growth of wheat seedlings under drought stress (17 days of ceasing irrigation) and induced significant recovery (10 days of reirrigation) for the seedlings represented by higher fresh and dry masses, water contents and survival (Salama, 2019). Similarly, Omar *et al.* (2017) found that these microbes: 1) tolerated PEG concentrations up to 50%; 2) possessed multi plant growth promoting traits (N_2 fixation, ACC-deaminase activity, P-solubilization and production of HCN, ammonia, and phytohormones (IAA, GA and ABA)); 3) maintained these traits in PEG-containing cultures; and 4) enhanced the growth of wheat seedlings growing *in vitro*, in a sterilized system (spermosphere model) under normal and PEG-stressed conditions.

The objective of the current study was to determine whether the positive impact of these PGPB will last till the yield stage or not? And if yes, how far these strains can diminish the drought-induced loss in wheat yield?

Materials and Methods

Plant material

Grains of wheat (*Triticum aestivum* L.) cultivar ‘Gemiza9’ (G9) were obtained from the Wheat Department, Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt. ‘Gemiza9’ is a relatively drought-sensitive, but high-yielding, rust-resistant and wide-cultivating cultivar (El-Shami *et al.*, 2000).

Microorganisms

The used PGPB were *Azospirillum brasilense* NO40 (B₁) and *Stenotrophomonas maltophilia* (B₂) isolated from rice (Omar *et al.*, 1989) and wheat rhizosphere (Omar *et al.*, 2017), respectively. They were allowed to grow in nutrient broth for 24 h at 28 °C with shaking, thereafter, the culture turbidity was adjusted to 0.3 ~ 10⁸ CFU/ml (using the spectrophotometer at 600 nm) to be used either for grain coating or liquid inoculation. For the grain coating, 30 ml of each culture was taken to inoculate bags of vermiculite carrier (50 g/bag) and incubated for 24 h, while for the liquid the fresh cultures were mixed with the irrigation water 20 ml/pot immediately after sowing, on the 8th day from sowing (just prior of drought initiation), after two months and after three months from sowing.

Plant growth and treatments

The experiment was conducted at the Experimental Green house of Wheat Department, Field Crops Research Inst., (ARC), Giza, Egypt, using sandy soil obtained from the Agricultural Research Station, Ismailia, ARC, Giza, Egypt. Its characters and composition are illustrated in Table 1. The wheat grains were selected for apparent uniformity of shape and size, surface-sterilized by HgCl₂ 0.1% for 5 minutes and rinsed 5 times with sterile tap water. Then, the grains were mixed with the inoculated carrier bags and Arabian gum solution on clean plastic sheets and left to be air dried for one hour in shadow before sowing. For the uninoculated treatments, a clean nutrient broth was added instead.

The experiment included 6 treatments × 8 replica as follows: 1) Cont. = unstressed-uninoculated control, 2) B₁ = unstressed and inoculated with *A. brasilense* NO40, 3) B₂ = unstressed and inoculated with *S. maltophilia*, 4) D = drought-stressed-uninoculated, 5) DB₁ = drought-stressed and inoculated with *A. brasilense* NO40 and 6) DB₂ = drought-stressed and inoculated with *S. maltophilia*.

Seven inoculated or uninoculated grains were sown per pot (25 cm height × 30 cm diameter containing 9 kg of soil) and were reduced to 5 plants/ pot after one month from sowing. The pots were left to grow at 20 ± 2 °C in a relative humidity of 65% and 16 h photoperiod at 450 μmol m⁻² s⁻¹ light intensity and watered with 70% of soil field capacity (SFC) a day after day till the 8th day. Up to this point, the drought stress was initiated by watering pots once every 10 days while the unstressed pots were irrigated normally once every 5 days. The mineral fertilizer (N, P and K) was applied according to the recommendations of the Egyptian Ministry of Agriculture.

Table 1. Some chemical and physical characteristics of the soil

pH	EC (dS/m)	Structure %			Mineral composition (mg/ Kg soil)						
		Sand	Silt	Clay	N	P	K	Fe	Mn	Zn	Cu
7.72	0.50	89.4	7.60	3.00	15.10	4.85	60.80	5.30	0.92	0.48	0.05

Sampling and parameters

Samples were collected after 120 days from sowing. Firstly, some productivity criteria were determined such as the percentage of spike formation/pot, spike weight (g), percentage of grain filling/plant, weight of grains/plant (g) and weight of 100 grains (g). Thereafter, the carbohydrate contents of the yielded grains were

estimated quantitatively using the methods described by Nelson (1944) and modified by Naguib (1963), while the contents of the total soluble proteins (TSPs) and the total free amino acids (TFAAs) were determined according to Bradford (1976) and Lee and Takahashi (1966), respectively. Moreover, the protein pattern of the yielded grains was studied using one dimensional Sodium- Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmli (1970). The gel bands' scanning was analyzed by the Gel Documentation System which determines molecular mass (MM) in kDa of each polypeptide band in relation to a standard marker (M) using Gel Proanalyzer version 3 Media Cybernetics Imaging Exports software (Gel Doc. 2001 BioRad System).

Statistical analysis

All analytical determinations were replicated at least three times and the presented data are the mean values. The obtained results were subjected to one analysis of variance ANOVA analysis to determine the significance between treatments using CoStat software (CoHort software, California, USA, 1998).

Results

Yield criteria

The results show that drought stress caused significant reductions in the percentage of spike formation, weight of spike, percentage of grain filling, weight of grains per spike and weight of 100 grains (Table 2). Clearly, the reductions were greater in the uninoculated- drought-stressed plants (D) than in the *A. brasilense* NO40 or *S. maltophilia*-inoculated-stressed ones. The percentages of spike formation and grain filling of the drought-stressed plants were raised by both strains to 99 and to $\geq 76\%$, compared with 86 and 47%, respectively in the uninoculated-stressed treatment (D). Also, the inoculated-drought-stressed plants had higher weights of spikes by about 2 folds of their uninoculated counterpart (treatment D). Due to the drought stress, the weight of grains per spike was declined by 76% in respect to the well-irrigated control; however, this decline was significantly controlled to only 24% in case of inoculation with *A. brasilense* NO40 and to 44% in the plants which were treated with *S. maltophilia*. Similarly, the weight of 100 grains was notably increased in the stressed-inoculated plants with the two PGPB by 67% than in the uninoculated-stressed ones (D). Moreover, the inoculation with *A. brasilense* NO40 significantly promoted the percentage of grain filling by 6%, the weight of spike by 23%, the weight of grain /spike by 22% and the weight of 100 grains by 10% under normal conditions than that of the uninoculated control.

Table 2. Effect of bacterial inoculation with *A. brasilense* NO40 (B₁) and *S. maltophilia* (B₂) on the yield criteria of *Triticum aestivum* (cv. 'G9') plants grown in sandy soil, under unstressed (irrigated every 5 days) and drought-stressed (irrigated every 10 days) conditions. Treatments means of 3 sets, each set contained 3 replicas (\pm SD). Cont. = the unstressed-uninoculated treatment; D=drought-stressed-uninoculated treatment, B (1-2) = the unstressed-inoculated treatments and DB (1-2) = the drought stressed-inoculated treatments.

Treatment	% spike formation/ pot	Spike weight (g)	% grain filling /plant	Grains weight/ spike (g)	Weight of 100 grains (g)
Control	100 \pm 0.0 ^a	0.53 \pm 0.01 ^b	89 \pm 2.3 ^a	0.37 \pm 0.02 ^b	2.9 \pm 0.06 ^b
B1	100 \pm 0.0 ^a	0.65 \pm 0.02 ^a	96 \pm 1.7 ^a	0.45 \pm 0.01 ^a	3.2 \pm 0.07 ^a
B2	100 \pm 0.0 ^a	0.57 \pm 0.02 ^{ab}	94 \pm 1.6 ^a	0.39 \pm 0.01 ^b	3.0 \pm 0.04 ^{ab}
D	86 \pm 3.1 ^b	0.18 \pm 0.01 ^d	47 \pm 2.6 ^c	0.09 \pm 0.01 ^c	1.5 \pm 0.02 ^d
DB1	99 \pm 1.7 ^a	0.36 \pm 0.06 ^c	79 \pm 2.5 ^b	0.28 \pm 0.01 ^c	2.5 \pm 0.20 ^c
DB2	99 \pm 1.7 ^a	0.38 \pm 0.05 ^c	76 \pm 5.2 ^b	0.21 \pm 0.02 ^d	2.5 \pm 0.20 ^c

Note: Treatments with identical letters are not significant at $P \leq 0.01$

The carbohydrate content of the yielded grains

The obtained results revealed that drought stress significantly affected the contents of carbohydrates in the yielded grains of wheat, where it significantly raised the amounts of the direct reducing sugars and sucrose while it notably reduced the starch content of the grains in the uninoculated and inoculated-stressed treatments (Figure 1). In response to drought stress, the direct reducing sugars were increased by 1.8-fold in the uninoculated treatment (D), and by 3.2 and 2.6 folds in the B₁- and B₂- treated-stressed plants, respectively, in respect to the uninoculated-unstressed control. The sucrose content of the grains was elevated by 77% in the uninoculated-drought-stressed treatment (D), but the highest amount of sucrose was recorded in the grains of the drought-stressed plants inoculated with *A. brasilense* NO40 (DB₁) that were about twice of that in the uninoculated-well-irrigated control. In contrast, the amount of starch in the grains was significantly decreased under stress; where the lowest accumulation of starch was recorded in the uninoculated treatment (D) which was 223 mg/g DM. Meanwhile, the inoculation of the plants with B₁ and B₂ notably enhanced the starch content of grains under stress by 48 and 32%, respectively, then that of the uninoculated-stressed counterparts (D). Under the well irrigation conditions, the bacterial inoculation increased the carbohydrate contents of grain; as the highest amount of the direct reducing sugars was obtained in the grains of *S. maltophilia*-treated plants (23 mg/ g DM), while treating plants with *A. brasilense* NO40 induced the maximum accumulation of sucrose and starch which were 222 and 530 mg/g DM, respectively, compared with 18, 150 and 417 mg/g DM respectively, in their uninoculated counterparts.

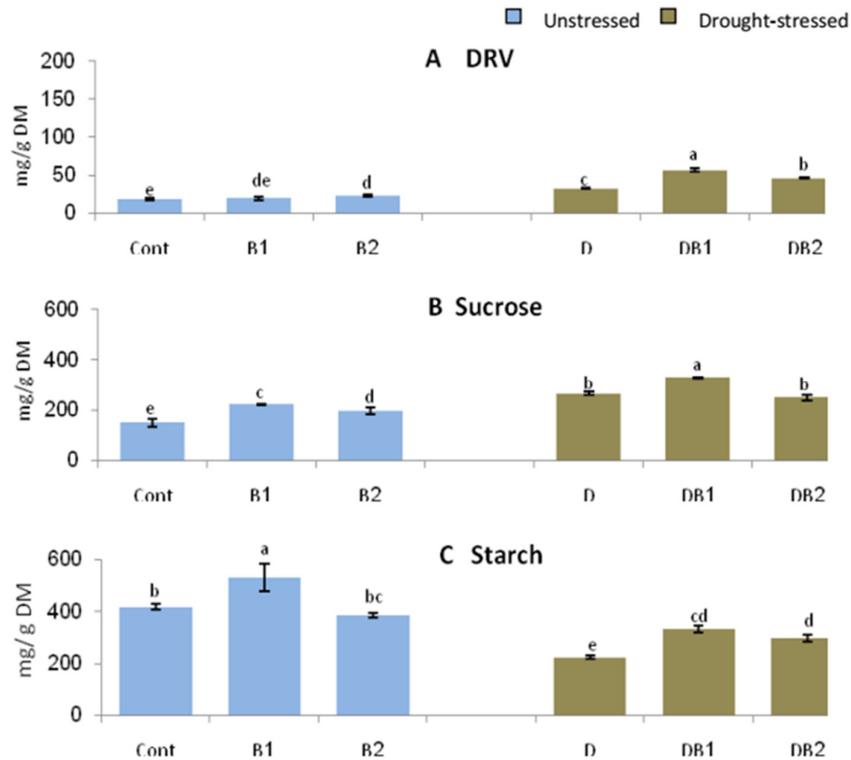


Figure 1. Effect of bacterial inoculation with *A. brasilense* NO40 (B₁) and *S. maltophilia* (B₂) on the contents of total reducing sugars (DRV) (A), sucrose (B) and starch (C) (mg/ g DM) of the yielded-grains of *Triticum aestivum* (cv. G9) plants grown in sandy soil, under unstressed (irrigated every 5 days) and drought-stressed (irrigated every 10 days) conditions. Error bars represent the standard deviation between 3 replicas. Treatments with identical letters are not significant at P ≤ 0.01. Cont. = the unstressed-uninoculated control; D= the drought-stressed-uninoculated treatment, B (1-2)= the unstressed-inoculated treatments and DB(1-2) = the drought-stressed -inoculated treatments

The contents of the total soluble proteins and total free amino acids of the yielded grains

Generally, the drought stress significantly increased the contents of both of TSPs and TFAAs in the yielded grains of all treatments (Figure 2). The largest increases of TSPs and TFAAs were recorded in the grains of the uninoculated-drought-stressed treatment (D) which were 2.3 and 4.6-fold of that of the well-irrigated control, respectively. Whereas, the yielded grains of the plants treated with B₁ and B₂ had lower accumulation of TSPs and TFAAs under stress by about 30 and $\geq 35\%$, respectively than the uninoculated counterpart (D). However, inoculating the unstressed plants with both PGPB slightly raised the amount of total soluble proteins to 42 mg/g DM compared with 35 mg/g DM in the uninoculated counterpart. Both bacterial strains notably increased the content of the total free amino acids in the grains of wheat plants. Among the unstressed treatments, the highest content of the TFAA was recorded in the *S. maltophilia*-treated plants which were 41% higher than that of the uninoculated control.

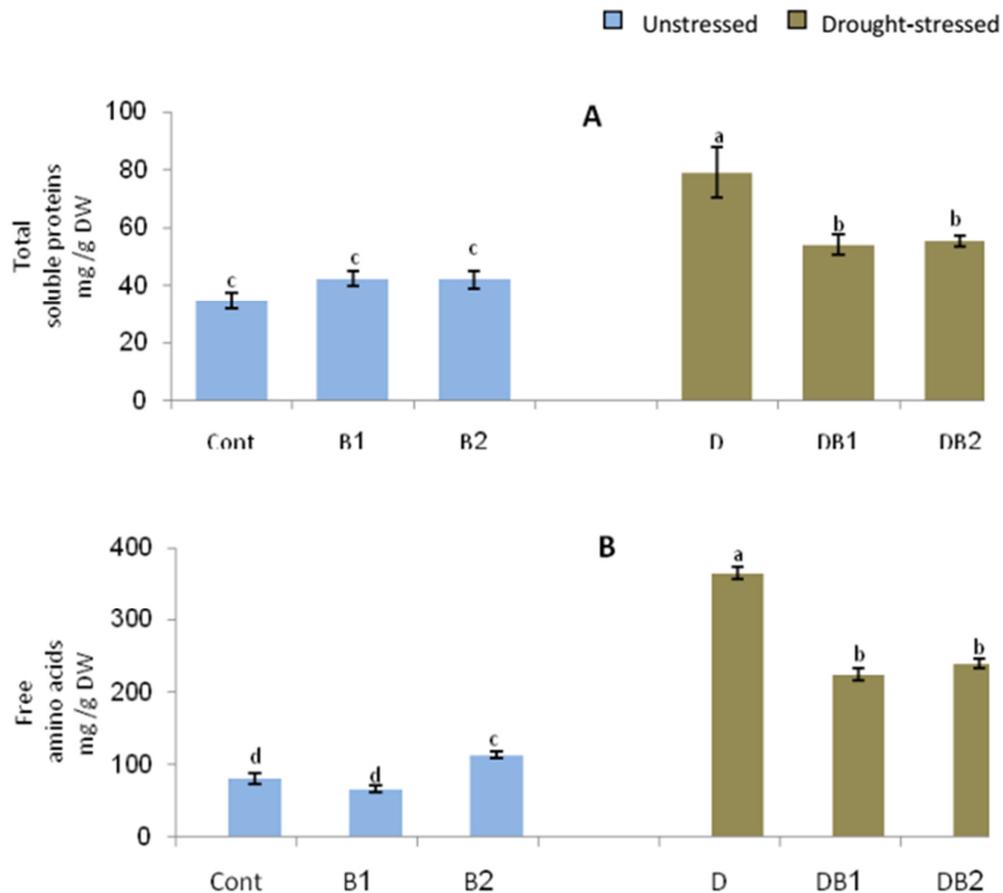


Figure 2. Effect of bacterial inoculation with *A. brasilense* NO40 (B₁) and *S. maltophilia* (B₂) on the contents of total soluble proteins (A) and total free amino acids (B) (mg/ g DM) of the yielded-grains of *Triticum aestivum* (cv. G9) plants grown in sandy soil, under unstressed (irrigated every 5 days) and drought-stressed (irrigated every 10 days) conditions. Error bars represent the standard deviation between 3 replicas. Treatments with identical letters are not significant at $P \leq 0.01$. Cont. = the unstressed-uninoculated control; D= the drought-stressed-uninoculated treatment, B (1-2) = the unstressed-inoculated treatments and DB (1-2) = the drought-stressed -inoculated treatments

Protein patterns of the yielded grains

The molecular masses (MM) of protein patterns shown in Table 3 provide the expression of the effects of drought stress and the inoculation with B₁ and B₂ in the yielded grains of wheat.

Table 3. Changes revealed by SDS-PAGE in protein patterns of the yielded-grains of *Triticum aestivum* (cv. 'G9') plants grown in sandy soil, under unstressed (irrigated every 5 days) and drought stressed (irrigated every 10 days) conditions without and with inoculation with *A. brasilense* NO40 (B₁) and *S. maltophilia* (B₂)

M (Kd)	MM(Kd)	Control	B ₁	B ₂	D	DB ₁	DB ₂
250		--	--	--	--	--	--
	139	+	+	+	-	--	--
	133	--	--	--	+	--	--
130	130	--	--	--	--	+	--
	129	--	--	--	--	--	+
	94	+	--	--	-	--	--
	91	--	+	--	--	--	--
	90	--	--	--	--	--	+
	89	--	--	+	--	--	--
	88	--	--	--	+	--	--
	87	--	--	--	--	+	--
95		--	--	--	--	--	--
72		--	--	--	--	--	--
	71	--	--	--	--	--	+
	70	--	--	--	+	+	--
	69	--	+	+	--	--	--
	62	+	--	--	-	--	--
	59	--	--	--	--	--	+
	56	--	--	--	+	+	--
55	55	--	+	--	--	--	--
	54	--	--	+	--	--	--
	51	+	--	--	-	--	--
	47	--	--	--	--	--	+
	46	--	--	+	+	+	--
	45	--	+	--	--	--	+
	44	--	--	--	+	--	--
	43	+	--	--	-	+	--
	42	--	+	+	--	--	--
	41	--	--	--	--	--	+
	40	--	+	+	+	+	--
	38	+	--	--	-	--	+
	37	--	+	+	+	+	--
36	36	--	--	--	--	--	+
	33	--	--	+	--	--	--
	32	--	+	--	+	+	+
28		--	--	--	--	--	--
	23	--	--	--	--	--	+
	22	--	--	+	+	--	--
	20	--	+	--	--	--	--

M= marker; MM = molecular mass; += present, -- = absent; Cont. = the unstressed and uninoculated control; D= the drought stressed and uninoculated treatment, B (1-2) = the unstressed and inoculated treatments and DB(1-2) = the drought stressed and inoculated treatments

Data revealed the following:

1) Drought stress induced synthesis of five new protein bands with MM 133, 88, 70, 56 and 44 in the stressed-uninoculated grains which were not detected in the control or in all of the inoculated-drought stressed seedlings, except for the protein bands with MM 70 and 56 that were also appeared in the grains of DB₁ treatment.

2) Drought induced the disappearance of six polypeptides having MM of 139, 94, 62, 51, 43 and 38 KDa, which were detected in the control. The inoculation returned some of them, as the bands with MM 43 and 38 KDa which reappeared in the DB₁ and DB₂ treatments, respectively

3) On the other hand, the bacterial inoculation not only relieved the deleterious impact of drought stress through the re-appearance of some bands which disappeared under drought stress, but also by the induction of newly synthesized bands, such as: the bands with MM 130 and 87 KDa in DB₁ and 129, 90, 71, 59, 47, 41, 36 and 23 kDa in DB₂ treatments which were undetected in the control or in the corresponding uninoculated-drought-stressed counterparts.

4) Under normal conditions, the bacterial inoculation resulted in newly synthesized proteins as the bands with MM 91, 55 and 20 KDa appeared only in *A. brasilense* NO40- inoculated plants and the bands with MM 89, 54, and 33 appeared only in *S. maltophilia*-treated plants, while the bands have MM 69 and 42 KDa appeared with both microbes.

Discussion

Crop yield is the final outcome of complexly interrelated crop growth and development processes strongly affected by water availability (Farooq *et al.*, 2009; Fahad *et al.*, 2017). The results showed that the drought stress resulted in harsh effect on wheat yield as indicated from the significant reductions in the all measured criteria of yield (spike formation%, weight of spike, grain filling%, weight of grains/spike and weight of 100 grains). These results are in conformity with those obtained by Darkwa *et al.* (2016) on *Vicia faba*. The drought-mediated decline in crop yields can be ascribed to various factors, especially those mainly determining the source and sink capacity (Blum, 2011; Wang *et al.*, 2017). This reduction in yield parameters under drought stress may be due to the premature leaf senescence, oxidation of chloroplast lipids and alterations in the assembly of pigments and proteins, leading to lowering the supply of carbon assimilate, reduced photosynthetic rate and consequently minimal biomass production, as well as diminished translocation of assimilates towards the developing seeds (Abid *et al.*, 2016; Kasim *et al.*, 2017). Wang *et al.* (2017) conducted a meta-analysis of 303 papers published before May 2015 studying the effects of drought stress on the morpho-physiological and biochemical characteristics, growth and biomass partitioning, and yield formation of diploid, tetraploid, and hexaploid wheat. This analysis indicated that the yield losses from drought stress significantly correlated to: 1) the reduction in the leaf area (as a key driver), the whole-plant assimilation rate, and leaf chl-a and b; 2) arrest in organs' differentiation and/or dysfunction of the differentiated reproductive organs (e.g. the number of flowers can be reduced by arrested differentiation or by abortion and deterioration of developed flowers under stress, while the declined number of grains developed from a given number of flowers can be a result of the stress-induced sterility of female or male organs, abortion of embryos (Blum, 2011) and/or the decrease of the endosperm cell number and metabolic activity; and greatly to 3) the disturbance in the resources' allocation and reallocation patterns.

The present study also reveals that the yielded grains under drought stress had lower content of starch and higher contents of the reducing sugars, sucrose, the total soluble proteins (TSPs) and the total free amino acids (TFAAs) in addition to having altered protein patterns than those of the unstressed plants. These results agreed with those of Qudisia *et al.* (2013) for maize. The total soluble protein content of the yielded seeds in the present results revealed a highly significant increase with drought, which was similar to the trend previously obtained in the vegetative stage reported by Kasim *et al.* (2017). This increase may be due to the induction of

stress proteins, which are particularly involved in the maintenance of cell redox and might act as antioxidant enzymes (Shu *et al.*, 2012).

Drought can often cause significant metabolism alteration in plants, for instance, the altered carbohydrates contents (reduced starch and increased sugars) which can be a result of: 1) declined starch production due to the reduced assimilate supply or the altered activities of the biosynthetic enzymes; or 2) enhanced starch degradation to sugars, as sugars can act as signaling and regulatory molecules; energy and carbon building blocks of protective proteins and compounds; and as compatible solutes, osmotic spacers, and ROS scavengers in order to increase cell turgor and to protect sensitive membranes and proteins (Thitisaksakul *et al.*, 2012; Dong and Beckles, 2019). Likewise, the increased levels of the free amino acids could be a result of diminished synthesis or increased breakdown of specific proteins, while other proteins (namely stress-induced proteins) may be induced or up-regulated (Farooq *et al.*, 2009; Aslam *et al.*, 2015; Ghatak *et al.*, 2017).

On the other hand, treating wheat plants with *A. brasilense* NO40 or *S. maltophilia* under drought stress significantly enhanced almost all their measured yield parameters, where the yielded grains had significantly higher contents of carbohydrates (the direct reducing sugars, sucrose and starch); lower amounts of total soluble proteins and the total free amino acids; and altered protein patterns compared with the uninoculated stressed treatments. Furthermore, under normal conditions, inoculating wheat plants with PGPB resulted in significantly improved yields with higher contents of carbohydrates, than those of the uninoculated control. These results agreed with those of Askary *et al.* (2009) and of Creus *et al.* (2004) who stated that even in stress conditions, *Azospirillum* inoculation increased the grain yield and mineral nutrition in plants under stress.

Five of the Heat Shock Protein families (HSPs) with MM ranging between 250 kDa and 15 kDa with roles to play in combating abiotic stresses in plants are represented in our study. Thus, the protein bands with MM 133 kDa may function as HSP 100 which play a crucial role in re-solubilizing protein aggregates via interactions with the sHSP chaperone system (Bosl *et al.*, 2006). The protein band with MM 88 kDa, may function as HSP 90 and regulates protein folding and plays a key role in signal transduction networks, protein degradation and protein trafficking (Park and Seo, 2015). The protein band with MM 70 kDa, belongs to HSP 70 and acts as a chaperone for newly synthesized proteins to prohibit their accumulation as aggregates as well as to maintain proper protein folding through their transfer to their final location (Park and Seo, 2015). The protein bands with MM 56 kDa are members of the HSP 60 family which help in protein folding and subunit assembly (Efeoglu, 2009). The protein bands with MM 44 kDa of the HSP40 family are known to induce HSP70 affinity for clients (Kampinga and Craig, 2010).

Taken together our previous studies in which *Azospirillum brasilense* NO40 and *Stenotrophomonas maltophilia* had enhanced the growth and performance of wheat seedlings under drought stress (Kasim *et al.*, 2013; Omar *et al.*, 2017) with the current results, we can assume that the uninoculated wheat plants showed a relatively low degree of drought tolerance, and may have favored survival over growth by maintaining highly activated defenses that had resulted in high deleterious effect on growth and productivity, unlike the inoculated plants that showed a relatively higher degree of drought tolerance by which they were able to deactivate the growth inhibition to some extent while maintaining a certain level of efficient protection against damage, which finally translated into higher yields under drought stress.

The augmented yield production and crop quality under both normal irrigation and drought stress conditions was reported in many plants treated with PGPB (Ngumbi and Kloepper, 2016; Etesami and Maheshwari, 2018). For instance, Treating plants with PGPB has been reported to: 1) induce favorable alterations in root growth and architecture (longer roots, longer and denser lateral roots and higher biomasses) (Naseem and Bano, 2014; Timmusk *et al.*, 2014); 2) improve shoot growth (higher shoots and heavier biomasses) (Kasim *et al.*, 2013; Grover *et al.*, 2014); 3) maintain relatively higher relative water contents than the drought-stressed and bacterially-untreated plants (Sandhya *et al.*, 2010; Naveed *et al.*, 2014; Naseem and Bano, 2014); 4) facilitate water and nutrient (e.g. Fe, P and N) acquisition (Desai *et al.*, 2012; Ngumbi and Kloepper, 2016); 5) modulate the plant hormones levels either by synthesizing phytohormones or by lowering

the plant-produced ethylene via the ACC-deaminase activity (Castillo *et al.*, 2013; Bresson *et al.*, 2013; Ngumbi and Klopper, 2016); 6) stabilize membranes (Gusain *et al.*, 2015); and 7) regulate each of expression of some drought-triggered genes (Kasim *et al.*, 2013; Sarma and Saikia, 2014), production of osmolytes (Bano *et al.*, 2013; Gururani *et al.*, 2013), and activities of different enzymes (Kohler *et al.*, 2008) resulting in promoted plant growth and productivity under drought stress (Vurukonda *et al.*, 2016; Forni *et al.*, 2017). Moreover, inoculating the salt-stressed barely with *A. brasilense* NO40 significantly ameliorated the adverse effect of salinity on growth and yield (Omar *et al.*, 2009), but no available studies illustrating the role of *S. maltophilia* in augmenting crop yields under abiotic stressed conditions.

However, the exact mechanisms by which the selected PGPB maintained and integrated their multiple plant growth traits to achieve their positive impact on the overall performance of wheat plants under drought stress remain largely speculative (Ngumbi and Klopper, 2016; Forni *et al.*, 2017). However, it is known that the PGPB have the ability to induce systemic tolerance by triggering several physical and chemical changes in plants that result in enhanced tolerance to abiotic stresses (Yang *et al.*, 2009). One or both of the following explanations can be postulated to explain the way in which this tolerance was attained in the present study:

1) The first explanation is that: priming and coating wheat grains with PGPB might have induced acclimation. It has been shown that certain beneficial microbes can pose some biotic stress on plant, that in turn activate the plant defensive metabolism before the stress onset, resulting in better or more rapidly mount defense responses, or both, to biotic or abiotic stress “the plant primed state” (Conrath *et al.*, 2006; Rajendra-Prasad *et al.*, 2016). The previous results of the less activated defenses (Salama, 2019) may imply that PGPB application enhanced the adaptation responses, rather than their direct activation as is the case in acclimation treatments in which plants are gradually exposed to an increasing stress situation. It is worth-noting that the less activated defenses may suggest that the inoculated plants were able to avoid the heavy consumption of resources for defense, and therefore maintained better growth and here better yield than the uninoculated plants.

2) The second explanation is that: the introduced PGPB might have earlier sensed the imposed stress and responded to it, because they are small, in intimate contact with soil water, and have semipermeable membranes (Schimel *et al.*, 2007), and accordingly, they might have affected the plant metabolism. For instance, it is known that soil microbes have the ability to influence rhizodeposition, not only respond to it (Phillips *et al.*, 2004). Likewise, it can be assumed that somehow the introduced PGPB could have provided some kind of early warning to the plant to be prepared to cope well with the imposed stress. This can be supported by the ability of both microbes to produce ABA and the possibility of being able to produce or trigger other alarming volatiles or compounds.

Conclusions

It seems reasonable to conclude that treating wheat plants with the PEG-tolerant *Azospirillum brasilense* NO40 or *Stenotrophomonas maltophilia* which possess multiple plant growth promoting traits and were compatible with systems into which they were introduced, can offer a promising, feasible and sustainable strategy to enhance wheat growth and productivity, not only under normal conditions, but also under drought stress. Accordingly, it is strongly recommended that farmers and agriculture companies in Egypt inoculate wheat grains and plants with such PGPB as *Azospirillum brasilense* NO40 and *S. maltophilia* as a means of combating drought stress. In practice, *Azospirillum brasilense* NO40 is already available and licensed by the Egyptian Ministry of Agriculture for inoculating wheat cultivated under drought stress conditions in sandy soils.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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