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EVALUATION OF CERTAIN LOCAL BACTERIAL STRAINS AS DIAZOTROPHS FOR WHEAT PLANTS

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ABSTRACT: *Nine identified bacterial strains were selected out of 48 isolates collected from various areas in the upper Western region of the Nile Delta in Egypt. Such 9 strains proved to be the most biofertilizing bacterial agents among all via laboratory examinations. A greenhouse pot experiment was carried out in order to ascertain the capability of those strains as effective diazotrophs for wheat plants. Influence of soil moisture content and salinity was included.*
Nitrogen contents in plant shoots and in rhizosphere soil, as well as dehdrogenase activity, had been determined at two growth periods, i.e. 60 and 120 days of planting. The results revealed that the strain "D 2" (Azotobacter chroococcum DSM 2286), "D9"(Enterobacter kobei CIP), "M3"(Bacillus megaterium EIF18), "N4" (Clostridium sp.), and "W2" (Klebsiella sp.), were, descendingly the most efficient strains, under all experimental variables.

Key wards: *Biofertilizers, N_2 fixation, Dehydrogenase activity, Wheat, Nitrogen uptake.*

INTRODUCTION

Recent studies have confirmed that a number of bacterial species, mostly associated with the plant rhizosphere, are beneficial for plant growth, yield and crop quality. They have been called plant growth-promoting bacteria and include strains of the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium and Serratia* (Bashan and de-Bashan, 2005 and Esitken *et al.,* 2010).

Soil salinization is a growing problem worldwide. It was estimated that 10% of the world's cropland and as much as 27% of the irrigated land may be already affected by salinity (Shannon, 1997). Another review quotes that one-third of the world's arable land resources are affected by salinity (Qadir *et al.,* 2000). Not surprisingly that, the gradual increase in salt content in irrigated soils has been considered as one of the main threats against crop production (Kotb *et al*., 2000). High NaCl (60 mol m^{-3}) in nutrient solution strongly affected the germination rate and root elongation, seedling and mature vegetative growth of both spinach and lettuce, but especially in lettuce (Kaya *et al*., 2002). At 80 mol m^{-3} NaCl, lettuce germinability was reduced to 50% (Odegbaro and Smith, 1969). Even when salt seemed to affect lettuce growth occured mainly through osmotic effects (Shannon, 1997). Moreover, salt–nutrient interactions could also account for the NaCl negative effects on plant growth and yield quality (Pardossi *et al.,* 1999), or for improving plant performance under saline conditions (Kaya *et al.,* 2002). On the other hand, the use of plant growth-promoting bacteria (PGPB) and mycorrhizal fungi to promote plant growth in saline soils was reported as a developing technology (Bacilio *et al.,* 2004). In general, inoculation with PGPB can enhance germination, seedling emergence and modify growth and yield of various cereal and non-cereal crops (Zahir *et al.,* 2004).

Water stress limits the growth and productivity of crops particularly in arid and semi-arid regions causing the most drastic economic losses in agriculture. This form of abiotic stress, affects the plant water relation at cellular and whole plant levels causing specific and unspecific reactions and damages. Inoculation of plants with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas (Marulanda *et al.,* 2007).

These beneficial microorganisms colonize the rhizosphere/endorhizosphere of plants and promote their growth through various direct and indirect mechanisms (Glick, 1995). There is a thin layer of soil immediately surrounding plant roots known as "rhizoplane", that is an extremely important for root activity and metabolism. (Garcıa *et al.,* 2001). Many environmental stresses including drought and salt stress impair electron transport system leading to the formation of activated oxygen (Chandra *et al.,* 1998). Activated oxygen forms, such as H_2O_2, O_2 and OH may accumulate during water deficit stress and damage the photosynthetic apparatus. Superoxide dismutase (SOD) and ascorbate peroxidase along with the antioxidant ascorbic acid and glutathione act to prevent oxidative damage in plants (Allen, 1995). Oxidative molecules initiate damage in the chloroplast and cause a cascade of damaging effect including chlorophyll destruction, lipid peroxidation and protein loss (Zhang and Kirkham, 1994).

Based upon the importance of atmospheric nitrogen fixing-growth promoting rhizobacteria, which, besides their diazotrophic capacity, stimulate plant growth by producing plant growth regulators or phytohormones, such as indole acetic acid (IAA), cytokinins, gibberellins, and many other organics. These compounds, not only promote the dinitrogen fixation process, but also participate in solubilization of inorganic phosphate and mineralization of organic materials (nutrients release), antagonistic effects against phytopathogenic microorganisms via synthesis of antibiotics or competition with detrimental microorganisms and eventionally for the faver of plant growth and crop production (Glick, 1995; Lucy *et al.,* 2004 and Marques *et al.,* 2010).

Therefore, the present work was designed to employ certain biofertilizing bacterial isolates, collected from the rhizosphere of different crops from some geographical locations at the upper Western region of the Nile Delta in Egypt, to ascertain their capability to tolerate salinity and /or drought. A greenhouse pot experiment was carried out in order to estimate the capacity of the bacterial strains for diazotrophy and dehydrogenase activity in an alluvial soil planted with wheat crop.

MATERIALS AND METHODS

1.Layout

The present work was planned to compare the biofertilizing capacity of nine mainly diazotrophic strains selected out of 48 isolates collected from the rhizosphere of different crops at various locations of Northen Nile Delta in Egypt. The 9 bacterial isolates were chosen referring to their highest capabilities for dinitrogen fixation, indole acetic acid (IAA) production, phosphate solibilization, tolerance to a different gradient of temperature, persistence against varying levels of either NaCl and $CaCO_3$. Such 9 isolates were then identified by 16sr DNA sequencing analysis. Pot experiment was carried out to practically ascertain the efficiency of the selected bacterial strains as asymbiotic N_2 fixers for wheat plants, under salinity and drought conditions. Overall microbial activity in the wheat rhisosphere soil was, likewise, performed.

2. Greenhous Pot Experiment

2.1. Soil used

Surface sample (0-30 cm) of an alluvial soil was collected from the Experimental Farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom. The sample was air-dried, ground to pass through a 2-mm sieve. A portion of the soil was subjected to analysis for pertinent physical and chemical properties and contents of some macro-and micronutrients, according to the methods described by Cottenie *et al.* (1982), Page *et al.* (1982) and Klute (1986). The obtained data were recorded in Table (1).

2.2. Inoculation process

Wheat seeds (*Triticum aestivum*, Gemeza 9) were inoculated with bacterial liquid medium of each strain (containing 5.6 x10^7 cfu) (Nelson and Knowles, 1978). Identification of the tested bacterial isolates is shown in Table (2). The bacterial strains were considered as main treatment.

Table (1): Physical and chemical properties and nutrient contents of the used soil.

a. Physical Properties				
Moisture Content, at the field capacity (%)	Fraction (%)			Textural Class
	Sand	Silt	Clay	
34.4	11.4	33.7	54.9	Clayey

b. Chemical Properties												
Organic Matter	$CaCO_3$	ESP	pH (1:2.5, soil: water Susp.)	EC (dSm^{1})	Soluble Ions (meq/100g soil)							
					Cations				Anions			
					Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
(%)				(1:5, soil : water extract)								
1.8	2.6	4.6	7.6	0.4	0.9	0.4	2.4	0.5	–	0.8	2.6	0.8

c. Nutrient Contents											
Major Nutrients						Minor Nutrients					
Total			Available			Total			DTPA extractable		
%			mg/kg			mg/kg					
N	P	K	N	P	K	Fe	Zn	Mn	Fe	Zn	Mn
0.15	0.10	0.60	58.1	9.2	270	155	37	134	43	7.50	9.20

Table (2): Genetic identification of the selected bacterial isolates to gene level.

Bacterial isolate	Identification*	Level (%)
D2	*Azotobacter chroococcum* DSM 2286	97
D9	*Enterobacter kobei CIP*	83
W2	*Klebsiella sp.*	98
W10	*Klebsiella oxytoca* P479	85
W11	*Stenotrophomonas sp.*	94
N4	*Clostridium sp*.	99
M3	*Bacillus megaterium EIF18*	98
M5	*Halomonas sp.*	95
M8	*Clostridium sp*.	78

* Based on partial sequencing of 16s rDNA gene and comparison with the National Center for Biotechnology Information.

3.2.4. Greenhouse work

Plastic pots, each contained 5 kg of the fine soil crumbs, were employed in this study. The potted soil was supplied with superphosphate (15.5% P_2O_5), at a rate of 200 kg fed^{-1}. (1.0g pot^{-1}). 324 pots were divided into 9 groups (36 pots for each group), representing the treatments of diazotrophic bacterial isolates (9 isolates). The bacterial agents were applied separately to wheat seeds, at the time of sowing. The diazotrophs inoculation treatments were considered as main treatment.

Directly before sowing, pots of each group was divided into three sub groups (12 pots per each sub group), to represent the moisture treatment of irrigation water (drought level), *i.e.* 40, 60 and 100 % of the field capacity 'FC" of the soil. The irrigation rates were considered as co-treatment. Furthermore, each sub group was divided into two sub sub groups (6 pots per each sub sub group) to represent the salinity treatments, *i.e.* 2000 or 4000 mg NaCl kg^{-1} soil. The salinity levels were considered as co-treatment in this study. In addition, 36 pots were involved in the experiment as control. Then, each pot was planted with 8 seeds of wheat plant (*Triticum aestivum cv.*) Gemeza 9. Moisture content had been kept constant by compensating the loss regulatory every three days. A randomized block design with six replicates was applied. Plants of each pot were thinned after 10 days of sowing to 5 plants per pot. Then all pots were fertilized with potassium sulfate (48% K_2O) at a rate of 100 kg fed^{-1}. (0.5g pot^{-1}) and also with ammonium nitrate (33% N) at a rate of 30 kg fed^{-1}. (0.15g pot^{-1}, as activating dose). Both potassium and nitrogen fertilizers were added with irrigation water. After 60 and 120 days of sowing (first and second samplings), whole plants of three replicates, at each time were taken carefully separately out of each pot, and the rhizosphere soil was then removed. Plant shoot materials were air-dried, oven- dried at 70 °C for 48 hrs, to obtain the dry weight, ground, digested by a mixture 1:3 concentrated perchloric acid : sulphoric acid and subjected for N determination. The rhizospere soil of each pot was collected at each sampling time for assay of dehydrogenase activity (DHA). The residual N content in the soil was determined at the end of experimental period.

3. Laboratory Determinations

3.1. Soil analysis

❖ Physical properties (Klute, 1986):

- Mechanical analysis was determined by the universal pipette method, using sodium oxalate as a dispersing agent.

❖ Chemical properties (Page *et al*., 1982):

- Organic matter content was assessed by means of Walkly and Black method.
- Soil pH value was measured, in the 1:2.5 soil-water suspension, using standard glass electrodes (pH meter).
- Calcium carbonate content was determined volumetrically, using a calcimeter.
- Total salinity (EC) was determined in the 1:5 soil/water extract, using an electrical conductivity meter (salt bridge).
- Soluble ions, i.e. the cations Ca^{2+}, Mg^{2+}, K^+ & Na^+ and the anions CO_3^{2-}, HCO_3^-, Cl^- & SO_4^{2-} were determined in the 1:5 soil- water extract, following the traditional standard methods.
- Total and available contents of nitrogen were determined, following the conventional method by means of a semi-micro-steam Kjeldahl distillation apparatus.
- Total and available contents Phosphorus was determined colourimetrically, by photoelectric colourimeter.
- Total and available contents Potassium was determined by flame photometer.
- Total and available contents of Zn, Fe and Mn were measured by atomic absorption spectrophotometer.

❖ Biochemical Assay (Casida *et al*., 1964):

Dehydrogenase activity (DHA) was determined colourimetrically, for the 2, 3, 5-triphenyl formazan (TPF) produced from the reduction of 2, 3,5 triphenyl tetra zolium chloride (TTC), using acetone for extraction.

3.2. Plant analysis (Cottenie *et al.*, 1982):

- Total nitrogen was determined in the acid digest of wheat plant shoots, following the conventional method by means of a semi-micro-steam Kjeldahl distillation apparatus.

4. Calculations

Raw results (analytical data of the replicates means of the various sub-treatments) were further calculated on the dry weight basis of the plants and/or the soil, as convenient.

Rates of the relative changes of the final results (as percent) "RC%" were calculated for the result tabulated for a particular sub-treatment, referring to the result of the specific control (without diazotrphic inoculation).

$$RC\% = \frac{\text{Result of a particular sub treatment} - \text{Result of the control}}{\text{Result of the control}} \times 100$$

RESULTS AND DISCUSSION

Salts and /or drought tolerant diazotrophic strains were practically examined in a greenhouse pot experiment. For this propose, wheat seeds were inoculated with the most potent 9 bacterial isolates that showed, in a preceding survey and characterization, high efficiency for nitrogenase activity, P solubilization, IAA production, high survival at each of different temperature degrees, salinity stress and $CaCO_3$ levels (Abdel-Whab, 2014). The inoculated seeds were planted in a clay soil. Nitrogen contents in both wheat plant shoots and soil and dehydrogenase activity in the rhizosphere soil were estimated, at two growth periods, *i.e.* 60 and 120 days after planting. Residual N content in soil was determined at the end of the experiment.

Nitrogen Content in Wheat Plant Shoots

Data listed in Table (3) show the concentrations and uptake of nitrogen and the rates of relative changes "RC %" of its concentrations, in the shoots of wheat plants, grown up from seeds initially inoculated with each of the selected 9 bacterial strains and exposed to varying moisture contents (100, 60 and 40 % of the field capacity of soil "F.C") and two salinity levels (2000 "C1" or 4000 "C2" mg NaCl /kg soil), at two sampling periods (60 and 120 days after sowing). Results showed that N concentrations and uptake by wheat plants significantly increased with all bacterial isolates compared with uninoculated plants.

At the first sampling time and salinity level "C1", the plants inoculated with the isolates D9, N4, and M3 attained the highest nitrogen concentration (Table 3), at all moisture contents tested. Whereas, under the salinity level "C2", the highest N concentrations were found in the plants inoculated with the bacterial isolates D2, D9 and N4 at the various moisture contents. On the other hand, the second sampling time and salinity level "C1", the bacterial isolates D2, W2, and N4 were responsible for the presence of the greatest nitrogen concentrations in wheat plant tissues. Also, at the salinity level "C2", the bacterial isolates D9, D2 and M3 were behind the highest nitrogen concentrations. No specific action for the varying moisture contents on such concern.

Rates of the relative changes "RC, %" calculated for the concentrations of N taken up by the plants (Table 3), at the first sampling period and salinity level "C1", the bacterial isolates D9, N4 and M3 were associated with the highest values of "RC%", at the different contents of moisture. Whereas, the values reported at the salinity rate "C2", the bacterial isolates D2, D9 and N4 were higher than those above noted. However, at the second sampling period and salinity level "C1", inoculated with the isolates D2, W2 and N4 gave higher values of "RC %" of N plant content. Whilst under the salinity level "C2", the bacterial isolates D2, D9 and M3 resulted in higher values of "RC %" of the N content, of the wheat shoot.

As an outlook at the results of the N contents in wheat plant shoots, as affected by the moisture contents in presence of either salt concentration (C1 or C2): the following orders are inferred,

- At 100 % F.C: D2>D9>M3>W2>M8>W10>N4> M5> W11
- At 60 % F.C: W2>D2>D9>M3>N4> W10> M8>W11>M5
- At 40 % F.C: M3> W 2>N4> D2>D9> W11>M8>W10> M5

Table (3): Effect of inoculation with selective bacterial strains (see table "2" for identification), moisture content and salinity levels on nitrogen concentration and uptake by wheat plant shoots and their rate of relative changes (RC, %*)of its concentration, at two growth periods.

Bacterial isolates	Salinity levels (mg NaCl/ kg soil)**	Moisture content (% of the field capacity of soil "F.C")																	
		100						60						40					
		Growth Period (days after planting)																	
		60			120			60			120			60			120		
		N content in wheat shoots, and RC rates																	
		Concentration (%)	Uptake (mg/kg)	RC, %	Concentration (%)	Uptake (mg/kg)	RC, %	Concentration (%)	Uptake (mg/kg)	RC, %	Concentration (%)	Uptake (mg/kg)	RC, %	Concentration (%)	Uptake (mg/kg)	RC, %	Concentration (%)	Uptake (mg/kg)	RC, %
Control***	C1	1.38	12.14	0	1.44	58.90	0	1.29	9.80	0	1.34	47.57	0	1.12	7.50	0	1.29	40.89	0
	C2	1.23	9.72	0	1.32	47.65	0	1.20	8.40	0	1.25	37.38	0	1.01	10.72	26	1.22	33.31	0
D2	C1	1.79	19.15	30	1.97	101.06	37	1.75	14.70	36	1.80	73.98	34	1.35	10.67	21	1.66	59.93	29
	C2	1.69	15.72	37	1.86	86.30	41	1.58	13.11	32	1.8o	63.72	44	1.34	10.59	20	1.61	51.20	32
D9	C1	1.81	19.55	31	1.91	98.37	33	1.69	14.37	31	1.72	70.86	28	1.31	9.56	17	1.58	57.04	22
	C2	1.69	16.06	37	1.86	86.86	41	1.56	11.54	30	1.73	60.72	38	1.30	9.36	16	1.47	47.19	20

Table (3): Continued

W 2	C1	1.72	18.06	25	1.84	90.53	28	1.62	14.26	26	1.93	80.29	44	1.24	8.43	23	1.73	62.97	34
	C2	1.67	15.20	36	1.84	80.22	39	1.58	13.27	32	1.73	67.12	38	1.23	9.59	22	1.61	57.96	32
W10	C1	1.56	15.44	13	1.87	90.88	30	1.48	12.28	15	1.79	71.06	34	1.23	9.47	10	1.63	57.54	26
	C2	1.44	12.82	17	1.79	72.85	36	1.37	10.55	14	1.72	59.34	38	1.22	8.54	21	1.58	47.56	30
W11	C1	1.61	15.46	17	1.8	87.48	25	1.53	13.01	19	1.87	70.87	40	1.17	8.07	16	1.51	52.70	17
	C2	1.56	13.73	27	1.68	65.86	27	1.41	10.86	18	1.65	57.59	32	1.01	6.36	0	1.4	41.44	15
N4	C1	1.78	17.62	29	1.92	96.38	33	1.79	14.86	39	1.87	77.61	40	1.25	9.50	24	1.79	64.80	39
	C2	1.66	15.11	35	1.77	73.81	34	1.58	12.96	32	1.73	65.91	38	1.25	8.88	24	1.61	58.12	32
M3	C1	1.72	17.72	25	1.84	92.55	28	1.61	14.33	25	1.75	72.45	31	1.29	9.80	28	1.68	60.98	30
	C2	1.51	14.04	23	1.84	82.80	39	1.56	12.95	30	1.73	67.30	38	1.28	9.73	14	1.63	58.84	34
M5	C1	1.63	15.16	18	1.86	80.91	29	1.55	12.71	20	1.84	65.50	37	1.28	9.60	14	1.59	54.22	23
	C2	1.55	13.49	26	1.73	65.57	31	1.37	10.69	14	1.65	54.62	32	1.27	9.14	26	1.37	40.69	12
M8	C1	1.70	16.49	23	1.86	79.42	29	1.46	11.83	13	1.86	67.33	39	1.26	8.82	25	1.66	53.29	29
	C2	1.58	13.59	28	1.79	69.09	36	1.46	11.10	22	1.72	56.24	38	1.26	9.83	25	1.45	42.78	19

* (RC, %): the difference between the value of a particular sub treatment and control, calculated as percent of that control.
**Salinity levels" : C1 and "C2" 2000& 4000 mg NaCl/kg soil.
*** Control: uninculated

Effect of inoculating wheat plants with selected bacterial isolates on the residual content of total nitrogen in soil

Data listed in Table (4) display the residual content of total nitrogen in the soil, at the experimental end, and its rates of relative changes "RC, %". These data reveal the effect of inoculating the wheat seeds with each of selected 9 bacterial isolates and left to grow up to 120 days, on a clay soil, under moisture contents of 100, 60 or 40 % of the soil field capacity "F.C" and salinity levels of 2000 "C1" or 4000 "C2" mg NaCl/kg soil. At plant harvest (120 days after sowing), the soil content of total nitrogen slightly increased as a result of the initial bacterial inoculation than the uninoculated plants. In the soil receiving the salinity level "C1", the bacterial isolates D2, N4 and M3 showed slight increases in the content of total N at any moisture treatment tested. Whereas, at the higher salinity level "C2", the same bacterial isolates were again the most effective, but with N4 and M3 exchanging position.

Rates of the relative changes "RC, %" calculated for the soil content of total N (Table 4) exhibit that, at the lower salinity level "C1", the bacterial isolates D2, N4 and D9 treatments were more effective as they produced high values of "RC %" of the total soil N at the moisture contents of 100, 60 and 40 % of F.C of the soil. Whilst, at the salinity level of "C2", the bacterial isolates D2, M3 and N4 were superior in regard of "RC" values. The data also denote that inoculation with other bacterial isolates, *i.e.* M8, W10 and W11 had no effect on soil content of total N at the end of experiment (Table 4).

The above mentioned results pointed out that inoculation with the bacterial isolates D2, D9, M3, W2 and N4 were the most efficient agents responsible for the highest values of N concentrations and uptake by wheat plants, with all experimental treatments. These findings are due to the high potency of those strains for nitrogenase activity, P-solubilization capacity, salt tolerance and IAA production rate. In such connection, work submitted by Islam *et al.* (2012), reveald that the biofertilizer *Azospirllium* strains "BM9" and "BM11" positively influenced N, P and K uptake in grain and straw of rice significantly. BM11 explored the highest N uptake in both grain (56.5 kg/ha) and straw (24.7) and K in straw (4.2 and 66.1 kg/ha, respectively), while "BM9" showed the highest P and K uptake in grain (13.1 and 11.7 kg/ha, respectively). The damaging effects of NaCl on wheat seedlings could be reduced by inoculation with *A. brasilense* Sp245 (Creus *et al.,* 1997), which partially reversed the negative effects of salt and osmotic stress on the relative elongation rate of shoots, such reduction was accompanied by a higher relative water content. *Azospirillum* could accumulate proline and glutamate in response to NaCl (Bashan and Holguin, 1997), and promote proline accumulation in maize exposed to water stress (Casanovas *et al.,* 2003), thus acting as an osmoprotectant. Plants inoculated with the PGPR generally had a higher N content than the uninoculated plants (Puente *et al.,* 2004). Farzana and Randizah (2005) observed that, the use of bio-fertilizer and bioenhancer, such as N_2 fixing bacteria and other beneficial micro-organisms, could reduce chemical fertilizer applications and consequently lower production cost. They also found that the isolates "UPMSP8", "UPMSP9" and *Azospirrlium lipoferum* SP7 significantly increased the plant growth and N, P and K uptake by sweet potato. These results are in accordance with those obtained by Egamberdiyeva, 2007, who reported that various bacterial inoculants (*Rahnella aguatilis* 6, *Pseudomonas fluorescens* PsIA12, *Pantoea agglomerans* strain "050309", strain "370320", strain "370308" strain "020315" and *Bacillus amyloliguefacines* Bc A12) differentially influenced the N,P and K contents of maize plant components. In the case of free-living diazotrophs, the additional provision of N to the plant was assumed to be significant in observed increases in yields; however, such organisms did not seem able to directly release fixed N to the plant, and this occurred only through the turnover of microbial biomass (Richardson*,* 2001).

Table (4): Effect of initial inoculation of wheat seeds with selective bacterial strains (see table "2" for identification), under varying soil moisture contents and salinity levels, on total N content residued in soil and its rate of relative changes (RC, %)*, at the end of experiment (120 days).

Bacterial isolates	Salinity levels (mg / kg soil)**	Moisture content (% of F.C)***						Bacterial isolates	Salinity levels (mg / kg soil)**	Moisture content (% of F.C)					
		100		60		40				100		60		40	
		N content (%)	RC , %	N content (%l)	RC , %	N content (%)	RC , %			N content (%)	RC , %	N content (%)	RC , %	N content (%)	RC , %
Control****	C1	0.34	0	0.34	0	0.33	0	W11	C1	0.37	9	0.40	19	0.35	5
	C2	0.32	0	0.31	0	0.32	0		C2	0.33	3	0.35	12	0.32	1
D 2	C1	0.41	21	0.41	21	0.35	6	N4	C1	0.40	16	0.42	25	0.38	15
	C2	0.37	17	0.36	16	0.35	11		C2	0.36	13	0.34	10	0.36	14
D9	C1	0.39	14	0.41	19	0.35	7	M3	C1	0.38	13	0.30	-	0.38	16
	C2	0.35	8	0.36	16	0.33	4		C2	0.35	8	0.38	22	0.33	4
W2	C1	0.39	15	0.34	0	0.35	5	M5	C1	0.34	0	0.37	8	0.36	10
	C2	0.37	16	0.36	15	0.32	1		C2	0.34	5	0.32	2	0.31	-
W10	C1	0.38	13	0.32	-	0.36	8	M8	C1	0.36	6	0.29	-	0.35	7
	C2	0.35	9	0.35	14	0.34	7		C2	0.33	4	0.27	-	0.34	5

* (RC, %): the difference between the value of a particular sub treatment and control, calculated as percent of that control.
**Salinity Levels": C1" and "C2": 2000 &4000 mg NaCl./kg soil.
***F.C.: Field capacity of the soil.
**** Control: uniculated.

Results of our present study, concerning the stimulation of N uptake by wheat plants, under the varying moisture contents and salinity levels confirm the earlier ones carried out by Bashan *et al.* (2006); El Zemrany *et al.* (2006); Adesemoye *et al.* (2009) and *Baudoin et al*. (2010).

The bacterial strains used stimulated the growth of wheat plants, via changes in root morphology and biomass, i.e. larger numbers of tips extending surface within the rhizosphere and augmentation of the polysaccharide enrichment of the inoculated roots, when compared to the uninoculated controls (El Zemrany *et al.*, 2006 & 2007). Consequently, such roots had a larger surface area to interact with soil particles, soil water, alteration in the composition of microbial community of the rhizosphere and modified root exudation (exude higher amount and type of organics in the rhizosphere).

Dehydrogenase activity "DHA" in soil

Data reported in Table (5) exert the values of dehydrogenase activity, and its rates of relative changes "RC, %", in the rhizosphere soil of wheat plants grown up from seeds inoculated with each of selected 9 bacterial isolates. The tested soil was moistened to 100, 60 or 40 % of the field capacity of soil "F.C" and salinized at 2000 "C1" or 4000 "C2" mg NaCl/kg. The enzyme assay had been carried out at two sampling periods, *i.e.* 60 and 120 days after sowing. Results showed that dehydrogenase activity in the rhizosphere soil of wheat plants considerably increased with all bacterial isolates as compared with the uninoculated controls. At the first sampling period and salinity level "C1", the bacterial isolates D9, D2 and M3, induced higher increases of the "DHA". Whereas, under the salinity level "C2", the bacterial isolates D9, D2 and N4 were the most positively effective in such concern. However at the second sampling period and salinity level "C1", inoculation with the bacterial isolates D2, M3 and N4 attained higher values of dehydrogenase activity "DHA". Whilst, under the salinity level "C2", the bacterial isolates M3, W2 and N4, produced higher values of the enzyme activity compared with control, under the assigned experimental treatments. Rates of the relative changes "RC, %" calculated for the dehydrogenase activity "DHA" in the rhizosphere soil of wheat plants inoculated with each of the selected 9 bacterial isolates, listed in Table (5) show that, at the first sampling period and salinity level "C1", the bacterial isolates D9, D2 and M3, resulted in higher values of "DHA", at all moisture contents applied. Under the salinity level "C2", the bacterial isolates D9, M3 and N4, gave the highest RC rates of the enzyme activity. At the second sampling period and salinity level "C1", the bacterial isolates D2, M3 and M3 gave higher RC values. But, under the salinity level "C2", the bacterial isolates M3, W2 and W2 were the highest.

Soil dehydrogenases are the major representatives of the oxidoreductase enzymes class (Gu *et al.,* 2009). Among all enzymes in the soil environment, dehydrogenases are of the most important, and are used as an indicator of the overall soil microbial activity (Quilchano and Marañon, 2002; Gu *et al.,* 2009 and Salazar *et al.,* 2011), because they occur intracellularly in all living microbial cells (Moeskops *et al.,* 2010; Zhao *et al.,* 2010 and Yuan and Yue, 2012). Moreover, they are tightly linked with microbial oxidoreduction processes (Moeskops *et al.,* 2010). Water availability strongly affects soil microbial activity, community composition (Geisseler *et al.*, 2011) and consequently soil enzymatic activities. As soils dry, the water potential increases, thus microbial activity as intracellular enzyme activity slows down (Geisseler *et al.,* 2011). In the case of wet soils, increased moisture could bring into soil solution soluble OM, which might be responsible for increase of bacterial population number (Subhani *et al.,* 2001). Founded significant negative relationships between DHA and pF are in agreement with our present results, that DHA is strongly affected with soil moisture. These strong correlations are undoubtedly connected with the fact that the metabolism and the survival of soil microorganisms are also strongly

Table (5): Dehydrogenase activity and its relative changes (RC, %)* in the rhizosphere soil of wheat plants, grown up from seeds initially inoculated with selected bacterial strains (see Table "2" for identification), and subjected to varying moisture and salinity levels, at two growth periods.

Bacterial strains	Salinity levels (mg/kg soil)**	Moisture content (% of F.C)***											
		100				60				40			
		Growth period (days after planting)											
		60		120		60		120		60		120	
		dehydrogenase activity and RC values											
		µg formazan g^{-1} soil $hour^{-1}$	RC %	µg formazan g^{-1} soil $hour^{-1}$	RC %	µg formazan g^{-1} soil $hour^{-1}$	RC %	µg formazan g^{-1} soil $hour^{-1}$	RC %	µg formazan g^{-1} soil $hour^{-1}$	RC %	µg formazan g^{-1} soil $hour^{-1}$	RC %
Control***	C1	55.09	0	35.82	0	47.81	0	18.06	0	21.70	0	17.09	0
	C2	41.97	0	22.02	0	30.34	0	15.53	0	19.40	0	14.55	0
D 2	C1	171.90	212	68.42	91	89.26	87	29.30	62	32.85	51	19.78	16
	C2	119.60	185	52.3	138	71.90	137	21.19	36	29.33	51	15.37	6
D9	C1	180.95	228	60.1	68	90.48	89	27.56	53	32.99	52	21.13	24
	C2	122.51	192	55.34	151	73.91	144	20.63	33	30.01	55	15.35	5

Table (5): Continued

W 2	C1	142.17	158	57.88	62	81.11	70	25.70	42	33.95	56	21.64	27
	C2	113.44	170	41.16	87	47.32	56	23.75	53	32.09	65	17.95	23
W 10	C1	122.00	121	41.30	15	68.32	43	25.68	42	31.08	43	18.13	6
	C2	88.65	111	34.03	55	44.27	46	17.67	14	25.38	31	15.00	3
W11	C1	112.42	104	50.06	40	58.93	23	19.02	5	33.37	54	17.90	5
	C2	73.93	76	35.36	61	46.74	54	17.26	11	29.75	53	14.70	1
N4	C1	163.11	196	63.39	77	80.31	68	24.72	37	33.97	57	21.66	27
	C2	100.19	139	55.47	152	77.50	155	21.53	39	32.72	69	17.97	5
M3	C1	154.85	181	60.89	70	86.30	81	31.35	74	33.97	57	20.01	38
	C2	93.27	122	56.65	157	79.30	161	21.54	39	32.53	68	16.48	13
M5	C1	120.68	119	50.98	42	67.92	42	18.54	3	31.69	46	17.13	0
	C2	84.66	102	35.96	63	39.46	30	15.55	0	26.50	37	14.93	3
M8	C1	77.43	41	43.71	22	80.10	68	18.45	2	31.55	45	19.94	17
	C2	66.88	59	37.44	70	46.74	54	17.98	16	27.68	43	14.92	3

* (RC, %): the difference between the value of a particular sub treatment and control, calculated as percent of that control.
**Salinity Levels": C1" and "C2": 2000 &4000 mg NaCl./kg soil.
***F.C.: Field capacity of the soil.
**** Control: uniculated.

impacted by the availability of water (Uhlirova *et al.,* 2005), which is essential for microbial survival and activity. Consequently, low water availability can inhibit microbial activity via lowering intracellular water potential and thus by reducing hydration and enzymes activity (Wall and Heiskanen, 2003). Periods of moisture limitation may affect microbial communities through starvation. Thus, the most common environmental stress for soil microorganisms is perhaps drought (Wolińska and Stępniewska, 2011). It had been shown in many studies that DHA is significantly influenced by water content and dropped with the decrease of soil moisture. For example Gu *et al.* (2009) observed higher DHA level (even by 90%) in flooded soil, rather than in non-flooded conditions. The higher DHA values in flooded conditions were also agreed with the results presented by Zhao *et al.* (2010) and Weaver *et al*. (2012). Results of the present study are in agreement with that of Pan *et al.,* 2013, who demonstrated that all enzyme activities tested in his study were negatively correlated with soil electrical conductivity (EC). Salinity depressed enzyme activities under laboratory conditions, as well as irrigation-induced salinity also detrimentally influenced soil enzyme activities (Rietz and Haynes, 2003).

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تقييم سلالات بكتيرية معينة كمثبتات نيتروجين جوي لنباتات القمح

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الملخص العربى

أختيرت تسع سلالات بكتيرية من ضمن 48 عزلة تم تجميعها من مواقع مختلفة في القطاع الغربي من شمال دلتا النيل. وقد سبق لهذه السلالات التسعة أن أثبتت إرتفاع قدرتها كأسمدة حيوية فيما بين جميع العزلات المختبرة معمليا. وأجريت تجربة أصص بالصوبة لتحديد قدرتها علي تثبيت النيتروجين الجوي لنباتات القمح، وذلك تحت تأثير معدلات مختلفة من الرطوبة والملوحة.

قدر محتوي المجموع الخضري لنباتات القمح و كذلك نشاط إنزيم الديهيروجينيز في ريزوسفير النباتات علي فترتي نمو هما 60 و120 يوما من الزراعة. كما قدر محتوي منطقة الجذور من النيتروجين المتبقي في نهاية التجربة. وأظهرت النتائج تفوق السلالات التالية علي الترتيب تنازليا، وذلك في كل قياسات التجربة :

"D 2"(*Azotobacter chroococcum* DSM 2286)> "D9" (*Enterobacter kobei CIP*) > "M3"(*Bacillus megaterium EIF18*), "N4" (*Clostridium sp.*) > "W2" (*Klebsiella sp.*).