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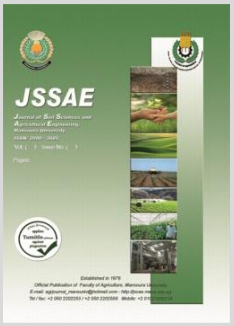
The Role of Salt-Tolerant Plant Growth Promoting Bacteria in Increasing the Resistance of Canola to Salt-Stress

Ghada A. Z. Ibrahim*

Soil Fertility and Microbiology Department, Desert Research Centre, Cairo, Egypt.



ABSTRACT

Salinity, a major environmental stress that inhibits agricultural productivity, has a negative impact on plant growth and development. The goal of this study was to use salt-tolerant bacteria to improve the plant's ability to resist salt-stress and to achieve high canola productivity in saline soil conditions. The rhizobacteria strains, *Acinetobacter radioresistens* (NBRC 102413) and *Enterobacter cloacae* (NBRC 102413) were evaluated for their ability to produce organic acids and phytohormones (IAA). A field experiment was conducted in the Agricultural Experimental Station in the Desert Research Center, Ras-Sudr, South Sinai Governorate, Egypt, during the winter season of 2018 / 2019. The researchers focused at how the inoculation with salt-tolerant bacteria and irrigation with three saline underground's water of (8.94, 11.12, and 12.1 dS m^{-1}) affected some growth and yield parameters, as well as the nutrients content and intracellular organic osmolytes produced by canola plants grown in saline-calcareous soil. *A. radioresistens* and *En. cloacae*, were shown to be able to survive in a saline-nutrient broth medium containing up to 12 % NaCl. The highest values of plant fresh weight, dry weight, seeds yield and oil yield, nutrients content and osmo-protectant molecules (glycinebetaine and choline), as well as rhizosphere microbial counts and dehydrogenase activity, were recorded in treatment of the irrigation with low saline-underground water (8.94 dS m^{-1}), while proline increased by using medium saline-underground water (11.12 dS m^{-1}).

Keywords: *Canola, Acinetobacter, Enterobacter, Salinity, Osmo-protectant molecules.*

INTRODUCTION

Calcareous soils encompass more than 30 % of the earth's surface, and they occur naturally in dry and semi-arid climates (Marschner, 1995). In Egypt, calcareous soils cover around 0.65 million feddans, or roughly 25 - 30 % of the total land area. Due to the availability of plant nutrients, high calcium carbonate levels impact soil qualities related to plant development. These soils pH is normally higher than 7, and can reach 8.5. The pH of these soils may reach 9 when sodium carbonate is present, $CaCO_3$ can build up in some soils, forming highly hard layers that are impervious to water and plant roots, while free lime may dominate a calcareous soil, it may also include considerable levels of iron (Fe), aluminium (Al), and manganese (Mn) as discrete minerals, coatings on soil particles, or complexed with soil organic matter. These metals act as strong sorption sites for P and are often more important than lime in limiting P solubility in calcareous soils, (Taalab *et al.*, 2019). Plant acclimatization to salinity, on the other hand, might be a tool to increase salt tolerance even in a sensitive genotype (Suzer, 2015). In this regard, Santangeli *et al.* (2019) explored the physiological processes underlying rapeseed cultivars' responses to progressive and chronic NaCl exposure (*Brassica napus* L.). Seedlings of the cultivars Dynastie (salt resistant) and SY Saveo (salt sensitive) were subjected to increasing soil salinity conditions over the course of 60 days. Salt-exposed plants of both cultivars had lower biomass, size, and number of leaves, according to the researchers. However, after 60 days, the sensitive cultivar had a lower relative reduction in biomass than the other cultivars.

Plant growth promoting bacteria (PGPB) are being used in a variety of studies to increase plant growth and induce systemic tolerance to different abiotic stressors in plants, such as salt and drought, by altering plant physiology (Egamberdieva *et al.*, 2016). Salt-tolerant PGPB helps plants survive in salt environments. Furthermore, the microbiome of plants that is naturally connected with them has the capacity to defend the host through stress avoidance, tolerance, and resistance responses. Recent advances in microbiome research have shown new approaches for revolutionary microbe-assisted technologies to improve plant salt tolerance and increase agricultural output in saline environments (Kumar *et al*., 2020). The rhizobacterial strain *En. cloacae* (PGLO9) was collected from the rhizosphere soil of a potato plant (*Solanum tuberosum* L.) to investigate functional potentialities of rhizobacteria in connection to plant growth boosting activities (Verma *et al*., 2018). Thanh and Diep, (2014) results showed that 3 isolates, including DTN1b (*Azotobacter vinelandii*), VTN2b (*Bacillus subtilis*) and VTN7 (*Enterobacter cloacae*) proposed as potential microbial biofertilizers for sustainable corn production because of their benefit and Biosafety.

Bybordi and Ebrahimian (2011) investigated the effect of salt stress on the activity of nitrogen and phosphorus metabolic enzymes in canola (*Brassica napus* L.). In compared to controls, they discovered that nitrate reductase activity was boosted in treated plants with low NaCl, but gradually reduced in plants exposed to high NaCl. They went on to suggest that high salt levels might cause a

*** Corresponding author.**
E-mail address: ghada_micro@yahoo.com
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drop in nitrate reductase activity, nitrate content, and total nitrogen content in canola, which could be a physiological response to minimise growth and surplus biomass in Canola. Phosphorus levels in leaves dropped substantially when exposed to salt. Omidi (2010) investigated the main and interaction effects of two factors Drought (D) and Genotype (G) studied were highly significant differences in shoot and root dry weight, proline content osmolyte, and enzyme activities of Guaiacol peroxidase (GAx), Ascorbate peroxidase (APx), and Catalase (Cat) in root and shoot of canola genotypes.

Canola (*Brassica napus* L.) is a major oilseed crop grown for edible oils and biodiesel fuel all over the world (Gharelo and Noparvar, 2018). Canola oil is a healthy vegetable oil because of its balance of omega 3-6-9 essential fatty acids, making it a healthy vegetable oil for cooking and the processed food sector all over the world. Calcareous soils encompass more than 30 % of the earth's surface, and they occur naturally in dry and semi-arid climates, (Marschner, 1995). Bandehagh *et al*., (2021) studied that the salinity reduces crop productivity significantly over the world. In the approaching decades, the production of salt stress-tolerant species will be critical to maintaining the food supply. Canola (*Brassica napus* L.) is a popular crop for food oils and biodiesel fuel manufacture. Despite the fact that much kinds of canola are salt-resistant, increased salinity values dramatically impair plant production and growth. Plant proteins and genomes are involved in developing a new phenotype in response to salt stress under saline conditions.

The aim of this study is increasing the productivity of canola cultivated in a saline soil by using some salt-tolerant PGPB (*Acinetobacter radioresistens* and *Enterobacter cloacae*) that produced some traits related to plant growth promoters.

MATERIALS AND METHODS

Isolation, purification, and preservation of rhizobacteria

Plants having economic value (Wheat, Barley and Mays) were collected from Desert Research Center Stations (Baloza 29° 25' N 31°15' E, Ras Sudr 29°35' 30' N, 32°42' 20° E and El Maghara 30°40' 30° 48 N, 33° 30' E), placed in sterilized plastic containers, and maintained at 10° C in ice box to isolate PGPB.

A rhizobacteria-colonizing-rhizosphere-soil isolation program was carried out. 5 gm fresh roots were rinsed under running water and surface sterilized in 5 % NaClO for 1 minute before being washed three times with sterilized distilled water. 1 ml of dilutions of 10^{-7}, 10^{-8} CFU mL^{-1} were placed on potato dextrose agar (PDA) plates and incubated at 28±2°C for 2 days, Schaad *et al.*, (2001). The pure isolates were stored in 20 % glycerol solution at -20° C for a long time. Somasegaran and Hoben (1994) reported the morphology, size, form, colour, and growth pattern of colonies after 24 hours of development on PDA plates at 28 ± 2° C for 2 days. Light microscopy was used to determine the size of the cells. The Gram reaction was carried out according to (Vincent and Humphrey's 1970).

Bacterial Isolates Identification

The most active isolates were identified at the molecular level using the partial 16S rRNA gene sequencing approach used by Sigma Scientific Services Co. (Berg *et al*., 2002). Direct genomic DNA extraction from colonies cultivated on NA medium was used to identify isolates. PCR was used to amplify the sequences of the bacterial 16S rRNA gene using forward and reverse primers:

F (5′AGA GTTTGA TCC TGG CTC AG-3′)
R (5′-GGT TACCTT GTT ACG ACTT-3′),

1 Taq & Go (MP Biomedicals, Eschwege, Germany), 1.5 mM $MgCl_2$, 0.2 mM of each primer, and 1 l of template DNA were used in the PCR (95° C, 5 min; 30 cycles of 95° C, 30 s; 57° C, 30 s; 72° C, 90 s; and elongation at 72° C, 5 min). Gene JETTM PCR Purification Kit was used to purify the PCR product (Thermo K0701). The forward and reverse primers were used to sequence the PCR result using an ABI 3730 x l DNA sequencer (Lane, 1991). The sequences obtained from bacterial isolates were analyzed using the Basic Local Alignment Search Tool (BLAST) analysis tools (Altschul *et al*., 1990) at the National Center for Biotechnology Information database (NCBI) Gene Bank database to identify the most similar 16S rRNA sequences available in the Gene Bank. *Acinetobacter radioresistens* strain NBRC 102413 accession number (NR 117677.1) and *Enterobacter cloacae* strain ATCC 13047 accession number (NR 118568.1) were identified.

The ability of the tested rhizobacterial isolates to exhibit some PGP-properties in vitro

To look for features associated to plant growth promoters in these isolates, *A. radioresistens* and *En. cloacae* were tested under *in vitro* conditions towards their efficiency for production of organic acids and phytohormones.

- **Determination of organic acids**

Voges-Proskauer (VP) test MRVP broth: ingredients per liter of de ionized water: 7.0 gm of buffered peptone, 5.0 gm of glucose and 5.0 gm of di-potassium phosphate, pH = 6.9, according to Anil Kumar *et al*. (2014).

- **Determination of phytohormones**

High-performance liquid chromatography (HPLC) Ultimate 3000 Thermo dionex, Germany, was used to examine plant hormones. HPLC using a photodiode array detector and data analysis software, (Hanifi and Elhadramy, 2007). These analysis estimated by Central laboratory of Desert Research Center.

- **Salinity tolerance test of isolated bacteria**

Bacterial growth determined in different salt concentrations (1, 3, 6, 9 and 12 % NaCl) on nutrient broth medium, after incubation for 24 hr. / 30° C. The turbidity absorption measured by Spectrophotometer (Jenway Model 6105 UV/ Vis spectrophotometer) at 600 nm (Jacobs and Gerstein, 1960).

- **Germination assay of canola *in vitro***

Canola seeds (*Brassica napus* L. family *Brassicaceae*) variety "Serw-4" was supplied by Oil Crops Research Department, Agricultural Research Center (ARC), Giza, Egypt. Field Crops Research Institute. Oil fatty acids in canola seeds contain less than 2 % erucic acid. Germination test was carried out to make sure of the viability of seeds. *In vitro*, ten seeds were cultivated in every Petri dish with three replicates. The irrigation water resources were obtained from three wells having three salinity degrees (8.94, 11.12 and 12.1 dS m^{-1}) from Ras-Sudr. The following growth metrics were measured after 7

days of planting: fresh & dry weights (gm / plant), plant height (cm) and germination assay (%).

Field experiment

Agricultural Experimental Station of Desert Research Center (DRC) conducted a field experiment during the winter growing season of 2018 / 2019. Ras-Sudr (located at 29°35′30″N for Latitude and 32°42′20″E for Longitude), South Sinai Governorate, Egypt, using immersion irrigation system. The study concerned with increasing the productivity of canola and its resistance to salinity stress by inoculation with tolerant salinity bacteria (*A. radioresistens* and *En. cloacae*) under saline-calcareous soil conditions.

The experimental design consisted of a split plot design with three duplicates, each having a 6 m^2 plot area (2 x 3m). The following saline water wells were used to irrigate the main plots: high salinity water well (12.1 dS m^{-1}), medium salinity water well (11.12 dS m^{-1}) and low salinity water well (8.94 dS m^{-1}). The sub-plots were assigned to the following bacterial inoculation treatments: control (uninoculated; watered just with water), *A. radioresistens* inoculation, and *En. cloacae* inoculation.

Canola seeds (variety "Serw-4") supplied by Oil Crops Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt were sown on November 2018, 2019. The inoculation process was done by adding 100 ml / plant from broth media of every microbe (has 10^7 CFU / ml) as a soil drench on canola plants for one time every month from planting to harvest.

Organic fertilizer (farmyard manure) at the rate of 20 m^3 fed^{-1} was applied to the experimental soil during soil preparation (15 days before sowing). Phosphorus was added during soil preparation at the rate of 200 kg / fed as calcium superphosphate (15 % P_2O_5). Potassium was added as potassium sulphate (48 % K_2O) at the rate of 50 kg fed^{-1}, while ammonium nitrate (33.5 % N) was added at the rate of 150 kg fed^{-1} in three equal doses after planting, after the plant has thinned and after 30 days from sowing.

Canola plants were uprooted at random from each plot to assay the following parameters: fresh and dry weights (gm / plant), seed yield weight (gm) and oil yield (%) as well as the total nitrogen, phosphorus and potassium in canola tissues and the Osmo-protectant molecules produced by canola (glycinebetaine, choline and proline). The rhizosphere microbial counts and soil dehydrogenase activity after harvesting were also estimated.

Analyses:

- Soil physical and chemical properties were estimated according to Piper (1950) and Jackson (1973).
- Some chemical properties of the used saline water wells were assayed according to Jackson (1973) and Page *et al.* (1982).
- The oven dried plant materials were wet digested using a mixture of pure $HClO_4$ and H_2SO_4 at a ratio of 1:1, according to Jackson (1973). Total nitrogen was determined using the micro-Kjeldahel method, phosphorus was determined Spectrophotometrically (Jenway Model 6105 UV/ Vis spectrophotometer) at 600 nm using ammonium molybdate and stannus chloride reagents, while potassium was determined Flamephotometerically (Jenway Model 6105), (Page *et al.*, 1982).
- The amino acids (glycinebetaine and choline) were estimated according to Grieve and Grattan (1983), while proline was estimated according to procedure of Bates *et al.* (1973).
- Rhizosphere microbial counts: modified Ashby's medium (Abd El-Malek and Ishac, 1968) for *Azotobacter chrococcum* was used for counting of nitrogen fixers by M.P.N technique and calculated using Cochren's tables (Cochran, 1950). King's medium (King *et al*., 1954) for counting *Pseudomonas* sp., while nutrient agar medium (Jacobs and Gerstein, 1960) used for total count.
- Dehydrogenase activity (DHA-ase) was assayed colourimetrically for the extraction of formed pink or red colored of 2,3,5 triphenyl Formazan (TPF) produced from the reduction of 2,3,5- triphenyl tetrazolium chloride (TTC) according to Casida (1977).

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the least significant difference at 0.05 level to compare the differences among means according to the procedures outlined by Snedecor and Cochran (1980) by using computer program of Statistix version 9 (Analytical software, 2008).

RESULTS AND DISCUSSION

Isolation of plant growth promoting bacteria

Table (1) shows the different sites were used to collect many plants for isolate bacterial saline resistance. Khanghahi *et al*. (2021) studied that the isolate and identify bacterial strains with biological nitrogen-fixing ability and potassium, phosphate, and zinc solubilization activities from a field of wheat, as well as to develop exact analytical methods for estimating the impact of varying temperatures, NaCl levels, and pH on the growth and activity of identified isolates *Pseudomonas* and *Acinetobacter*.

Table 1. Evaluation of bacteria isolates as plant growth promoting bacteria

Bacterial source			Parameters measured		Bacterial counts*10^2 CFU/g root
Isolation localities	Plant	Bacterial isolates code	Stem fresh weight (gm)	Root fresh weight (gm)	
Control			0.42	0.32	0.24
Ras-Sudr	Barley	RB	0.55	0.36	18.2
Ras-Sudr	Maize	RM	0.60	0.43	19.7
Baloza	Barley	BB	0.52	0.35	16.3
El-Maghara	Wheat	EW	0.57	0.45	17

Identification of selected bacteria

On the basis of the consensus sequences for the 16S rRNA gene, phylogenetic trees were constructed using sequences from the three bacterial isolates (Figs. 1 and 2). Identification of bacterial strains using 16sRNA revealed that the three strains were belonged to the *A. radioresistens* strain NBRC 102413 and *En. cloacae* strain ATCC 13047. They deposited in Gene bank with the accession numbers *A. radioresistens* strain NBRC 102413 accession number (NR-117677.1) and *En. cloacae* strain ATCC 13047 accession number (NR_118568.1), respectively. Nhu and Diep (2017) studied that the bacterial isolates were tested in-vitro for plant growth promoting properties including nitrogen fixation, phosphate solubilization and IAA production

together with producing siderophores, nitrogen-fixing and phosphate-solubilizing bacteria showed high degrees of similarity to those of the GenBank reference strains (between 97% and 100%), *Bacillus subtilis* and *Acinetobacter* sp. Hafeez *et al*., (2018) estimated that the halotolerant phosphate solubilizing *Enterobacter cloacae* HFZ-H4 strain to be used as biofertilizer formulation and study of their shelf life. Bio-formulations of *Enterobacter cloacae* HFZ-H4 strain in fly-ash, saw dust and rice husk ash were screened for the better survival as bacterial bio-fertilizers formulation. From results of this study it can be concluded that the halotolerant strain of *Enterobacter cloacae* HFZ-H4 formulations in fly ash could be a better option for the growth and yield of the crop in sodic / saline soil.

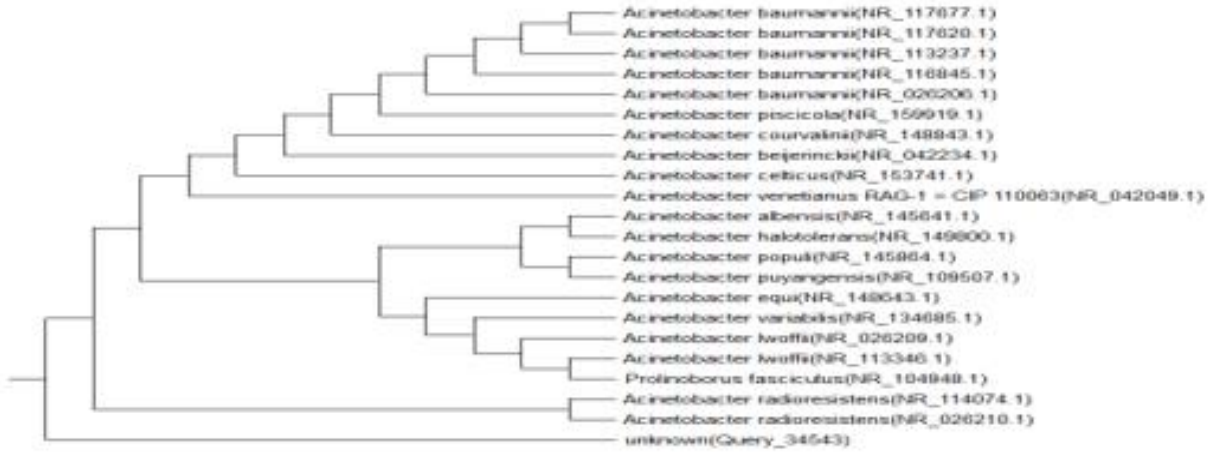

Fig. 1. The identify tree based on the 16S rRNA sequences of *Acinetobacter radioresistens* strain NBRC 102413 with Related 16S rRNA sequences found in Gen Bank database.

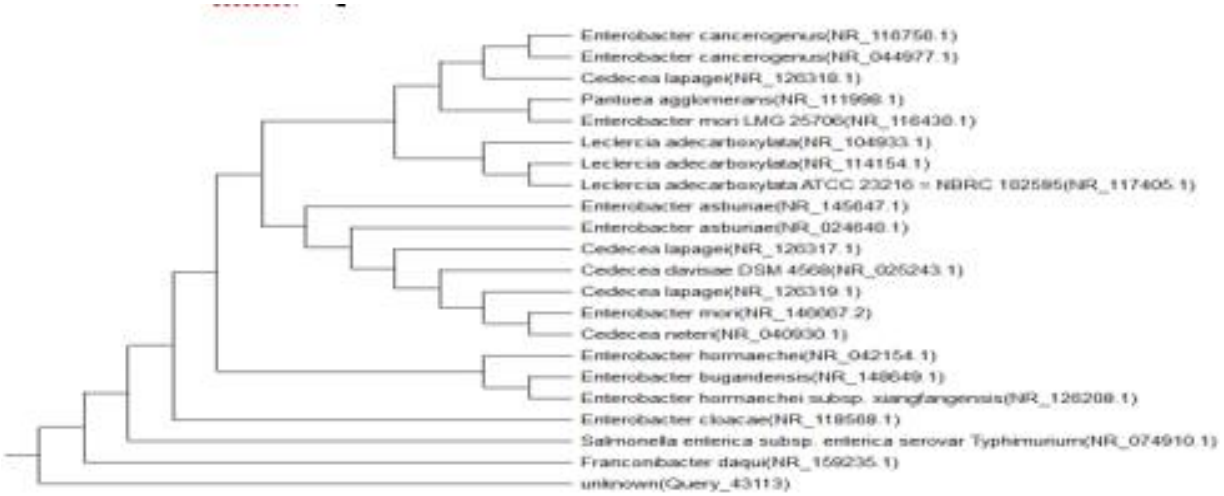

Fig. 2. The identify tree based on the 16S rRNA sequences of *Enterobacter cloacae* strain ATCC 13047 with related 16S rRNA sequences found in Gen Bank database.

The ability of the tested rhizobacterial isolates to exhibit some PGP-properties in vitro

Some of PGP-related properties of the tested bacterial isolates were estimated. In general, both of the tested bacteria were apparently able to trigger some PGP-properties under *in vitro* conditions.

The ability of *A. radioresistens* strain NBRC 102413 and *En. cloacae* strain ATCC 13047 to produce organic acids was determined. Hence, the efficiency of tested isolates to produce a particular groups of organic acids, which lead to a low soil pH and helps in facilitating the transformation and absorption of minerals, particularly under calcareous soil conditions. In this concern, Adeleke *et al*. (2017) reported that the organic acids in soil produced from many sources one of them is microorganisms. Posso *et al*. (2017) confirmed that it has been sure that rhizosphere microorganisms that produced organic acids are effective method to release the unavailable phosphorus to available mineral compounds in soil. They added that bacteria varied in their capability production and type of metabolized organic acids, the most spread were citric and gluconic acid."

Figure (3 and 4) shows the ability of both tested isolates to produce indole acetic acid (IAA). The quantitative amounts of produced IAA were 0.1198 and 0.0061 ppm for *Ac. radioresistens* and *En. cloacae*, respectively. In fact, many investigators consider the indole secretion, by PGPRs, as a vital mechanism to clarify the plant promotion by stimulate root growth and provides it with more branching and larger surface area (Verma *et al.*, 2010; Mohite, 2013; and Majeed *et al.*, 2015).

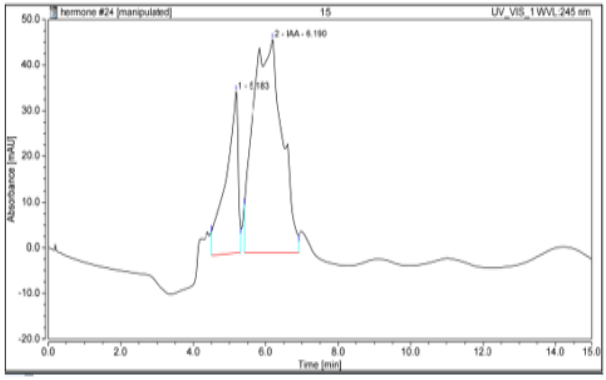

Fig. 3. Phytohormones in *Acinetobacter radioresistens*

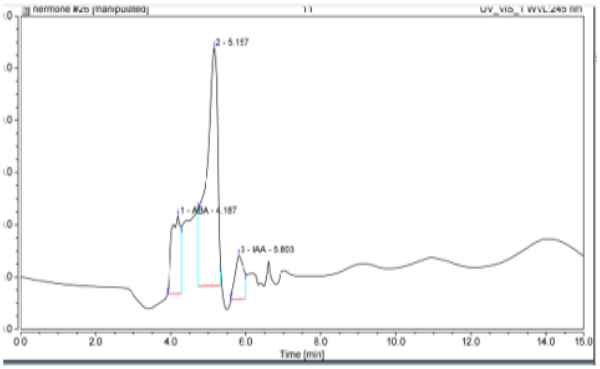

Fig. 4. Phytohormones in *Enterobacter cloacae*

Hence, it could be demonstrated that both tested rhizobacteria were able to exhibit some PGP-properties, which may display several modes of beneficial action.

Salinity tolerance test of isolated bacteria *in vitro*

Figure (5) shows bacterial growth under different salt concentrations (1, 3, 6, 9 and 12 % NaCl) on nutrient broth medium, after incubation for 24 hr. / 30° C. Obtained results showed that the two strains used were able to live in saline nutrient broth medium up to 12 % for *A. radioresistens* and *En. cloacae*. In this respect, Ji *et al.* (2020) found that the physiological and biochemical characteristics of *Enterobacter cloacae* HG^{-1} strain isolated from saline-alkali soil showed highly salt-tolerant (10 % NaCl).

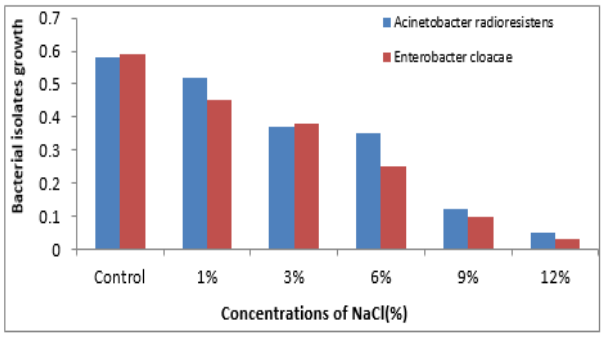

Fig. 5. Salinity concentration tolerance of bacterial isolates used in canola field experiment

Germination assay of canola seeds *in vitro*

Figure (6) shows the effect of irrigation of canola seeds by three wells of water (well 1, well 2 and well 3) with three saline degrees (8.94, 11.12 and 12.1 dS m^{-1}) on the fresh and dry weights (gm / plant), length (cm) and germination (%) after 7 days from planting. Obtained results revealed that canola seeds irrigated by well1 of water (8.94 dS m^{-1}) gave fresh and dry weight values higher than control treatment. The highest value of plant lengths (cm) was recorded by using well1 of water (8.94 dS m^{-1}) and well2 of water (11.12 dS m^{-1}). While, the germination (%) of canola seeds was decreased gradually with increasing the salinity of water. Shahbaz *et al.* (2011) found that seeds of six cultivars of canola (*Brassica napus* L.) were germinated under various levels of salinity (0, 50, 100, 150 and 200 mM NaCl solutions), while the increase in NaCl concentrations progressively inhibited seed germination. They added that seedling growth parameters were affected by salt stress, particularly at 150 and 200 mM.

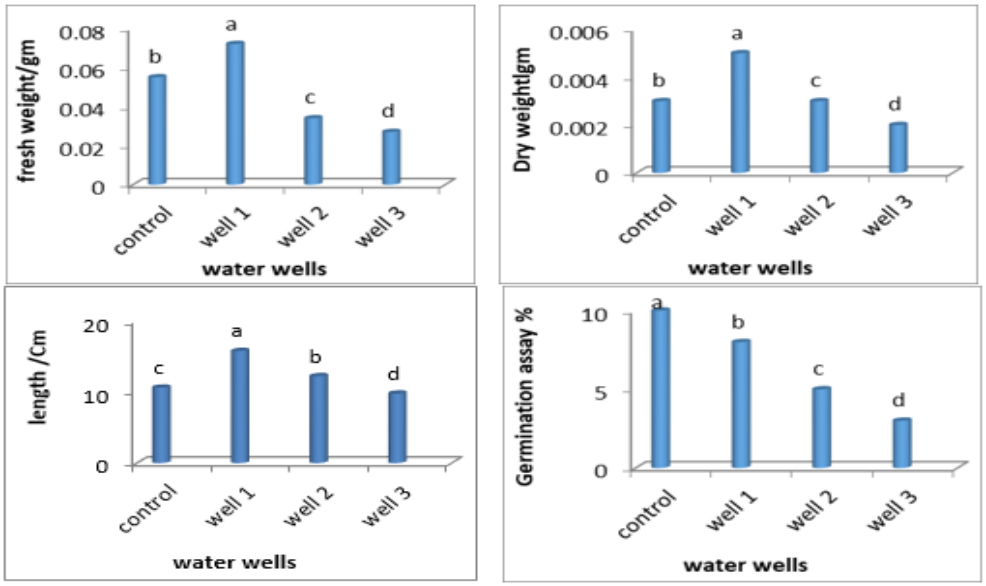

Fig . 6. Seedling germination assay of canola *in vitro* (after 7days) irrigated by three wells of water with three salinity degrees in Ras-Sudr

Field experiment

The salinity was in the soil and irrigation water. The main physical and chemical properties of the experimental soil and the initial chemical analysis for three wells water used in the field experiment are shown in Tables (2 and 3).

Table 2. Some physical and chemical properties of the experimental soil at Ras-Sudr station

Soil depth (cm)	Particle size distribution (%)				$CaCO_3$ (%)	EC (dS m^{-1})	pH
	Sand	Silt	Clay	Texture grade			
0-30	77.44	12.60	9.96	Sandy	27.0	4.20	7.50
30-60	80.10	10.30	9.60	loam	32.0	4.80	7.76
Soluble cations and anions in saturated soil extract (meq/l)							
Soil depth (cm)	Soluble anions				Soluble cations		
	Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	SO_4^-	Cl^-
0-30	31.40	0.47	8.60	2.10	0.00	2.80	10.67	29.10
30-60	34.50	0.42	9.20	4.50	0.00	2.95	14.92	30.75

Table 3. Some chemical properties of the studied three wells water at Ras- Sudr

Wells	EC (dSm-1)	pH	Soluble cations (meq/l)				Soluble anions (meq/l)			
			Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	SO_4^-	Cl^-
Well water1	8.94	6.17	53.3	1.90	17.1	16.3	0.00	4.60	27.80	56.2
Well water2	11.12	6.26	65.6	1.53	27.9	15.4	0.00	8.3	39.83	62.3
Well water3	12.1	7.00	67.1	1.95	32.2	19.4	0.00	10.6	47.05	63.0

Data recorded on some growth and yield parameters, nutrients content and amino acids produced by canola plants as affected by inoculation with tolerant salinity bacteria (*A. radioresistens and En. cloacae*) and irrigation by three wells of saline water (8.94, 11.12 and 12.1 dS m^{-1}) under saline-calcareous soil conditions. Also, the estimated rhizosphere microbial counts and soil dehydrogenase activity after harvesting will be presented and discussed as follows:

a) Plant growth and yield characters

Canola plant parameters, i.e., fresh and dry weights (Ton / Feddan), seed yield weight (Ton / Feddan) and oil yield (%) as affected by inoculation with tolerant salinity bacteria and irrigation by three wells of saline water are presented in Table (4). All tested characters were significantly affected by inoculation and irrigation by three wells of saline water. It is clear that the inhibition effect increased with the increase in the salinity of well water. The reduction in plant growth could be according to the osmotic effect as a result of salt stress that caused an increase in growth inhibitors and decreased growth promoters. These results are in line with Rady *et al.* (2013) and Gharelo and Noparvar (2018). Regardless of irrigation with saline well water, the inoculated plants surpassed the uninoculated plants (control) in all tested growth and yield parameters with a relative superiority of *A. radioresistens* over *En. cloacae*. Many researchers have demonstrated the importance of inoculation with resistant saline bacteria in increasing plant growth and yield (Khalifa *et al*., 2016; Lafi *et al*., 2017 and Ji *et al.*, 2020). They found that *A. radioresistens* and *En. cloacae* had features that promoted plant development and adaption to the environment, and that they generated substantial increases in all growth metrics for all major plant species when compared to uninoculated plants.

The results show that canola plants watered by a low saline well (8.94 dS m^{-1}) and infected with resistant salinity bacteria outperformed all other treatments, achieving the maximum growth and yield characteristics. As a result, canola plants inoculated with *A. radioresistens* or *En. cloacae* and irrigated by a low saline well had higher fresh weight (187.53 and 162.67 %), dry weight (112.32 and 109 %), seed yield weight (368.14 and 306.8%), and oil yield (90.24 and 84.39 %) than uninoculated plants irrigated by the same saline. Under conditions of salt stress, inoculation with tolerant salinity bacteria could indeed produce both organic acids and IAA. Hence, it is plausible that rhizobacteria may provide a useful way to reduce the adverse effects of salinity stress on plants and balances the relative content of IAA in canola to enhance their salt tolerance and increase canola growth under salt stress. The previous findings were in harmony with those reported by (Yang *et al*., 2009; Yao *et al.*, 2010; Egamberdieva *et al.*, 2016 and Li *et al.*, 2017).

Table 4. Effect of bacterial inoculation and irrigation by three wells of saline water on some growth and yield parameters of canola plants grown under saline-calcareous soil conditions

Treatments		Growth parameters (Biological yield)			
		Fresh weight (Ton / Feddan)	Dry weight (Ton / Feddan)	Yield weight of seeds (Ton / Feddan)	Yield of oil (%)
High saline plot	Control	19.43c	5.85b	9.63c	15.6c
	Acinetobacter radioresistens	49.57a	7.92a	16.46a	24.8a
	Enterobacter cloacae	35.18b	6.99ab	14.48b	19.3b
	LSD (0.05)	2.8115	1.6150	1.9781	2.9835
Medium saline plot	Control	33.65c	10.36b	15.14c	18.3b
	Acinetobacter radioresistens	90.11a	18.43a	55a	33.5a
	Enterobacter cloacae	80.92b	17.73a	27.12b	31.2a
	LSD (0.05)	2.8161	2.8538	3.4490	2.7547
Low saline plot	Control	50.13c	19.55b	10.58c	20.5b
	Acinetobacter radioresistens	144.14a	41.51a	49.53a	39a
	Enterobacter cloacae	131.68b	40.86a	43.04b	37.8a
	LSD (0.05)	2.8160	2.8160	3.4801	1.7302

*** Values followed by the same letter do not differ significantly at 5% probability level.**

b) Nutrients content of canola plants

Data in Table (5) shows the effect of inoculation with tolerant salinity bacteria and irrigation by three wells of saline water on the N, P and K percentages of canola plants grown under saline-calcareous soil conditions. It's obvious that the decrease in the content of chemical nutrients with increase in the salinity of well water. As mentioned before, the inoculated plants surpassed the uninoculated ones (control) with a relative superiority of *A. radioresistens* over *En. cloacae*. Generally, results behaved in a similar manner as in canola growth and yield parameters and confirmed that canola plants irrigated by low saline well (8.94 dS m^{-1}) and inoculated with tolerant saline bacteria exceeded all other tested treatments and gave the highest values of N, P and K percentages in canola tissues. The greatest values were (2.92 and 2.47 % N), (3.63 and 2.65 % K), and (0.48 and 0.46 % P) were found in canola plants treated with *A. radioresistens* or *En. cloacae* and watered with low saline well. In fact, the ability of the studied tolerant saline rhizobacteria to enhance mineral solubilization under calcareous soil conditions and/or synthesis of siderophores and plant hormones (such as auxins or gibberellins), which positively affect root

development and, as a result, their function in the uptake of both water and nutrients, could be attributed to the improvement in nutrient percentages in canola tissues. These findings followed the same pattern as those reported by (Verma *et al*., 2010; El Sayed and Hagab, 2020 and Ji *et al*., 2020).

Table 5. Effect of bacterial inoculation and irrigation by three wells of saline water on the N, P and K percentages of canola plants grown under saline - calcareous soil conditions

Treatments		Nutrients concentration		
		Nitrogen (%)	Phosphorus (%)	Potassium (%)
High saline well	Control (uninoculated)	1.36c	0.265c	0.074c
	Acinetobacter radioresistens	2.55a	0.308a	1.95a
	Enterobacter cloacae	1.73b	0.280b	0.146b
	LSD (0.05)	0.0682	0.0118	0.0232
Medium saline well	Control (uninoculated)	1.48c	0.283c	0.153c
	Acinetobacter radioresistens	2.71a	0.466a	2.25a
	Enterobacter cloacae	1.93b	0.334b	1.33b
	LSD (0.05)	0.0200	0.03460	0.0314
Low saline well	Control (uninoculated)	1.6c	0.349c	1.139c
	Acinetobacter radioresistens	2.92a	0.480a	3.633a
	Enterobacter cloacae	2.47b	0.465b	2.65b
	LSD (0.05)	0.1165	0.0116	0.0232

*** Values followed by the same letter do not differ significantly at 5% probability level.**

c) Some biochemical compounds (intracellular organic osmolytes) produced by canola

Osmo-protectant molecules (glycinebetaine, choline and proline) produced by canola under saline conditions are shown in Table (6). It's clear that the content of canola plants from such intracellular organic osmolytes amino acids took the same trend as in plant growth and nutrients content data. content of canola plants from such intracellular organic osmolytes took the same trend as in plant growth and nutrients content data. Canola plants inoculated with *A. radioresistens* and *En. cloacae* individually and irrigated with low saline well water (EC of well1 = 8.94 dS m-1) achieved the highest production of glycinebetaine (235.0 and 180.0 µmol / gm dry weight) and choline (610.0 and 780.0 µmol / gm dry weight) in canola tissue, respectively. Canola plants inoculated with *A. radioresistens* and *En. cloacae* individually and irrigated with medium saline well water (EC of well 2 = 11.12 dS m-1) achieved the highest production of proline (4.87 and 2.75%) in canola tissue, respectively. While, uninoculated plants irrigated by the same saline well produced 37.0 µmol glycinebetaine / gm dry weight, 420 µmol choline / gm dry weight, and 0.8 % proline. In fact, proline, glycinebetaine and choline are amino acids that can accumulate in low concentrations under optimal conditions. Under salt stress, such osmolytes are metabolites that biosynthesized and accumulate inside the cell in high concentrations as a common physiological response in plants when exposed to diverse environmental factors that cause stress, such as salinity. Plants accumulate proline, glycinebetaine and choline to mitigate detrimental effects of salt stress (Salinas *et al.,* 2013 and Escalante-Magana *et al.*, 2019).

It is obvious that inoculation with tolerant salinity bacteria play an important role in the alleviation of salt stress on canola plants through their positive effect on biochemical contents of plant. In this respect, Li *et al*., (2017) and Ji *et al.* (2020) found that the proline concentrations in the leaves of inoculated plants increased to 12.43 % over than uninoculated plants. They added that the physiological analyses of proline content and antioxidant enzyme activity were increased by *Enterobacter cloacae* inoculation at NaCl concentrations of 50 and 100 mM. While, Salinas *et al.* (2013), Khalid *et al*., (2015) and Wei *et al.* (2017) confirmed that the proline, betaine, and choline is an important osmo-protectant molecules, related with secondary metabolism, protect cells against oxidative damage and have ameliorative effects on the growth by altering ion homeostasis (regulate ion channel and transporters), photosynthetic and antioxidant capacity.

Table 6. Effect of bacterial inoculation and irrigation by three wells of saline water on some biochemical compounds produced by canola plants grown under saline-calcareous soil conditions

Treatments		Amino acids		
		Glycinebetaine (µmol/gm dry weight)	Choline (µmol/gm dry weight)	Proline (%)
High saline well	Control (uninoculated)	39c	30c	0.5b
	Acinetobacter radioresistens	181a	40b	2.7a
	Enterobacter cloacae	63b	120a	1.4ab
	LSD (0.05)	1.9979	3.4605	1.6353
Medium saline well	Control (uninoculated)	50c	73c	0.8c
	Acinetobacter radioresistens	190a	95b	4.7a
	Enterobacter cloacae	67b	124a	3b
	LSD (0.05)	3.4605	1.9979	1.6353
Low saline well	Control (uninoculated)	37c	420c	0.4c
	Acinetobacter radioresistens	235a	610b	1.7a
	Enterobacter cloacae	180b	780a	2.5b
	LSD (0.05)	1.9979	3.4605	1.1650

***Values followed by the same letter do not differ significantly at 5% probability level.**

d) Rhizosphere microbial counts and dehydrogenase activity (DHA-ase)

Rhizosphere is a zone where there is an increase in microbial and enzyme activity. The measurement DHA-ase enzyme activity in soil has often been used as a parameter to evaluate the overall microbial activity in soil. Data in Table (7) shows some aspects of rhizosphere properties after harvesting canola, affected by inoculation with tolerant saline bacteria and irrigation by three wells of saline water. As general, all tested rhizosphere properties were significantly

affected by different treatments under study and behaved in a similar manner as in aforementioned parameters. Irrespective of the salinity of well water, an increase in all recorded total microbial counts and rhizosphere DHA-ase activity was observed upon inoculation. Total bacteria numbers and DHA-ase activity were lower in the uninoculated treatments. In fact, rhizosphere soil microbiological characteristics, as measured by total microbial counts and dehydrogenase enzyme activity, showed a stronger response to applied resistant saline bacteria, with *A. radioresistens* outperforming *En. cloacae*. These findings are consistent with those of (Ashrafuzzaman *et al*., 2009 and Abdel Latef *et al*., 2021). They discovered that rhizobacterial inoculation (PGPRs) increased the abundance and activity of the microbial population in the canola rhizosphere.

Irrespective of inoculation, the highest total microbial counts and dehydrogenase activity were obtained in plots irrigated by low saline well water followed by medium saline well and then high saline well water. Hence, there are a negative correlation between the salinity and the rhizosphere properties. In this respect, Omer (2017) reported that soil salinity as a vital stress factor harms the microbial process, diminishing bacterial diversity and controlling microbial wealth, composition, and functions.

Treatments comprising tolerant saline bacteria (*A. radioresistens* or *En. cloacae*) in combination with irrigation with low saline well water confirmed their synergistic interaction to increase the total microbial counts and DHA-ase activity of rhizosphere soil. For instance, total count of bacteria was increased to (111.11 and 55.56 %), and the increase in the counts of nitrogen fixers was (30.63 and 14.41%), the increase in the count of *Pseudomonas* sp. was (37.88 and 21.21 %), while the increase in DHA-ase activity was (103.57 and 60.71 %) over that obtained under uninoculated plants irrigated by the same saline well. The result substantiates the findings of (Siczek *et al*., 2017; Cordero *et al*., 2020 and Abdel Latef *et al.*, 2021). They concluded that under salt stress the total microbial count, nitrogen fixer count and dehydrogenase activity in the canola and pea rhizosphere was enhanced in response to inoculation with growth promoting rhizobacteria.

Table 7. Effect of bacterial inoculation and irrigation by three wells of saline water on some aspects of rhizosphere properties after harvesting canola plants

Treatments		Total count of bacteria (CFU/ g soil X 10^5)	Nitrogen fixers (MPN/ g soil X 10^3)	*Pseudomonas* sp. (CFU/ g soil X 10^5)	Dehydrogenase activity (µg TPF /g dry soil/ 24h)
High saline well	Control (uninoculated)	52	89	56	12
	Acinetobacter radioresistens	67	120	75	22
	Enterobacter cloacae	57	106	65	14
Medium saline well	Control (uninoculated)	74	95	60	20
	Acinetobacter radioresistens	250	137	82	43
	Enterobacter cloacae	110	118	74	31
Low saline well	Control (uninoculated)	90	111	66	28
	Acinetobacter radioresistens	190	145	91	57
	Enterobacter cloacae	140	127	80	45

*** Values followed by the same letter do not differ significantly at 5% probability level.**

CONCLUSION

Previous results showed that the inhibition effect in all studied canola parameters increased with the increase in the salinity of water well. It is worth to note that canola plants exhibited significant response to rhizobacteria inoculation with a relative superiority of *A. radioresistens* over *En. cloacae*. As general, canola plants irrigated by low saline well water (8.94 dS m^{-1}) and inoculated with tolerant saline bacteria exceeded all other tested treatments and gave the highest values of fresh and dry weights, seed yield weight and oil yield, nutrients content and osmo-protectant molecules as well as rhizosphere microbial counts and dehydrogenase activity in soil, except osmo-protectant molecule proline increased by irrigated with medium saline well water (11.12 dS m^{-1}). Thus, there is considerable evidence that the plant growth promoting rhizobacteria could be considered a premium tool used for improving the quality and productivity of canola and may provide a useful way to ameliorate saline stress. However, this trial is in need to be repeated by using different canola varieties under different saline soil conditions to reach the level of recommendation.

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دورالبكتيريا المتحملة للملوحة والمعززه لنمو النبات فى رفع مقاومة الكانولا للإجهاد الملحى

غادة أمين زكي إبراهيم

قسم خصوبة وميكروبيولوجيا الأراضى – مركز بحوث الصحراء

تأثر نمو النبات بالملوحه (وهو إجهاد بيئي كبير)، مما يحد من الإنتاج الزراعي. لذلك، فقد تم تقييم قدرة سلالتين من الرايزوبكتيريا المنتجة للمواد المشجعة للنمو (PGPR) وهما:*A. radioresistens* و *En. cloacae* ، فلهما أهمية اقتصادية فى انتاج بعض الخواص المتعلقة بتشجيع النمو النباتى. ولهما القدرة على النمو في البيئة المغذية المحتوية على تركيز ملحي من كلوريد الصوديوم يصل إلى 12 ٪ لكلاهما. وقد تم اختبار الأنبات لبذور الكانولا معملياً. كذلك أجريت تجربة حقلية خلال شتاء موسم 2018 / 2019 م بمحطة التجارب الزراعية التابعة لمركز بحوث الصحراء برأس سدر، محافظة جنوب سيناء، مصر باستخدام نظام الرى بالغمر. وقد اهتمت الدراسة بتأثير التلقيح بالبكتيريا المتحملة للملوحة والري بثلاث آبار من المياه المالحة (8.94 و 11.12 و 12.1 ديسيسيمنز/ م) على بعض صفات النمو والمحصول والمحتوى من المغذيات المعدنية، وقد تم تسجيل أعلى قيم للوزن الطازج والوزن الجاف ومحصول البذور والزيت والمحتوى من العناصر المغذية والاحماض الامينية وكذلك الأعداد الميكروبية الكلية ونشاط انزيم الديهيدروجينز لريزوسفير التربة باستخدام مياه البئر 1 (8.94 ديسيسيمنز/ م) فى تجربة الزراعه. وتم قياس الأحماض الأمينية (الجلايسوبيتين والكولين والبرولين) التى تنتجها نباتات الكانولا داخل الخلايا تحت ظروف التربة الجيرية الملحية. اشارت النتائج المعملية الى قدرة البكتيريا المختبرة على إنتاج الأحماض العضوية والأندول اسيتيك اسيد (IAA). وقد اظهرت نتائج التجربة الحقلية أن نباتات الكانولا قد استجابت معنوياً للتلقيح البكتيرى مع تفوق نسبي للبكتيريا *A. radioresistens* على *En. cloacae*. كما زاد تأثير التثبيط في جميع قياسات الكانولا المدروسة مع زيادة ملوحة مياه الآبار، فيماعدا الحمض الامينى (برولين) الذي ازداد بالري بمياه الآبار ذات الملوحة المتوسطة (11.12 ديسيسيمنز/ م).