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ALLEVIATION OF SALINITY STRESS IN WHEAT BY ACC DEAMINASE-PRODUCING *BACILLUS ARYABHATTAI* EWR29 WITH MULTIFARIOUS PLANT GROWTH PROMOTING ATTRIBUTES

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Abstract

Salinity is one of the most crucial stressors that restrain crop productivity. Endophytes with 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity possess the potential to ameliorate salt stress in plants by degrading the precursor molecule of ethylene, ACC, whose concentration is elevated in salt-stressed plants. The present investigation reports the efficiency of salt-tolerant endophyte *Bacillus aryabhattai* EWR29 exhibiting ACC deaminase activity as bioinoculant to improve the growth of the wheat plant under salinity stress conditions. The strain EWR29 manifested multifarious plant growth-promoting traits including, phosphate solubilization, and production of indole acetic acid (IAA), siderophore, HCN, and exopolysaccharides (EPS). Besides, it endures salinity (12%) and, drought stresses (“0.49 MPa) and showed a strong biofilm-formation potential. Moreover, *acdS* gene coding for ACC deaminase was sequenced and characterized at the molecular level. Inoculation of wheat plants with *B. aryabhattai* EWR29 alleviated the negative effects of salinity stress and promoted significant increases in growth criteria compared with un-inoculated plants grown at the same salinity levels. The bioinoculant induced up-regulation of the antioxidant enzymes peroxidase (POD) and superoxide dismutase (SOD), and decreased proline content in treated plants indicating the mitigation of salt effect. The results indicated the potential used of *B. aryabhattai* EWR29 as bioinoculant to enhance the growth of wheat plants cultivated in salt-affected agriculture fields and alleviate salt stress.

Key words : ACC deaminase, Endophyte, Halotolerant, Salinity, Stress.

Introduction

Salinity is one of the major agricultural dilemmas facing soil fertility and crop productivity in arid and semi-arid soils. In Egypt, soil salinization is a rising problem particularly, at the northern regions of the Nile Delta and Sinai (Nawar *et al.,* 2015; Hammam and Mohamed 2018). Being one of the most deleterious abiotic stresses, soil salinity has adverse effects on the yield of crops through affecting their physiological, biological, and metabolic processes (Ma *et al.*, 2018). Under high salinity, the increased sodium and chloride concentrations lead to decrease in K^+ and Ca^{2+} uptake by plants, enzyme inactivation, inhibition of protein synthesis, decrease in the rate of photosynthesis and respiration (Munns 2002). Considered as one of the major threats toward wheat, salinity reduces tiller number per plant, spike length, spikelets per spike, and grain size; leading to stunted growth and lower crop productivity (Negrão *et al.*, 2017). Under normal conditions, plants synthesize ethylene as a volatile plant hormone at low levels, awarding a wide range of profitable influences on plant fitness; however, the sharp increase in ethylene production during biotic and abiotic stresses has adverse consequences on plant health (Tao *et al.*, 2015). The stress-induced ethylene is synthesized by utilizing 1-aminocyclopropane-1-carboxylic acid (ACC) as an immediate precursor (Houben and Van de Poel 2019). Thus, it has been suggested that ACC deaminase-producing bacteria associated with plants enhance plant growth during stress conditions by decreasing the internal concentrations of stress-induced

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ethylene through degradation of ACC into α-ketobutyrate and ammonia (Gontia-Mishra *et al.,* 2014). Hence, application of salt-tolerant bacterial strains possessing ACC deaminase activity as bioinoculants may contribute to mitigating plant stress-induced ethylene and promoting plant growth in salt-affected soils (Singh and Jha 2017; Sarkar *et al.,* 2018; Orozco-Mosqueda *et al.,* 2019; Orozco-Mosqueda *et al.,* 2019). Therefore, recent efforts shed light on the potentiality of endophytic bacteria that dwell inside plants to enhance plant growth under challenging conditions (Afzal *et al.,* 2019). In this trend, various endophytes have been found to exert profound beneficial effects on host plants and improve their growth and fitness under salinity and drought stresses (Rho *et al.,* 2018; Cherif-Silini *et al.,* 2019). Not only protect plants during stress conditions but also endophytes can enhance plant growth by direct and indirect mechanisms. The growth promotion achieves by production of indole acetic acid (IAA) as a plant hormone (Andreozzi *et al.,* 2019; Borah *et al.,* 2019), and the iron chelators (siderophores) that enhance the bioavailability of iron (Ribeiro *et al.,* 2018; Kumawat *et al.,* 2019), and phosphate solubilization (Chen *et al.,* 2019; Emami *et al.,* 2019). Moreover, some salt-tolerant endophytes secrete exopolysaccharides (EPS) and form biofilms, reducing the salt stress of plants (Wang *et al.,* 2017; Abbas *et al.,* 2019). The present study addressed the isolation of an efficient ACC deaminase producing endophyte, *B. aryabhattai* EWR29, from wheat roots and characterization of its potential for growth-promotion and salt stress alleviation.

Materials and Methods

Isolation and screening of ACC deaminase-producing endophytes

Healthy wheat plants were gently uprooted and their roots were washed under running tap water to remove adhering soil particles. Root sections of 2 –3 cm long were excised using a sterile scalpel and surface sterilized by sequential immersion in 70% alcohol for 1 min, 2.5% sodium hypochlorite for 2 min, and 0.1% $HgCl_2$ for 1 min and washed in four changes of sterile phosphate buffered saline (PBS). Aliquots from the final wash were checked for sterility and surface-sterilized samples were triturated in PBS under aseptic conditions. Subsequently, the triturates were serially diluted and used for isolation of ACC-utilizing bacteria by plating on DF salt minimal medium supplemented with ACC as sole nitrogen source (Penrose and Glick 2003). After incubation for 48 h at 28 ± 2°C, bacterial colonies were picked and purified twice on the same culture medium and screened for ACC deaminase production on minimal ACC medium agar containing 0.005% bromothymol blue (Patil *et al.,* 2016). Then, discrete colonies showing intense blue color, indicating high ACC deaminase activity were selected for future investigations. Quantification of ACC deaminase activity of isolated endophytes was conducted according to by the PCR plate ninhydrin–ACC assay (Li *et al.,* 2011). The quantity of released α-ketobutyrate was measured and the activity of ACC deaminase was expressed in nmol of α-ketobutyrate/mg protein/h (Penrose and Glick 2003). Based on the quantitative screening of ACC deaminase, a promising strain designated EWR29 was selected for further investigations.

Identification of the most promising ACC deaminase-producing strain

Gram's nature, morphology, endospore formation, and biochemical tests were determined following the standard procedures. Enzyme activities of EWR29 and reaction to various substrates were determined by API ZYM (bioMérieux) and API 20NE (bioMérieux) according to the manufacturer's instructions. Further, the identity of the most potent bacterial strain EWR29 was confirmed via amplifying of 16S rRNA gene by polymerase chain reaction (PCR) using 27F and 1492R universal primers. The amplified PCR product was purified and sequenced at Macrogen (Seoul, South Korea). The BLASTn search program (http://www.ncbi.nlm.nih.gov) was used to look for nucleotide sequence homology. The 16S rRNA gene sequence of the most promising strain was submitted to GenBank and accession number was assigned. The sequence obtained was then aligned by ClustalW using MEGAX software (Kumar *et al.,* 2018) and a neighbor-joining (NJ) tree with bootstrap value 1000 was generated using the software.

Assessment for plant growth-promoting traits

Quantitative estimation of tri-calcium phosphate solubilization was assayed in the Pikovskaya's broth (Pikovskaya 1948) and the concentration of soluble phosphate was estimated by stannous chloride method at 600 nm (King 1932) against the standard curve of KH_2PO_4. While IAA production in the presence of L-tryptophan (200 µg/ml) was determined by spectrophotometric method using Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution) at 530 nm against standard curve of IAA (Bric *et al.,* 1991; Goswami *et al.,* 2013). Moreover, quantitative estimation of HCN production was conducted following the modified colorimetric methemoglobin method (von Rohr *et al.,* 2009). Briefly, overnight bacterial cultures were prepared in Luria-Bertani broth supplemented with 5 g/L glycine. Then cells were collected by centrifugation at 5000 × *g* for 15 min and extracellular HCN concentration was determined using the methemoglobin reagent at 424 nm against KCN standard curve. The extracellular polysaccharide (EPS) production was determined as described previously (Sarkar *et al.,* 2018). *In vitro* biofilm formation ability of strain EWR29 was assayed using the method mentioned previously (O'Toole and Kolter 1998). Also, quantitative estimation of siderophore production was conducted using the modified microplate method (Arora and Verma 2017). The bacterial strain was cultured in iron-free Fiss minimal medium for 48 h at 28 ± 2°C. After centrifugation at 5000 × *g* for 15 min, CAS assay solution was added to the cell-free supernatant (1:1 v/v), mixed and allowed to stand for 20 min; un-inoculated minimal medium was used as blank. The reduction in the blue color intensity was estimated at 630 nm in 96 well microplate and percent siderophore unit (psu) was calculated according to the following formula:

$$\text{Siderophore production (psu)} = [(A_r - A_s)/A_r] \times 100$$

Where, A_r = absorbance of reference (CAS solution and un-inoculated broth), A_s = absorbance of sample (CAS solution and cell-free supernatant of sample).

The potential of strain EWR29 to tolerate drought and salt was investigated following microtiter plate method. To assess drought tolerance polyethylene glycol (PEG, MW 6000) was employed to develop an artificial drought stress. Briefly, 250 μL nutrient broth amended with different concentration of PEG ranging from 0–30% was added into each well of the microtiter plate followed by inoculation with 10 μl bacteria suspension ($OD_{610\ nm}$ = 0.45). In another set, different concentrations of NaCl (1-30%) were incorporated to the nutrient broth, followed by inoculation with 10 μL bacteria suspension. Afterward, the inoculated microtiter plates were incubated at 28 ± 2 °C for 48 h on a rotary shaker at 200rpm and the bacterial growth was monitored by reading the absorbance at 600 nm.

PCR amplification, cloning, and sequencing of ACC deaminase gene (*acdS*)

Partial *acdS* gene was amplified by PCR using degenerate primers DegACC52 (52 -GGBGGVAAYAARMYVMGSAAGCTYGA) and DegACC32 (52 -TTDCCHKYRTANACBGGRTC) as described earlier (Hontzeas *et al.,* 2005). The purified amplicon was cloned into pGEM-T Easy Vector (Promega) and transformed into *E. coli* JM109 competent cells following the manufacturer's protocol. The recombinant plasmid was extracted from a white transformant using *GeneJET Plasmid Miniprep Kit* (Thermo Fisher Scientific, USA) and sequenced with the universal primers M13F and M13R. The forward and reverse DNA sequence reads were assembled to obtain the consensus sequence by using Codoncode Aligner software (V12.12) and the partial *acdS* gene sequence was submitted to GenBank and accession number was assigned. BLAST analysis was conducted by submitting the deduced amino acid sequences to NCBI BLASTp server. Multiple amino acid sequence alignment analysis was performed using Clustalw online tool (https://www.genome.jp/tools-bin/clustalw) and visualized using CLC sequence viewer.

Evaluation of *B. aryabhattai* EWR29 efficacy on the growth of wheat under salinity stress

Seeds of salt-sensitive bread wheat (*Triticum aestivum* L.) cultivar Gemmiza-9 used in this study were obtained from Wheat Research Department (Field Crops Research Institute, Agricultural Research Center, Giza, Egypt). Wheat seeds were surface-sterilized by with 70% ethanol for 2 min followed by three times successive washings with sterile distilled water. Then, seeds were immersed in 1.5% sodium hypochlorite solution for 3 min followed by six rinses with sterile distilled water. To prepare bacterial inoculum, strain EWR29 was grown in nutrient broth at 30°C for 24 h in a shaking incubator (200 rpm). Cells were collected by centrifugation at 5000 rpm for 10 min and washed twice in a sterile potassium chloride solution (0.9%, w/v) and the pellets were re-suspended in the same solution to adjust the absorbance to 0.6–0.7 at 600 nm corresponding to 10^6 CFU/mL (Cherchali *et al.,* 2019). Surface-sterilized seeds were treated with the bacterial suspension by soaking for 1 h under aseptic condition while control seeds were soaked in sterile potassium chloride solution (0.9%, w/v) for the same period. Afterward, seeds were sown in plastic pots filled with sterilized soil and Hoagland media supplemented with NaCl (50, 100, 150 mM) was used for providing nutrient as well as imposing the salt stress at every alternate day (Singh and Jha 2016). The experiment was conducted for 45 days, and seedling growth was measured by recording shoot length, root length, fresh weight, and dry weight. The activity of antioxidant enzymes peroxidase (POD), and superoxide dismutase (SOD) in fresh leaves of wheat was assayed according to method described by Khan *et al.,* (2016), while proline content was estimated following the method of Khan and Bano (2016).

Statistical analysis

All results presented here were the means of 3 replicates. Data were analyzed by one-way analysis of variance (ANOVA) utilizing Duncan method at 5% level of significance by using IBM SPSS software version 22.

Results

Isolation and screening of ACC deaminase-producing endophytes

A total of 34 ACC-utilizing bacterial endophytes recovered from healthy wheat roots showing intense blue color were subjected to quantitative estimation of ACC deaminase by determination of α-ketobutyrate production. All the assessed isolates showed variation in ACC deaminase activity in the range of 32.69–24210 nmol α-ketobutyrate per mg of cellular protein per hour. The highest ACC deaminase activity (24210 nmol/mg protein/h) was exhibited by bacterial strain EWR29 that selected for further investigations.

Identification of the ACC deaminase-producing strain EWR29

The selected strain EWR29 was identified based on its morphological, biochemical, and molecular characteristics. Results revealed that EWR29 is Gram-positive rod-shaped and spore-forming bacterium. EWR29 Showed positive activity for Voges–Proskauer test, nitrate reduction, oxidase, catalase, chymotrypsin, β-galactosidase, aesculin hydrolysis, urease, and gelatinase. Negative response was observed in indole production, H_2S production, nitrate utilization, methyl red test, and β-glucuronidase (Table 1). Further, the strain was identified as *Bacillus aryabhattai* EWR29 based on its 16S rRNA gene sequence homology. The 16S rRNA gene sequence of isolate has been deposited in Genbank under the accession number MH938083.1.

Assessment for plant growth-promoting traits

The strain EWR29 was screened for its various PGP traits, *in vitro* (Table 2). On quantification of phosphate solubilization, it solubilized 135.12 µg/ml. The production of IAA was quantified by supplementing the growth media with L-tryptophan (49.43 ± 4.16 µg/ml). The strain produced 174.3 ± 8.43 µM HCN and showed a feasible production of EPS **(**1273.35 ± 28.52 µg/ml). Also, biofilm formation was assayed on microtiter plates using crystal violet. The strain EWR29 showed strong biofilm development with an optical density of 1.65 ± 0.14 at 590 nm and found to be an efficient siderophore-producer (89.62 ± 3.2 psu). Besides, it tolerated PEG concentration as high as 20% ("0.49 MPa) and endured salt concentration up to 12%.

PCR amplification, cloning, and sequencing of ACC deaminase gene (*acdS*)

The strain EWR29 yielded a 738-bp DNA fragment by use of the degenerate primer pair, cloned into pGEM-T Easy Vector, and sequenced. Partial sequence of *acdS* was deposited in the GenBank database under accession number and MH939181.1 while the accession number QBY21423 was assigned to the deduced amino acid sequence. BLAST analysis of the cloned gene confirmed the presence of *acdS* gene. It showed 99.18% similarity with that of *B. thuringiensis* (AGQ42751.1), followed by 97.56% similarity with *B. mycoides* and 96.34% similarity with *Lysinibacillus xylanilyticus*. The multiple sequence alignment of *B. aryabhattai* EWR29 *acdS* gene with those of other bacterial species was depicted (Fig. 2).

Evaluation of *B. aryabhattai* EWR29 efficacy on the growth of wheat under salinity stress

In general, a significant decrease was observed in all investigated growth parameters of un-inoculated plants with increasing NaCl concentration (Table 3). The least adverse impact of salinity was recorded at 50 mM NaCl however dramatic reduction plant growth was obvious at higher concentrations of NaCl. Inoculation of wheat plants with *B. aryabhattai* EWR29 exhibited remarkable growth-promotion potential toward the treated plants under normal and saline-stressed conditions. The inoculated plants that exposed to 100mM NaCl exhibited 34.6, 87.8, 46.1, and 33.1% increments in their shoot length, root length, fresh weight, and dry weight, respectively, compared to un-inoculated plants grown at the same conditions. At 150mM NaCl, the bioinoculant efficiently promoted the growth of wheat seedlings where shoot length, root length, fresh weight, and dry weight were enhanced by 57.4, 51.5, 65.3, and 39.8%, respectively, compared to un-inoculated plants. Results revealed significant increases in POD, SOD, and proline levels in leaves of salinity-stressed plants (Table 4). Application of salinity stress is directly related to levels of POD, SOD, and proline. Compared with the control plants, salinity stress increased POD and SOD activities. However, inoculation of the plants *B. aryabhattai* EWR29 significantly elicited the two antioxidant enzymes compared to Un–inoculated plants. With increasing NaCl concentration, POD, SOD activities increased progressively, especially in inoculated plants (Table 4). On the other hand, proline concentrations were much lower in inoculated plants under salinity stress compared to those in un-inoculated plants at the same salinity conditions. *B. aryabhattai* EWR29 reduced proline level by 38.5 and 49.8% in treated-plants at 100 and 150 mM NaCl, respectively, compared to un-inoculated plants grown in the same salinity stress.

Discussion

Application of ACC deaminase-producing endophytes could be a promising approach to ameliorate stress in plants. The present study addresses the screening of ACC deaminase-producing endophytes isolated from wheat roots and the potential of improving wheat growth under salinity conditions. Of 34 ACC deaminase-producing endophytes isolated wheat roots, one strain designated EWR29 was selected based on its superior activity. According to its phenotypic characteristics and phylogenetic analysis, the strain was identified as *B. aryabhattai*. Due to formation of stress-resistant endospores, *Bacillus* is considered as one of the most important genera regarding the formation of stable bioinoculants that survive harsh environmental conditions and persist in soil for long times (Kumar *et al.*, 2014; Dheeman *et al.*, 2017). Besides, the admirable ACC deaminase activity of *B. aryabhattai* EWR29 provides another beneficial trait that could alleviate the deleterious effects of abiotic stresses. Nowadays, the crucial role of ACC deaminase in enhancing the tolerance of plants to stress conditions is widely accepted. The possession of ACC deaminase enables bacteria to diminish ethylene levels in stressed plants by cleaving the plant ethylene precursor, ACC, into ammonia and α-ketobutyrate (Win *et al.*, 2018; Orozco-Mosqueda *et al.*, 2019; Yoolong *et al.*, 2019). Concerning plant's response to stress, an elevated level of ethylene is observed in response to salinity, extreme temperatures, flooding, drought, heavy metals, and ultraviolet light (Sharma *et al.*, 2019). These elevated levels of stress-induced ethylene may lead to detrimental effects such as suppression of root and shoot elongation, restriction of leaf expansion and induction of epinasty in stressed plants (Chen *et al.*, 2009). Thus inoculation of plants with ACC deaminase-producing bacteria may reduce stress-induced ethylene and help the plant to withstand stress conditions. In agreement with our findings, various ACC deaminase-producing *Bacillus* species were described (Tian *et al.*, 2018; Marwa *et al.*, 2019; Tahir *et al.*, 2019). Apart from *Bacillus*, ACC deaminase-producing species belonging to genera *Pseudomonas*, *Paenibacillus*, *Kocuria*, *Leclercia*, and *Lysobacter* were also reported (Laborda *et al.*, 2018; Afridi *et al.*, 2019; Orozco-Mosqueda *et al.*, 2019; Kang *et al.*, 2019). Phosphate solubilization is another merit of *B. aryabhattai* EWR29 that proved to release of soluble phosphate from insoluble inorganic complex considered as a possible mechanism of plant growth promotion. Similar observations regarding phosphate solubilization efficacy of various plant-associated bacteria have been reported (Sαnchez-Cruz *et al.*, 2019; Macedo-Raygoza *et al.*, 2019; Ferchichi *et al.*, 2019). In fact, phosphorus is the second limiting nutrient for crop plants after nitrogen. However its ample quantities in most agricultural soils, the vast majority phosphorus is present in non-soluble forms due to phosphorus fixation by iron, calcium, and aluminum (Shen *et al.*, 2011; Ch'ng *et al.*, 2017). Diverse phosphate-solubilizing bacteria were affirmed to solubilize phosphate and increase the available phosphate for plants through organic acids, siderophores, protons, and hydroxyl ions production (Alori *et al.*, 2017). Therefore, the utilization of phosphate-solubilizing bacteria could be an eco-safe alternative to the synthetic phosphate fertilizers that provoke environmental hazards as groundwater pollution and waterway eutrophication (Huang *et al.*, 2017). *B. aryabhattai* EWR29 produced significant amounts of IAA (49.43 µg/ml), which might promote seedling growth and development. IAA production is a common hallmark of plant-associated bacteria and its role in root hair formation and stimulation of root cell elongation is well-documented (Verma *et al.*, 2018; Gang *et al.*, 2018). Recently, bacterial IAA was found to enhance seed germination, root length elongation, and seedling growth in wheat under saline stress (Sorty *et al.*, 2016). HCN-producing bacteria such as *B. aryabhattai* EWR29 are thought to exert antagonistic potential against phytopathogens. Owing to its toxicity, HCN is perceived biocontrol agent that may protect plants against pathogenic invasion during stressful conditions (Das *et al.*, 2017; Cucu *et al.*, 2019). However, recent study supposed that the beneficial role of HCN is through increasing of phosphorus availability by metal chelation (Rijavec and Lapanje 2016). The ability of *B. aryabhattai* EWR29 to produce EPS that might alleviate salt stress indirectly, as it binds excess Na^+ ions, thereby reducing the harmful impact of salt and restrict restricts sodium uptake (Ashraf *et al.*, 2004; Chanratana *et al.*, 2017). In a similar study, EPS produced by *Enterobacter* sp. was found to play a very important role in the protection of rice grown under salinity conditions by decreasing the amount of sodium available for plant uptake and reduce the salt stress of plants (Sarkar *et al.*, 2018). Furthermore, the plant-protective role of EPS against desiccation has been reported (Naseem *et al.*, 2018). Moreover, *B. aryabhattai* EWR29 exhibited a strong biofilm formation aptitude that could improve its colonization potential. It is believed that the competitiveness and colonization power of beneficial plant-associated bacteria may be enhanced through the capability of biofilm development (Ansari and Ahmad 2019). In a recent study, biofilm produced by *Proteus* sp. DSP1 and *Pseudomonas* sp. DSP17 posed a barrier for metal entrance into the plant root and protected plants from metal stress (Raklami *et al.*, 2019).

Table 1: Phenotypic characteristics of *B. aryabhattai* EWR29.

Test	Result	Test	Result
Gram stain	+	Oxidase	+
Endospore formation	+	Catalase	+
Indole production	-	Chymotrypsin	+
Voges–Proskauer test	+	β-Glucuronidase	-
Nitrate reduction	+	β-Galactosidase	+
H_2S production	-	Aesculin hydrolysis	+
Citrate utilization	-	Urease	+
Methyl red test	-	Gelatinase	+

Symbol: + represents positive result; - represents negative result.

Table 2: Characterization *B. aryabhattai* EWR29 for plant growth promoting traits.

Characteristic	Result
Phosphate solubilization (µg/ml)	135.12 ± 12.71
IAA production (µg/ml)	49.43 ± 4.16
HCN production (µM**)**	174.3 ± 8.43
EPS production (µg/ml)	1273.35 ± 28.52
Biofilm formation (OD at 570 nm)	1.65 ± 0.14
Siderophore production (psu)	89.62 ± 3.2

Symbol: ± represents standard deviation.

Table 3: Effect of different salinity levels on antioxidant enzyme activity in the foliage of *B. aryabhattai* EWR29-inoculated and un-inoculated wheat plants.

Salinity	Treatment	SL (cm)	RL (cm)	FW (g)	DW (mg)
Control	Un-inoculated	34.3 ± 3.1^{b}	18.2 ± 1.6^{b}	8.3 ± 0.7^{b}	464 ± 36^{b}
	Inoculated	42.7 ± 4.2^{a}	23.6 ± 2.1^{a}	10.4 ± 0.8^{a}	696 ± 45^{a}
50 mM	Un-inoculated	32.5 ± 3.8^{b}	15.9 ± 1.9^{c}	7.6 ± 0.4^{b}	412 ± 51^{b}
	Inoculated	38.6 ± 2.9^{ab}	19.7 ± 2.3^{b}	9.9 ± 0.6^{a}	488 ± 72^{b}
100 mM	Un-inoculated	24.8 ± 3.2^{c}	13.2 ± 1.4^{c}	6.5 ± 0.7^{b}	347 ± 64^{c}
	Inoculated	33.4 ± 2.3^{b}	24.8 ± 1.9^{b}	9.5 ± 0.4^{a}	462 ± 49^{b}
150 mM	Un-inoculated	20.2 ± 3.7^{c}	10.3 ± 1.4^{d}	4.9 ± 0.5^{c}	311 ± 55^{c}
	Inoculated	31.8 ± 2.6^{b}	15.6 ± 2.4^{c}	8.1 ± 0.4^{b}	435 ± 38^{b}

The same letter in each column indicates no significant difference according to Duncan's multiple range test ($p < 0.05$). Symbol: ± represents standard deviation. SL= Shoot length, RL= Root length, FW= Fresh weight, DW= Dry weight.

The endophyte *B. aryabhattai* EWR29 also secretes significant amounts of siderophores that estimated quantitatively (89.62 psu). This result agreed with recent studies that described siderophores production by various *Bacillus* spp. (Palyzovα *et al.*, 2019; Saleh *et al.*, 2019; Ferreira *et al.*, 2019). Siderophores are low molecular weight iron chelators produced by various microorganisms. By chelating iron, siderophores-producing organisms make it available for their growth and enhance the iron uptake by the associated plants (Priyanka *et al.*, 2017; Sah *et al.*, 2017). Not only benefit plants by iron acquisition, but also siderophores discourage phytopathogens growth. Many reports are available on the antifungal potential of siderophores against phytopathogenic molds and the siderophore-mediated competition for iron that provides indirect plant growth promotion via biocontrol against phytopathogens (Sulochana *et al.*, 2014; Zeng *et al.*, 2018). Results clarify the ability of *B. aryabhattai* EWR29 to withstand high salinity (12%) and drought (“0.49 MPa) that extends its prospect for supporting plant growth in soils having such elevated salinity and drought conditions. In a similar context, drought-tolerant *Pseudomonas fluorescens*, *P. migulae*, and *Enterobacter hormaechei* isolated from foxtail millet were also able to produce EPS (Niu *et al.*, 2018). The drought-tolerant plant-associated bacteria could thus be advantageous over others to thrive in drought environment and fulfill beneficial effects on plants. Notably, EPS-producing plant-associated bacteria possess a water-retention capacity and cementing properties. Hence shield bacteria against desiccation and protect their host plants from drought stress through improving soil structure (Sandhya *et al.*, 2009; Ghosh *et al.*, 2019). Thus, inoculation of plants with EPS-producing rhizobacteria possessing multiple growth-promoting activities may improve the efficacy of bacterial inoculants in arid or semiarid environments. At the molecular level, *B. aryabhattai* EWR29 was confirmed to harbor *acdS* gene that codes for ACC deaminase as it shares high sequence similarities with that of other bacterial species. Multiple sequence alignment unveiled the conserved features of *acdS* gene of *B. aryabhattai* EWR29 compared to that of related bacteria. These findings regarding the conservation of *acdS* gene agreed with those described in various ACC deaminase-producing organisms suggesting the horizontal gene transfer (Hontzeas *et al.*, 2005; Nascimento *et al.*, 2014; Singh *et al.*, 2015). The beneficial role of *acdS* gene on plant growth is not restricted to decrease ethylene level only, but also it improves the overall plant growth-promoting activity of the harboring bacteria. In a recent study, a significant reduction of plant growth promotion activity was observed in *acdS*-disrupted *Rahnella aquatilis* HX2 (Peng *et al.*, 2019). To evaluate the stress-mitigation potential of ACC deaminase-producing *B. aryabhattai* EWR29, the growth criteria and physiological traits were monitored in bacterized wheat plants under normal and stress conditions and compared with those of non-bacterized plants. Under normal conditions, wheat plants inoculated with *B. aryabhattai* EWR29 significantly promoted the

Table 4: Effect of different salinity levels on antioxidant enzyme activity in the foliage of *B. aryabhattai* EWR29-inoculated and un-inoculated wheat plants.

Salinity	Treatment	POD (μmol/gFW/min)	SOD (μmol/gFW/min)	Proline (μmol/gFW)
Control	Un-inoculated	0.53 ± 0.08^{e}	0.41 ± 0.04^{g}	12.17 ± 0.54^{f}
	Inoculated	0.49 ± 0.11^{e}	0.78 ± 0.06^{f}	13.55 ± 0.92^{f}
50 Mm	Un-inoculated	0.76 ± 0.07^{d}	1.03 ± 0.09^{e}	21.78 ± 0.81^{c}
	Inoculated	0.98 ± 0.09^{c}	1.68 ± 0.13^{d}	15.12 ± 1.08^{e}
100 mM	Un-inoculated	0.93 ± 0.13^{c}	1.79 ± 0.18^{d}	29.64 ± 1.32^{b}
	Inoculated	1.43 ± 0.06^{b}	2.42 ± 0.21^{b}	18.22 ± 0.76^{d}
150 mM	Un-inoculated	1.18 ± 0.10^{c}	2.15 ± 0.16^{c}	38.63 ± 1.46^{a}
	Inoculated	1.89 ± 0.12^{a}	3.82 ± 0.26^{a}	19.37 ± 1.03^{d}

The same letter in each column indicates no significant difference according to Duncan's multiple range test ($p < 0.05$). Symbol: ± represents standard deviation.

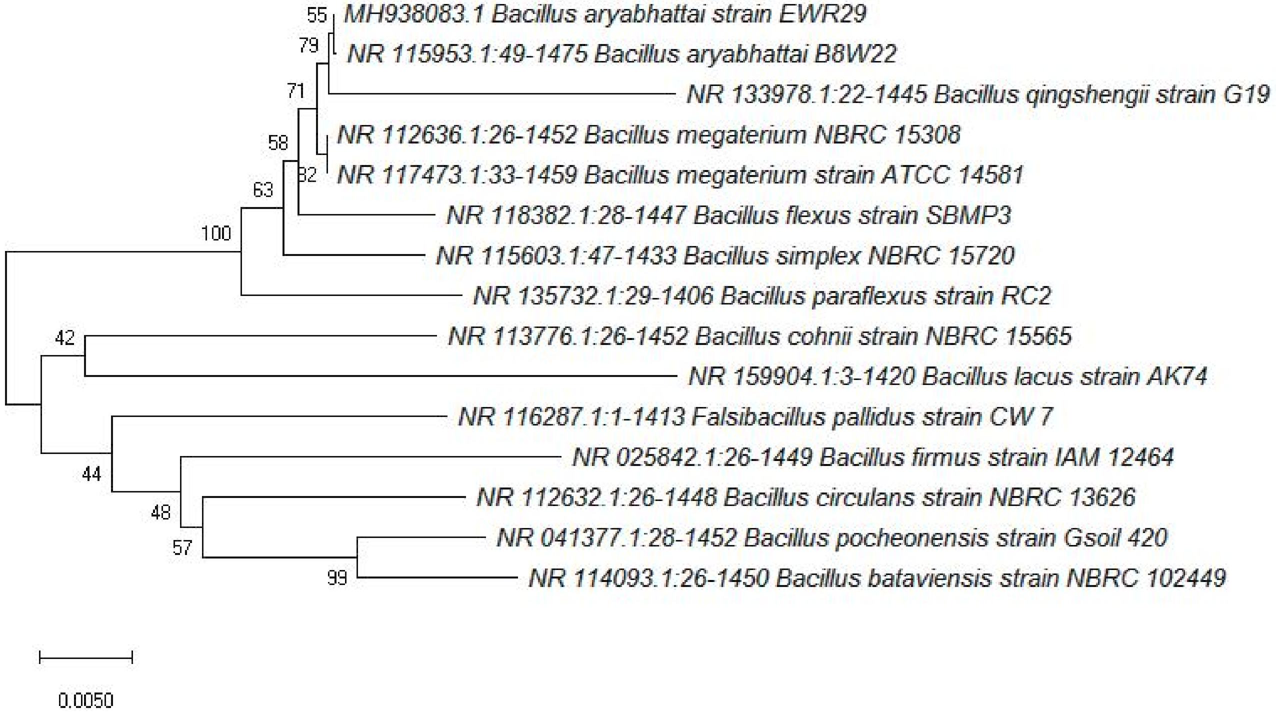

Fig. 1: Phylogenetic tree showing the relationships between *B. aryabhattai* EWR29 and the most closely related species.

length of shoot and root as well as biomass inoculated plants compared to un-inoculated control. Such an obvious increase in the overall performance of inoculated plants can be correlated with the multifarious plant growth-promoting attributes exhibited by *B. aryabhattai* EWR29, especially phosphate solubilization and IAA production. Similar increases in growth of plants treated with various endophytes have been reported (Aghai *et al.,* 2019; Kearl *et al.,* 2019; Eke *et al.,* 2019). Our results revealed a severe reduction in all assessed growth parameters in non-bacterized wheat plants upon exposure to salinity stress. This reduction in growth, increased as salt concentration increases that may be due to induced nutrient and osmotic imbalance in plants, thereby affecting the plant's physiological traits (Evelin *et al.,* 2019). Our results accentuated the protective impact of ACC deaminase-producing *B. aryabhattai* EWR29 against salinity stress. It is worth to mention that inoculated wheat plants exhibited significant enhancement of growth characteristics under salinity stress compared with un-inoculated plants. Inoculation of wheat plants with *B. aryabhattai* EWR29 alleviates the harmful effect of salt even at 150 mM NaCl, where no significant difference was observed in shoot length, plant fresh weight, and dry weight compared with non-treated control grown at normal salinity conditions. This manifest protection against salinity may be through virtue of ACC deaminase activity in addition to EPS production and biofilm formation capacity of *B. aryabhattai* EWR29. The biofilm produced by some organisms traps nutrients and water from the soil and minimizes sodium ions uptake by plants (Nadeem *et al.,* 2014). In agreement, salt-tolerant endophytes *Kocuria rhizophila* and *Cronobacter sakazakii* with ACC deaminase activity ameliorated plant salinity stress and increased the morphological traits of wheat plants (Afridi *et al.,* 2019). Also, it has been reported that ACC deaminase-producing

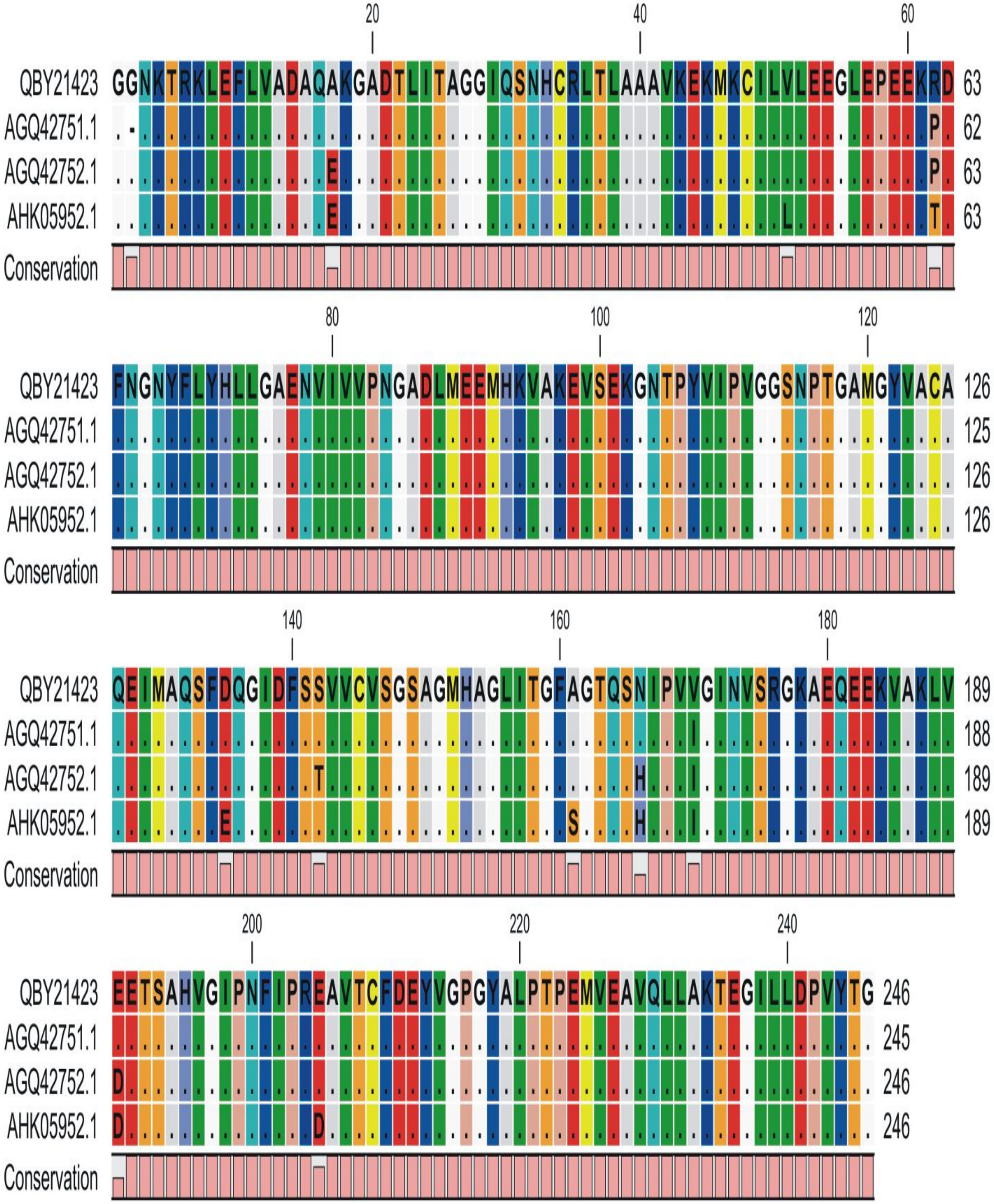

Fig. 2: Multiple sequence alignments of *B. aryabhattai* EWR29 *acdS* gene (Accession number QBY21423) with that of other bacterial species.

The identical residues are indicated as dots. Bars height at the bottom of the figure indicates the level of residue conservation (from 0 to 100%) at each position.

strains *Arthrobacter protophormiae* (SA3) and *Dietzia natronolimnaea* (STR1) facilitated salt stress tolerance in wheat crop. This effect was suggested to be conferred by enhancing IAA content, reducing abscisic acid and ACC content, modulating the expression of a regulatory component (CTR1) of ethylene signaling pathway and DREB2 transcription factor (Barnawal *et al.,* 2017). In a similar study, the colonization of wheat plants by *Stenotrophomonas maltophilia* SBP-9 augmented their resistance and improved the growth and ionic balance of plants, allowing them to cope with imposed salinity stress (Singh and Jha 2017). Regarding the impact of salinity stress on antioxidant enzymes, our results demonstrated a significant increase in POD and SOD activities upon plant exposure to salt compared with control in the absence of the bioinoculant. Whereas *B. aryabhattai* EWR29 inoculation further stimulated the antioxidant system that may lead to accelerated elimination of toxic reactive oxygen species (ROS). It is believed that ROS levels increase in salinity-stressed plants, leading to oxidative-mediated damage of plants (Mαthé *et al.,* 2019; JóŸwiak and Politycka 2019; Parvin *et al.,* 2019). The enzyme SOD exerts a pivotal role of defense against ROS by catalyzing the dismutation of $O^{''}_2$ to H_2O_2 and O_2 while POD metabolizes H_2O_2 to water (Rao *et al.,* 1996). Hence, the induction of stress tolerance in plants has been reported by various beneficial bacteria through triggering the cellular antioxidant mechanisms such as POD and SOD (El-Esawi *et al.,* 2018; Razzaghi Komaresofla *et al.,* 2019). It has been suggested that the ameliorative role of *B. subtilis* against salt stress in chickpea accomplished via the reduction of ROS and the upregulation of antioxidant systems (Abd-Allah *et al.,* 2018). Similar enhancements in antioxidant enzymatic activities were observed in wheat plants inoculated with biofilm-producing *B. pumilus* isolate FAB10 under salinity conditions, suggesting the ability of the plant to alleviate salinity-mediated oxidative stress (Ansari *et al.,* 2019). Concerning proline content, our results revealed its dramatic increases in a concentration-dependent manner in un-inoculated plants. In contrast, the proline content was significantly reduced in plants inoculated with *B. aryabhattai* EWR29 indicating the mitigation of salinity stress. Similar higher contents of the osmoprotectant agent, proline, were observed in salt-stressed plants demonstrating its antioxidant role as one of the adaptation mechanisms under salt stress (Sun *et al.,* 2019; Desoky *et al.,* 2019). In harmony with the present findings, inoculation of plants by various bacterial strains reduced proline content under salinity conditions, suggesting their role in salinity stress alleviation (Abd-Allah *et al.,* 2018; Sapre *et al.,* 2018; Ansari *et al.,* 2019; Razzaghi Komaresofla *et al.,* 2019). Remarkably, the promising endophyte, *B. aryabhattai* EWR29, displayed multiple beneficial traits and reduces salinity stress in wheat plants.

Conclusion

The present study highlights the potential application of ACC deaminase-producing *B. aryabhattai* EWR29 as a bioinoculant to enhance wheat growth and mitigate salinity stress through possessing promising multifarious plant growth-promoting attributes including IAA production, phosphate solubilization, siderophore production, EPS production, and biofilm formation potential. The spore formation and drought resistance merits of the reported halotolerant strain may be prototypical characteristics for plant growth promotion, particularly under abiotic stress. Furthermore, the findings established the alleviation of salt effect and promotion of wheat plant growth under salt stress through eliciting the antioxidative enzymes. Hence, *B. aryabhattai* EWR29 could be recommended as a bioinoculant to enhance the growth of wheat plants cultivated in salt-affected agriculture fields and alleviate salt stress.

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