MERIT RESEARCH JOURNALS
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Merit Research Journal of Agricultural Science and Soil Sciences (ISSN: 2350-2274) Vol. 8(5) pp. 072-081, May, 2020
Available online http://meritresearchjournals.org/asss/index.htm

Original Research Article

Mitigating the effect of salinity stress through integrated application of ACC-deaminase containing rihizobacteria and biogas slurry to improve the productivity of wheat crop

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Abstract

Soil salinity is a devastating environmental stress for sustainable agriculture around the globe. To rescue plant growth, ACC deaminase containing PGPR provide the great potential against salinity stress in dry lands. Biogas slurry is an important contribution to soil fertility and better crop yields. For this, wheat seeds were inoculated with *Alcaligenes faecalis*, *Bacillus cereus* and *Lysinibacillus fusiformis*; and biogas slurry was applied at different salinity levels (4, 6 and 9 dS m^{-1} EC) in soil. The 9 dS m^{-1} salinity stress level adversely reduced the plant growth which reflects the NaCl toxicity. Rhizobacterial inoculation improved the shoot and root length up to 40 and 34 % with their respective control. When the wheat plants were exposed to salinity stress, the sodium (Na^+) ions accumulation was significantly reduced in ACC-deaminase containing PGPRs-treated plants with respect to control, suggesting that the high level of tolerance to negative influences of Na^+ ions concentration. Hence, biogas slurry with the PGPR strain *Lysinibacillus fusiformis* was the most effective combination for improving growth and yield of wheat under saline condition. In conclusion, combined use of biogas slurry and PGPR in agricultural is a sustainable strategy for the alleviation of salinity stress in wheat.

Keywords: EC, Organic amendment, Pot, Soil, Yield

INTRODUCTION

Soil salinity is a huge devastating environmental stress which is a major threat to sustainable agricultural (Nemati et al., 2011; Ashraf, 2013). More than 800 million hectare land area is impaired by soil salinity and sodicity around the world (Munns and Tester, 2008; Cheng et al., 2012). Owing to high evaporation rate and low rainfall, salinity creates a low water potential in the soil, making it difficult for plants to acquire water (Porcel et al., 2012; Shrivastava and Kumar, 2014).

Salinity induces various biochemical and physiological alterations in plants which contribute to a reduced yield (Krasensky and Jonak, 2012; Haghighi and Pessarakli, 2013; Liu et al., 2015). A high percentage of soluble salt in the root zone caused deleterious effects on germination, mineral imbalance, root turgor pressure and stomatal closure (Zhu et al., 2004; Parida and Das, 2005; Dudley et al., 2008). Moreover, plants ultimately die due to senescence of leaves, generation of reactive oxygen species and ionic toxicity (Kravchik and Bernstein, 2013; Ahmad et al., 2016). The soil microbial activities also adversely affected due to increased soil salinity (Singh and Jha, 2016).

Ethylene is important for plant growth and development, as well as in the fruit ripening process, but an excess amount of ethylene might decrease seed germination and root growth (Belimov, et al., 2001; Saravanakumar and Samiyappan, 2007). Under abiotic or biotic stress conditions, plants synthesize ethylene which at higher concentration termed as 'stress ethylene' inhibits elongation of plant shoot and root, causes chlorosis and leaf abscission, suppresses leaf expansion or promotes epinasty (Yang et al., 2009).

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize on plant roots and improve plant growth via nutrient solubilization, biological nitrogen fixation, nutrient uptake, biosynthesis of phytohormones and up-regulating the conserved salinity responsive mechanisms (Kang et al., 2014). A possible strategy to cope with the low productivity of saline lands is microbial assisted amelioration of salt-induced damage (Dodd and Pérez-Alfocea, 2012). Therefore, use of PGPR could be a beneficial step in the regulation of nutritional balance in plants subjected to salt stress (Kohler *et al*. 2009; Nadeem et al. 2009). Under stress conditions, plant roots exude 1-aminocyclopropane^{-1}-carboxylic acid (ACC) where ACC-deaminase containing rhizobacteria can sequester ACC to into ammonia and α-ketobutyrate (Glick, 2007).

The demand for wheat is growing approximately at the rate of 2 % per year worldwide (Rosegrant and Cline 2003). As in context of world economy and nutritive value (carbohydrates up to 55%), wheat is considered one of the most important cereal crop which fulfils the 20% food requirement of world (Bos et al., 2005). It is a widely cultivated crop covering 17% of the world arable land whereas more than 50% of that area comes in developing countries of Asia. However, 32% of the wheat cultivated area of the developing world experiences the drought (FAO, 2012).

Another important practice to mitigate the effect of salinity stress on crops is the application of organic conditioners, which can both ameliorate and increase the fertility of saline soils (Melero et al., 2007). Salt-affected soils generally exhibit poor structural stability due to their low organic matter content. Use biogas slurry as organic matter can also be used to ameliorate salinity stress by reducing Na^+ uptake in plants (Ahmad et al., 2014). Biogas is an excellent organic fertilizer which derives from the anaerobic decomposition of organic waste in the biogas digester (Chen et al., 2012). Biogas slurry is an important contribution to soil fertility and better crop yields (Nasir *et al*., 2010). Some constituents of biogas slurry could increase the resistance to abiotic stress and maintain soil pH, electrical conductivity and fertility (Islam et al., 2010; Ahmad et al., 2014).

It is very likely that using of PGPR in combination with biogas slurry may improve soil fertility management strategy for enhancing agricultural productivity, particularly under salt-affected conditions. Therefore, the present study was designed to explore the potential of integrated use of salt tolerant PGPR and biogas slurry to boost wheat growth under saline environment.

MATERIAL AND METHODS

Collection of rhizobacterial strains

Three ACC-deaminase containing rhizobacterial isolates *Alcaligenes faecalis*, *Bacillus cereus* and *Lysinibacillus fusiformis* were examined for inducing salt tolerance in wheat, obtained from Soil Microbiology and Biochemistry Laboratory, Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan.

Preparation of bacterial inocula and seed bacterization

The respective rhizobacterial inoculum was prepared in 250-mL Erlenmeyer flasks by using DF salt minimal medium (Dworkin and Foster, 1958) containing ACC as substrate. Each flask containing DF salt minimal broth was inoculated with a loopful of respective strains and left for three days at 100 rpm at 28°C temperature in an incubator. Sterilized distilled water was added to achieve the 0.45 nm optical density of the respective inoculum by using spectrophotometer at 540 nm wavelength to obtain uniform population of rhizobacteria (10^7-10^8 CFU mL^{-1}) prior to seed inoculation. Wheat seed dressing was done with the obtained suspension of inoculum was mixed with sterilized peat (200 mL kg^{-1} peat), sugar solution (10%) and clay. In the case of the uninoculated control, the seeds were coated with sterilized DF salt minimal media, peat and sugar solution.

Pot experimental set-up

A pot experiment was conducted by using rhizobacterial strains and biogas slurry to mitigating the effect of drought stress on wheat crop. For this purpose, salt stress was induced by adding NaCl salt to maintain salinity levels (4, 6 and 9 dS m^{-1} EC) in soil by using following formula (USDA Salinity Lab. Staff, 1954).

$$Weight\ of\ salt\ (gram) = \frac{TSS\ (me\ L^{-1}) \times Eq.wt.of\ salt}{1000} \times kg\ of\ soil$$

The total soluble salt (TSS in me L^{-1}) is obtained from a graph between EC and TSS (USDA Salinity Lab. Staff, 1954). The biogas slurry (BGS) was collected from the biogas plants, air-dried. The BGS and respective salt were mixed with the sieved soil, prior to pot filling (10 kg per pot^{-1}). Urea, triple super phosphate and sulfate of potash were applied as N, P_2O_5 and K_2O, at the rate of 120, 90, 60 kg ha^{-1}, respectively. Full dose of potassium and phosphate fertilizers were applied as basal, whereas, nitrogen was applied in three splits viz. before sowing, at tillering and at booting stage). In each pot, five wheat seeds were sown and three plants pot^{-1} were maintained by thinning after 15 days of sowing. Pots were arranged in a wire house at ambient light and temperature using completely randomized design in factorial set (Steel et al., 1997). All the treatments were replicated thrice.

Preparation and analysis of biogas slurry

The BGS was obtained through biogas plant installed at Chah Kangan Wala Mouza Ferozpur, Multan and was air dried on a plastic sheet. Two levels of BGS were maintained (i.e. control and 600 kg ha^{-1}) according to treatment plan, before sowing in pots. By following the standard protocol as presented by Ryan et al., (2001) the concentration of organic carbon (38.5%), total nitrogen (1.45%), phosphorus (1.75%) and potassium (1.04%) in the BGS was analyzed. The EC of BGS was noted as 2.95 dS m^{-1} and pH was 7.5.

Pre-sowing soil analysis

Air dried sieved soil was obtained from research area. The soil sample was characterized for various physico-chemical attributes. The textural class (sandy clay loam), EC_e (3.12 dS m^{-1}), pH (7.8), saturation percentage (34.5%), organic matter (0.89%), CEC (5.18 $cmol_c$ kg^{-1}), available phosphorus (8.1 mg kg^{-1}), total nitrogen (0.045%) and extractable potassium (113 mg kg^{-1}) soil were determined.

Measurement of physiological, growth and yield parameters of plant

For proline content determination, leaf fresh tissue was ground with 10 mL sulfosalicylic acid (3%) and then centrifuged. The supernatant was mixed with ten mL acetic acid and ninhydrin acid and boiled. Then toluene was added and extracted and absorbance was noted at 520 nm by spectrophotometer (Bates et al. 1973). The leaf samples were taken in polypropylene centrifuge tubes for determination of Na^+. Liquid nitrogen was added in centrifuge tubes and placed at freezing temperature (–20°C) (Akhtar et al., 1998). The sap was collected and centrifuged and the suspension was analyzed for these ions concentrations by using flame photometer (Ryan et al., 2001). The data regarding growth and yield parameters were collected at physiological maturity and crop harvest.

Statistical Analysis

An analysis of variance technique (ANOVA) was based on completely randomized design (CRD) with three factor factorial arrangement (Steel et al., 1997). However, Tukey's (HSD) test was used to compare various significantly treatments mean at probability of 5% (p<0.05). The computer based statistical software "Statistix 9®" was used for analysis (Analytical Software, USA).

RESULTS

Effect on growth attributes

The data regarding the effects of rhizobacteria containing ACC-deaminase activity and BGS on the plant height and number of tillers of wheat plants exposed to salinity stress are showen in Figure 1a and b. At normal EC, the *Bacillus cereus* + BGS treatment revealed a significant increase in plant height (up to 40.6%) of wheat plants over uninoculated control. Similarly, at 6 and 9 dS m^{-1} EC, the strain *Bacillus cereus* + BGS showed a significant increase in plant height i.e., up to 24.1 and 39.6%, respectively, over respective uninoculated controls. Data regarding the number of tillers, showed that the *Bacillus cereus* + BGS treatment significantly increased the number of tillers i.e., up to 60.0 and 62.5 % at normal and 6 dS m^{-1} EC, over respective uninoculated controls.

Effect on yield attributes

Data regarding the 100-grain weight (Figure 2a), the *Bacillus cereus* + BGS treatment showed a maximum increase (up to 44.9) at normal EC and 40.0% at 6 dS m^{-1}

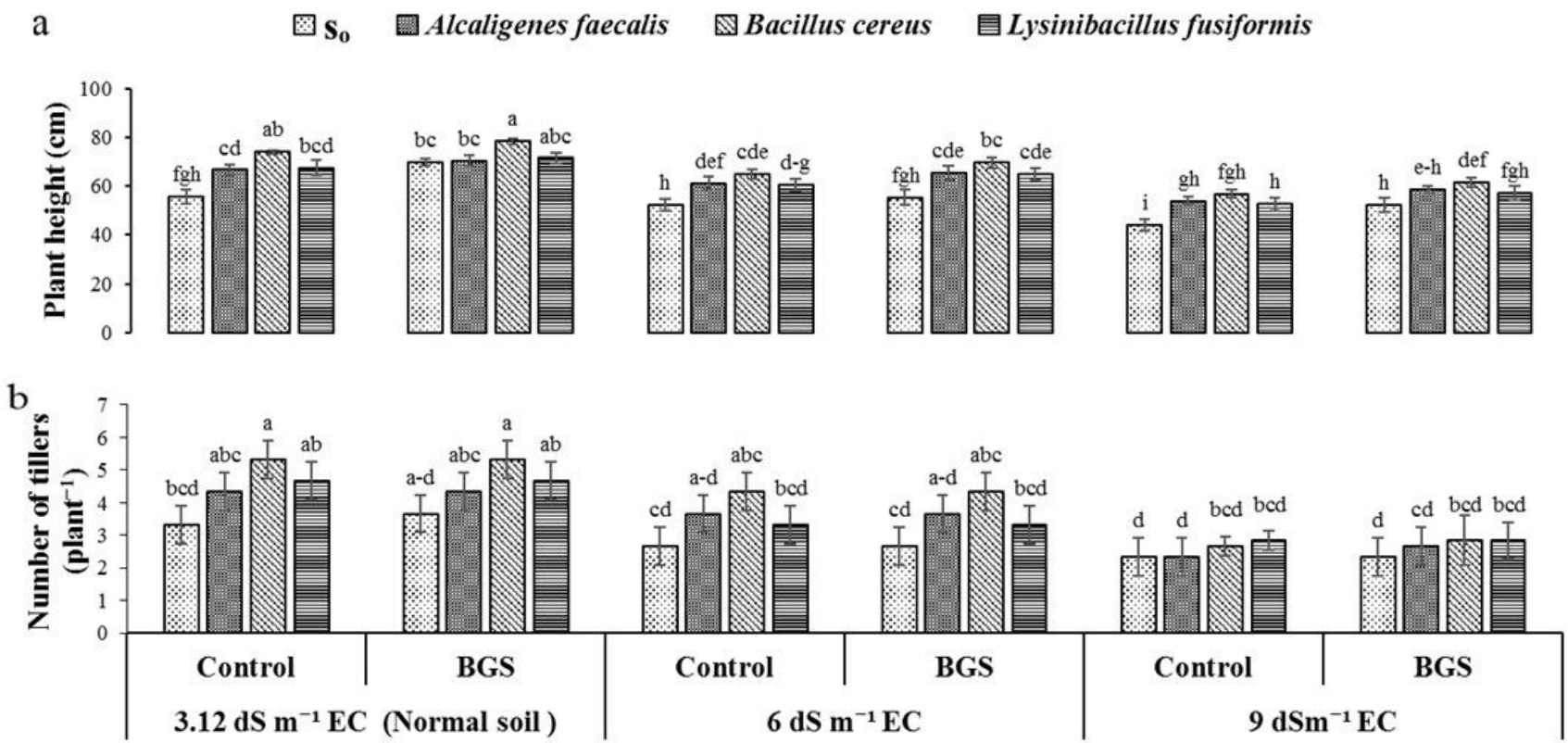

Figure 1 (a and b). Effect of rhizobacteria and BGS on plant height (cm) and number of tillers (plant^{-1}) of wheat plants under salinity stress conditions. The treatments, sharing the similar letters do not have HSD with each other at $p \leq 0.05$ (± Standard deviation; n=3). Where, S_o is no rhizobacteria and BGS is biogas slurry.

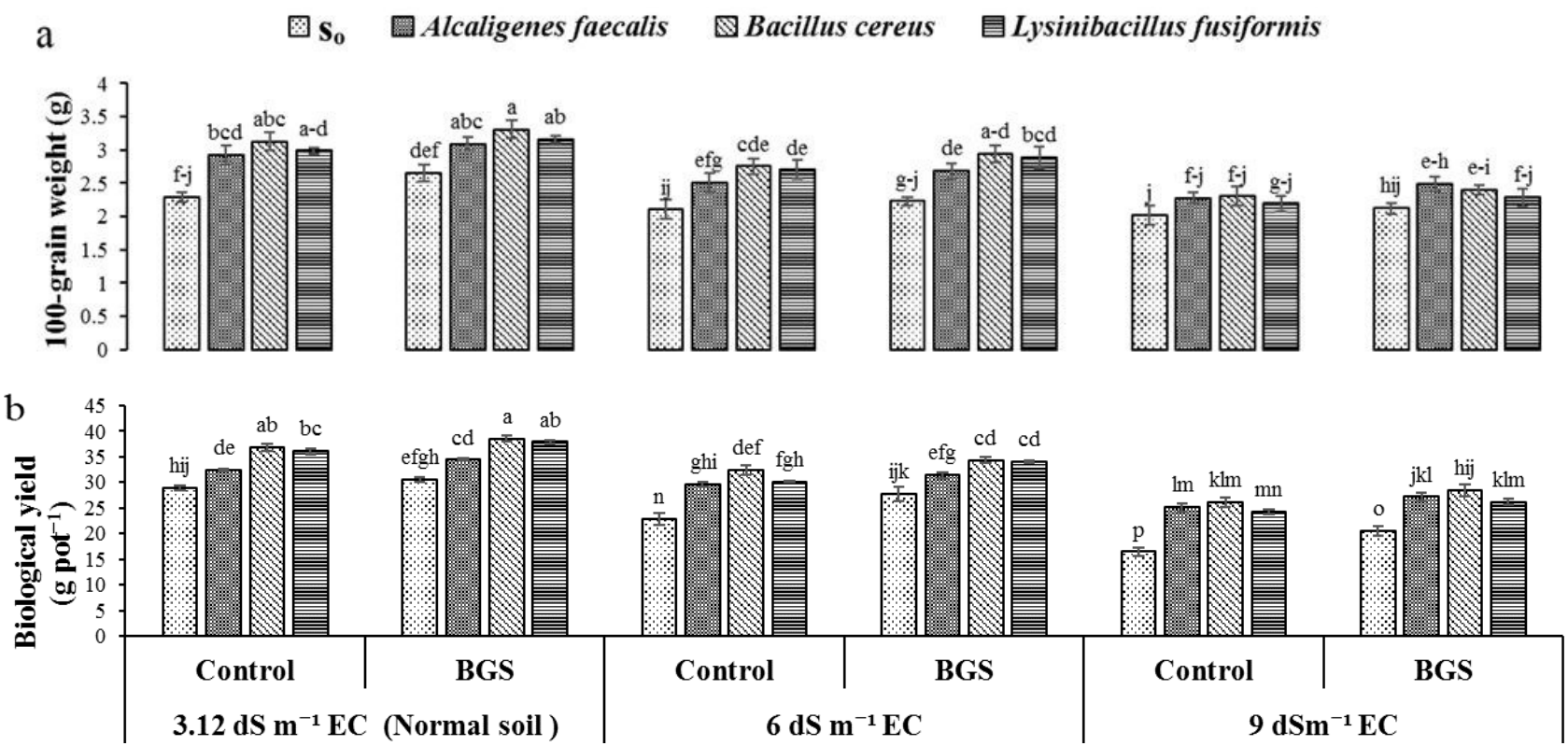

Figure 2 (a and b). Effect of rhizobacteria and BGS on 100-grain weight (g) and biological yield (g pot-1) of wheat plants under salinity stress conditions. The treatments, sharing the similar letters do not have HSD with each other at $p \leq 0.05$ (± Standard deviation; n=3). Where, So is no rhizobacteria and BGS is biogas slurry.

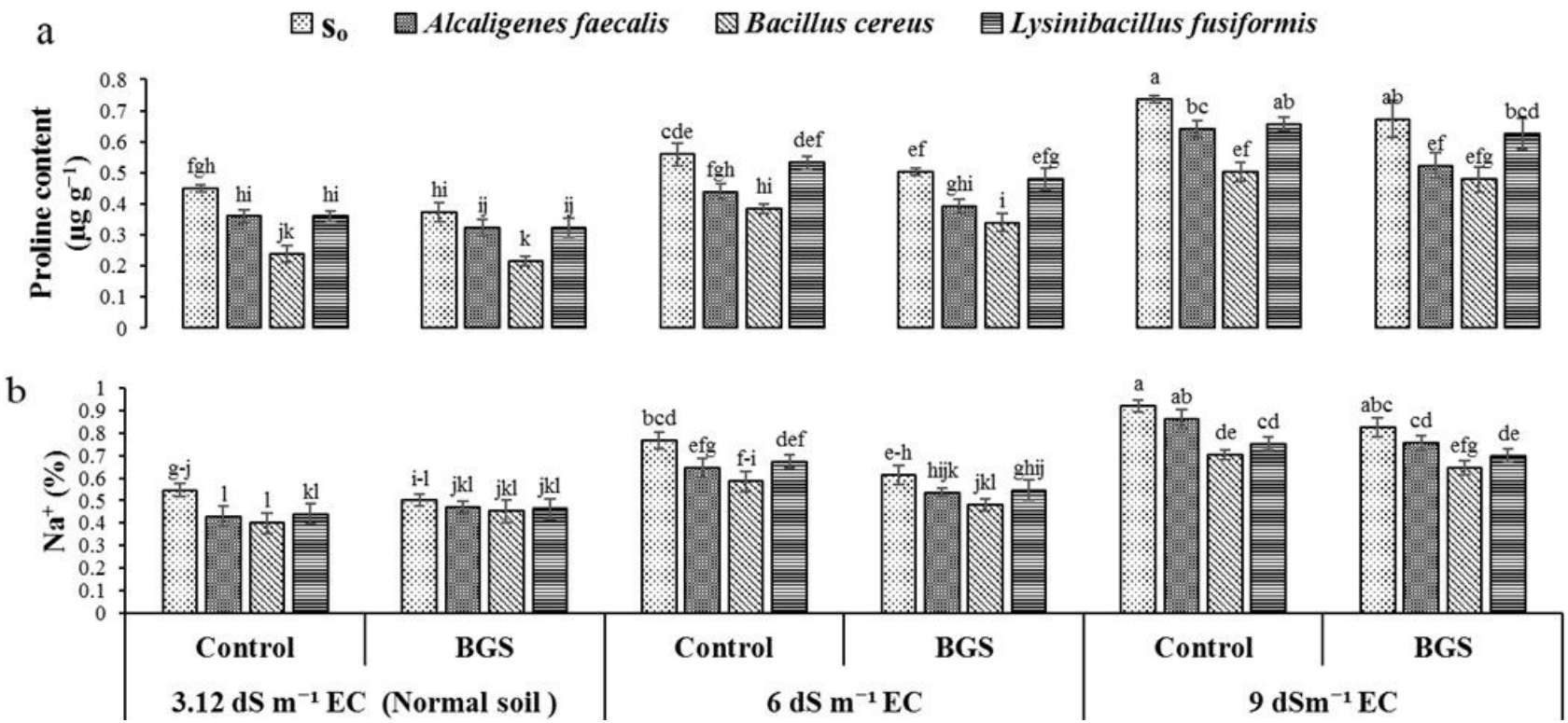

Figure 3 (a and b). Effect of rhizobacteria and BGS on proline (µg g-1 FW) and Na+ (%) of wheat plants under salinity stress conditions. The treatments, sharing the similar letters do not have HSD with each other at $p \leq 0.05$ (± Standard deviation; n=3). Where, So is no rhizobacteria and BGS is biogas slurry.

EC, over respective uninoculated controls. However, the *Lysinibacillus fusiform* + BGS treatment showed a significant and maximum increase in 100-grain weight i.e., up to 44.9 at 9 dS m^{-1} EC level, over respective uninoculated control.

The data showed that the biological yield of uninoculated control treatment was significantly reduced with increasing the salinity stress (Figure 2b). However, the application of ACC deaminase containing rhizobacterial inoculation and BGS mitigated the effect of salinity stress. At 9 dS m^{-1} EC, the *Bacillus cereus* + BGS treatment showed a significant increase in biological yield i.e., up to 72.8%, over the respective uninoculated control.

Effect on physiological parameters

Effect on proline contents

The rhizobacterial inoculation significantly reduced the proline contents as compared to corresponding uninoculated controls. At normal, 6 and 9 dS m^{-1} EC levels, the maximum proline contents reduction was observed i.e., up to 51.2, 39.3 and 34.8% in *Bacillus cereus* + BGS treatment, respectively, over respective uninoculated controls.

Effect on Na^+ ion concentration

When the wheat plants were exposed to salinity stress, the sodium ions (Na^+) accumulation was reduced in ACC deaminase containing rhizobacteria -treated plants with respect to uninoculated control (Figure 3). At 6 and 9 dS m^{-1} EC levels, the maximum and significant reduction in sodium ions (Na^+) accumulation in wheat leaf sap i.e., up to 37.4 and 29.7% was observed in *Bacillus cereus* + BGS treatments, respectively over respective uninoculated controls.

DISCUSSION

In this study, to evaluate the acclimatize effect of three ACC deaminase containing PGPR strains i.e. *Alcaligenes faecalis, Bacillus cereus* and *Lysinibacillus fusiformis* on the physiology, growth and yield of wheat under salinity stress conditions through integrated application of biogas slurry. These rhizobacteria have been rarely exploited to date in improving the wheat growth under stress conditions. Our results suggested the salinity stress exerted sever influences on the growth of uninoculated wheat plants, whereas, the rhizobacterial inoculation better the growth, physiological and yield attributes of wheat plants.

The combined effects of ACC deaminase containing PGPR strains and biogas slurry help the wheat plants to mitigate the salinity stress by improving the plant physiological processes, thereby improving growth and yield attributes. The rhizobacterial inoculation and organic amendment have recently been shown to mitigate salinity stress in plants through various mechanisms (Thomas et al. 2013). However, their combined effect has not been illustrated. In the present study, for the first time in the literature, the independent and combined effects of the combined effects of ACC deaminase containing PGPR strains and biogas slurry on ameliorating salinity stress in wheat plants were investigated.

Soil salinity negatively affects the plant growth by causing osmotic and ionic stress (Azooz, 2004). Rhizobacerial inoculation improves the growth and development of plants through various mechanisms mainly by producing plant growth regulators. Auxin is an important plant hormone and its application can alleviate the adverse effects of soil salinity on plant growth (Kaya et al., 2013). This study highlight the importance of using auxin producing growth promoting rhizobacteria for inducing salt tolerance in maize. The ability of these bacterial strains to tolerate higher levels of salinity may be due to changes in morphology and metabolism, which makes them able to cope and adopt saline conditions (Sgroy et al., 2009; Zahir et al., 2010).

This could be explained by the fact that application of biogas slurry increased soil porosity and reduced its bulk density, thereby resulting in better growth and root proliferation in saline soils. On the other hand, inoculation with rhizobacterial bacterial strains also had a positive effect on root growth and morphology, presumably because of phytohormone production (particularly of auxin) and, consequently, improved nutrient and water uptake. Similar effects of PGPR inoculation on plant growth promotion have been observed by various researchers in other crops, including mung bean (Ahmad et al., 2011), and peanut (Dey et al., 2004).

Salt tolerance of plants can be indicated by K^+/Na^+ (Hamida-Sayari et al., 2005). It was found in our study that salinity stress caused the ionic imbalance in plants. It resulted in higher intake of Na^+ than K^+. This increased uptake of Na+ results in reduction of K^+/Na^+ (Nadeem et al., 2010). Uptake of higher concentration of K^+ as compared to Na+ is vital to maintain higher K^+/Na^+ in cells (Kavitha et al., 2012). In our study, we found that rhizobacterial inoculation /co-inoculation significantly

decreased the Na^+. This might be due to lowered uptake of Na^+ due to longer roots caused by inoculation/coinoculation with rhizobacterial strains as reported previously (Yue et al., 2007; Ahmad et al., 2012).

The application of BGS as an organic amendment enhanced biomass yield and nitrogen uptake (Möller et al., 2008; Arthurson, 2009) by improving the physical properties of soil by increasing the moisture retention and hydraulic conductivity and reducing the bulk density of the soil (Garg et al., 2005; Barbosa et al., 2014). Due to the high concentration of inorganic nitrogen, BGS supplies more plant available nitrogen than other organic fertilizers (Odlare et al., 2008). The BGS also contains large concentrations of soluble inorganic phosphorus and thus may represent a valuable phosphorus fertilizer (Bachmann et al., 2011). The rhizobacteria might have accelerated the decomposition of BGS that resulted in release of nutrients. The availability of nutrients in combined application could improve the growth and yield parameters of wheat crop.

CONCLUSION

In conclusion, ACC deaminase containing PGPR strains i.e. *Alcaligenes faecalis*, *Bacillus cereus* and *Lysinibacillus fusiformis* improved the physiology, growth and yield of wheat under marginal saline land conditions through integrated application of biogas slurry. The present study revealed that biogas slurry with the PGPR strain *Lysinibacillus fusiformis* was the most effective combination for improving growth and yield for wheat under saline conditions. Our research suggests that the combined use of BGS and PGPR in agricultural is a sustainable strategy for the alleviation of salinity stress in wheat.

ACKNOWLEDGMENTS

This study is a part of PhD thesis of the second author.

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