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UNRAVELING THE POTENTIAL OF LIQUID ENDOPHYTIC BACTERIAL INOCULANTS FOR ENHANCING FORAGE SORGHUM GROWTH UNDER SIMULATED SALINE STRESS CONDITIONS: AN *IN-VITRO* EVALUATION

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SUMMARY

Salinity is one of the most severe abiotic stresses that limit global crop productivity by affecting the growth of plants. However, the need of the hour is to expand the agricultural areas to saline regions for food security amidst growing human populations. Plant growth-promoting bacteria (PGPB) offer a promising solution to enhance plant growth in saline environments. The present study evaluates the plant growth-promoting characteristics of potential plant growth-promoting bacteria (PGPB) *i.e. Burkholderia seminalis, Stenotrophomonas maltophilia*, and *Enterobacter* sp. (JJG_Zn) under varying levels of simulated salinity stress (0 mM, 50 mM, 100 mM, and 150 mM). All the three PGPBs exhibited plant growth-promoting traits i.e Indole-3-acetic acid (IAA) production, gibberellic acid production, Phosphate solubilization, ammonia production, siderophore production, and ACC deaminase activity even at the highest concentration *i.e.* at 150 mM of saline stress. Furthermore, the beneficial effect of bacterial inoculants of aforesaid bacterial cultures on saline stress tolerance in forage sorghum through bio priming was also evaluated under in vitro conditions. The dual inoculation of *B. seminalis* + *S. maltophilia* (T_5) was found to improve the germination parameters *viz*., germination percentage, germination speed, mean germination, and physiological traits such as shoot length, root length, fresh and dry shoot and root weight, and seed vigor index I and II over the uninoculated controls across different salt concentrations. Thus, the study suggests that bio-priming of forage sorghum seeds with potential liquid bacterial inoculants may mitigate the detrimental effects of saline soil and promote seedling growth and development. Therefore, these promising bacterial cultures can be further exploited under the salinity-affected regions across agro-climatic zones. Also, a thorough biosafety analysis of these cultures should be conducted, considering the 'one health' concept.

Key words: *Burkholderia seminalis, Enterbacter* sp. (JJG_Zn), liquid bacterial inoculants, *PGPR,* salinity, *Stenotrophomonas maltophilia*

Soil salinity is one of the most vicious abiotic stresses of global magnitude. It poses a significant challenge to crop growth and development. Soil salinity is a measurement of salt concentration within the soil, typically represented as electrical conductivity (EC). According to the United States Department of Agriculture's Salinity Laboratory (USDA), the soil is categorized as saline when its electrical conductivity (EC) measures 4 dS/m or higher. Soil salinization can be attributed to two main factors: primary salinization resulting from natural processes and secondary salinization caused by anthropogenic activities (Ondrasek *et al.,* 2011).

Salinity stress is also often referred to as hyperionic stress. It leads to the accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant tissues when exposed to soils with high NaCl levels, causing harmful effects. The entry of both Na^+ and Cl^- ions into plant cells disrupts the ion balance significantly, and excessive uptake can lead to significant physiological disruptions (Gupta and Huang 2014). Elevated concentrations of Na^+ can also inhibit the absorption of essential potassium (K^+) ions, which are crucial for growth and development. In severe cases, it ultimately reduces productivity and plant mortality (James *et al.,* 2011).

It is estimated that each year an additional 10% of land is becoming salinized, and by the year 2050, nearly 50% of the arable land in the country could be affected by salt (Kumar and Sharma 2020).

The Indian government has set a goal of rehabilitating 26 million hectares of degraded lands, which includes areas affected by salt, by the year 2030 to ensure food security for the population (Kumar and Sharma 2020). Therefore, the salinity-affected area may be utilized for fodder crop production. In this context, sorghum (*Sorghum bicolor* (L.) Moench) is a promising fodder crop which is a moderately salt tolerant crop (Devi *et al*., 2018), due to its C_4 metabolism, it can maintain photosynthetic activity and dry matter production even in challenging conditions like high temperatures, drought, and salinity (Reddy *et al.,* 2019). In Punjab, it is a prime *kharif* fodder crop and is cultivated over an area of 2.72 lakh ha area. In addition, it stays greener and palatable for a longer duration than maize and *bajra* fodders. It contains approximately 9.0% crude protein and 55.6% total digestible nutrients (Anonymous 2022), thereby satisfying the livestock's supreme fodder quality requirements, which are important contributors to the Indian economy.

To mitigate the effects of soil salinization various methods are recommended that include the application of substances such as gypsum ($CaSO_4 2H_2O$), salicylic acid, silicon, epigallocatechin-3-gallate, kinetin, Ag-nanoparticles, spermidine, and aspirin (Khan *et al*., 2020 Ahmad *et al.,* 2019, Alam *et al.,* 2019, Zhang *et al.,* 2018, Kaur *et al.,* 2018, Hussain *et al.,* 2018, Ahammed *et al.,* 2018) to alleviate abiotic stresses in many plants. However, the environmental hazards related to these practices owing to their chemical nature and high cost of production cannot be overlooked. Therefore, plant growth-promoting bacteria (PGPB) could be alternative potential agents in alleviating salinity stress by boosting plant water absorption capacity, increasing essential nutrient uptake, and accumulating osmolytes (OS) such as proline, glutamate, glycine betaine, soluble sugars, polyols, choline, and O-sulphates (Ha-Tran *et al.,* 2021). Thus, the present study's primary objective is to explore the potential of PGPBs in the mitigation of saline stress in fodder sorghum under in vitro conditions.

METHODOLOGY

Procurement of test cultures

The pure cultures of *Burkholderia seminalis, Stenotrophomonas maltophilia* and *Enterobacter* sp. (JJG_Zn) were procured from the School of Organic Farming, Punjab Agricultural University, Ludhiana, Punjab, India.

Estimation of plant growth-promoting features

The standard protocols were used for the quantitative assay for various plant growth-promoting traits and are described in Table 1. The experiment was performed in the month of June 2022.

Seed source, surface sterilization, and bio-priming of seeds

The fodder sorghum seeds of variety PSC4 were procured from the seed store, at Punjab Agricultural University, Ludhiana, Punjab, India. The seeds were then washed with tap water to remove the dust and other debris followed by surface sterilization with 70% ethanol for 1 minute for the removal of surface microorganisms (epiphytes) followed by dipping in 0.1% mercuric chloride ($HgCl_2$) for 2 minutes. The disinfected seeds were subsequently rinsed with sterile distilled water about 3-4 times to avoid any interference of disinfectant residues. The sterilized seeds were then bio-primed by soaking for 2 hours in liquid bacterial inoculant of each bacterial culture as per the treatment. A total of 5 treatments of liquid bacterial inoculants were used and are described in Table 2.

In-vitro evaluation of fodder sorghum seeds bio-primed with liquid bacterial inoculants for germination and plant growth promotion under NaCl-simulated saline-stressed conditions

The experiment was conducted in June 2022. Salinity stress was induced by supplementing the agar gel assembly with different concentrations of sodium chloride (NaCl). The agar gel assembly was prepared by autoclaving 0.85% agar in 25×150mm test tubes amended with the required amount of NaCl (0 mM, 50 mM, 100 mM,150 mM). Each test tube was then inoculated with bio-primed forage Sorghum seeds aseptically with the help of sterile forceps. Afterward, the test tubes were incubated under suitable conditions for 14 days. The 0mM, 50mM,100mM, and 150 mM saline stress corresponds to 0.0052 ds/m, 2.99 ds/m, 4.62 ds/m, and 7.09 ds/m saline stress respectively.

Parameters studied : The following parameters were studied as follows:

Germination Indices: Forage sorghum seeds were considered germinated upon the emergence of the radicle up to 2 mm and germination was observed daily for 14 days. Various seed

TABLE 1
Analysis of the various plant growth-promoting traits in accordance with the standard protocols

S. No.	PGP Trait	References
1.	Indole-3-acetic acid (IAA) production	Gordon and Weber (1951)
2.	Ammonia production	Dye (1961)
3.	Phosphate solubilization	Jackson (1973)
4.	Siderophore catecholate	Arnow (1937)
5.	Siderophore hydroxamate	Csaky (1948)
6.	Gibberellic acid production	Borrow *et al.,* (2008)
7.	ACC deaminase activity (qualitative)	Govindaswamy *et al.,* (2008)

germination indices have been used to assess the in-vitro germination of sorghum seeds. Germination percentage (GP) was calculated according to Scott *et al.,* (1984), Germination speed (GS) was calculated using the formula given by Khan and Ungar (1998), and Mean germination time was estimated by the method of Ellis and Roberts (1981) and seed vigor index was determined by the two methods of Abdul-Baki and Anderson 1973).

Plant Growth Parameters: After 14 days, the root and shoot length of the seedlings were recorded with a millimeter scale. Fresh root and shoot weight was measured with an electronic balance. For dry root and shoot weights, samples were placed at 60°C for 48 hours in the oven and then the dry samples were, measured with a precision electronic balance.

Statistical analysis of data

All the data was analyzed using analysis of variance (ANOVA) to detect significant differences between the means. Means that differed significantly were compared using Duncan& Multiple Range Test (DMRT) with a probability level of 0.05 using SPSS statistical software version 20.0.

RESULTS AND DISCUSSION

Assay of Plant growth promoting (PGP) characteristics of bacterial cultures

The data about the study of plant growth-promoting features of all three bacterial cultures at different saline concentrations revealed that at 50mM NaCl, the amount of IAA (Trp+)(37.15, 43.01 and 67.01 µg/mL), IAA (Trp-) (18.00, 20.00 and 34.00 µg/mL), ammonia production (30.78, 36.03 and 38.30 µg/mL), phosphate solubilization (18.18, 18.81 and 19.70 µg/mL), siderophore catechol (127.93, 146.23 and 158.07µg/mL), siderophore hydroxamate (116.10, 157.49 and 169.07µg/mL), and gibberrelic acid (95.08, 96.46 and 100.22µg/mL) production by *Bukholderia seminalis, Stenotrophomonas maltophilia* and *Enterobacter* sp. (JJG_Zn) was higher as compared to 100 and 150Mm NaCl concentrations (Tables 3,4 and 5). In addition, *Enterobacter* sp. (JJG_Zn) produced the maximum amount of all PGP traits followed by *S. maltophilia* and *B. seminalis*. It was observed that although a significant effect of NaCl was observed on the production of PGP traits by the three PGPBs. However, all three bacterial cultures retained their plant growth-promoting activity even under high saline concentrations i.e 150mM NaCl. Furthermore, the ability of the cultures to utilize ACC as the sole source of nitrogen was evaluated based on growth on plates containing substrate ACC, and the results were recorded after 72 hours of incubation. At the varied NaCl concentrations, all three PGPB were able to utilize ACC in DF salt media thus all three bacterial cultures displayed a positive ACC deaminase activity.

TABLE 2
Treatments utilized for the saline stress *in vitro* assay

S. No.	Treatments
1.	Uninoculated (Control)
2.	Liquid bacterial inoculant of *Burkholderia seminalis*
3.	Liquid bacterial inoculant of *Stenotrophomonas maltophilia*
4.	Liquid bacterial inoculant of *Enterobacter sp.* (JJG Zn)
5.	Dual inoculation:-Liquid bacterial inoculant of *B. seminalis+S. maltophilia*

The tolerance to salinity might be due to the exopolysaccharide (EPS) production by the plant growth-promoting bacteria that can chelate various cations, including Na^+. Arora *et al.,* (2010) also reported that under salinity stress, the bacteria secrete EPS, which binds to Na+ ions and thereby reduces their toxicity in the soil.

Germination indices

In the present investigation, an overall significantly higher germination percentage as well as

the germination speed was observed in the treatment T_5 i.e dual inoculation with liquid bacterial inoculants (LBI) of *B.seminalis* and *S.maltophilia* under unstressed as well as salt stress conditions whilst the lowest was recorded in the treatment T_1 (Tables 6 and 7). The increase in seed germination percentage may be attributed to the release of phytohormones like gibberellic acid and IAA by the inoculated bacteria, which regulated cell division and promoted germination thereby mitigating environmental stresses such as salt stress. The findings of the present investigation aligned with Lee *et al.,* (2021) who also reported significant improvements in wheat growth parameters, including germination percentage, with the application of *Bacillus megaterium* strain PN89 under saline conditions.

Mean germination time (MGT)

MGT is defined as the length of the lag period from the start of imbibition to radicle protrusion. The present study revealed that the treatment T_5 i.e dual inoculation with liquid bacterial inoculants (LBI) of *B.seminalis* and *S.maltophilia* resulted in the lowest MGT under non-stressed as well as salt stress conditions followed by the treatment T_4 which was statistically significantly lower than the control (3.69) (Table 8). The reduction in mean germination time (MGT) may be due to the release of gibberellic acid by the inoculated plant growth-promoting bacteria (PGPB) and enzymatic activities during seed development and germination. α-Amylase, an essential enzyme during seed germination, facilitates starch breakdown to provide germinating seeds with necessary carbon sources and energy (Thu *et al.,*2020). These findings aligned with Dehnavi *et al.,* (2022), who also documented significant variations in MGT of Sorghum genotypes under non-stressed conditions and noted an increase in MGT with rising saline concentrations.

Seedling length: Shoot length and root length

The seedling length measurement comprises both the shoot and root length of the fodder sorghum seeds. In the present study, the seedling length of bio-primed fodder sorghum seeds under non-stressed conditions showed a significant increment over the respective control (Tables 9 and 10). Under non-stressed conditions, the maximum shoot length was documented in treatment T_5 (15.52cm) which was at par with treatment T_4 (15.43cm). All the treatments were statistically significant over the treatment T_1

TABLE 3
Quantitative estimation of PGP traits of bacterial cultures at different saline concentrations

Bacterial strains	IAA (µg/mL) (trp)				IAA (µg/mL)				Ammonia production (µM/mL)			
	0 mM	50 mM	100 mM	150 mM	0 mM	50 mM	100 mM	150 mM	0 mM	50 mM	100 mM	150 mM
Burkholderia seminalis	40.20±0.30^{a}	37.15±0.33^{b}	33.39 ±0.26^{c}	31.12±0.19^{d}	20.33±0.33^{a}	18.00±1.66^{a}	12.00±1.10^{b}	9.00±0.83^{b}	33.0±3.04^{a}	30.78±±2.84^{ab}	24.69±1.24^{bc}	21.18±0.31^{c}
Stenotrophomonas maltophila	48.11±0.48^{a}	43.01 0.47^{b}	40.02±0.36^{c}	37.37±0.44^{d}	23.44±0.45^{a}	20.00±1.84^{a}	14.00±1.29^{b}	10.00±0.92^{b}	38.12±3.52^{a}	36.03±3.32^{a}	30.16±0.81^{ab}	25.07±0.39^{b}
Enterobacter sp.(JJG_Zn)	73.87±0.16^{a}	67.01±0.217^{b}	60.05±1.22^{c}	60.04±0.64^{c}	37.13±0.69^{a}	34.00±3.14^{a}	25.00±2.30^{b}	15.00±1.38^{c}	42.62±3.94^{a}	38.30±3.53^{a}	33.65±0.54^{ab}	28.34±1.37^{b}

The values of the experiment are means of three replications ± standard error. Means with the same letter along the row are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3 where a>b>c.

TABLE 4
Quantitative estimation of PGP traits of bacterial cultures at different saline concentrations

Bacterial strains	Phosphate solubilisation (µg/mL)				Siderophore catechol (µg/mL)				Siderophore hydroxamate (µg/mL)			
	0 mM	50 mM	100 mM	150 mM	0 mM	50 mM	100 mM	150 mM	0 mM	50 mM	100 mM	150 mM
Burkholderia seminalis	19.05±1.75^{a}	18.18±0.32^{a}	18.16±0.19^{a}	17.59±0.48^{a}	135.64±1.56^{a}	127.93±1.09^{b}	120.30±0.89^{c}	114.66±1.23^{d}	119.74±1.50^{a}	116.10±0.90^{a}	106.86±1.12^{a}	100.00±0.16^{a}
Stenotrophomonas maltophilia	20.31±1.28^{a}	18.81±0.21^{ab}	18.54±0.27^{ab}	17.88±0.09^{b}	154.90±2.26^{a}	146.23±2.09^{b}	140.52±1.10^{bc}	135.50±2.19^{c}	164.37±0.93^{a}	157.49±2.49^{b}	148.41±1.10^{c}	139.77±2.06^{d}
Enterobacter sp.(JJG_Zn)	22.39±2.06^{a}	19.70±0.44^{ab}	19.53±0.34^{ab}	18.03±0.10^{b}	165.04±1.45^{a}	158.07±1.32^{b}	151.63±1.00^{c}	147.07±1.22^{d}	176.44±1.49^{a}	169.07±1.29^{b}	160.21±1.00^{c}	151.30±1.32^{d}

The values of the experiment are means of three replications ± standard error. Means with the same letter along the row are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a>b>c>d.

TABLE 5
Quantitative estimation of PGP traits of bacterial cultures at different saline concentrations

Bacterial strains	Gibberrelic acid production (µg/mL)				ACC (qualitative)			
	0 mM	50 mM	100 mM	15 0mM	0 mM	50 mM	100 mM	150 mM
Burkholderia seminalis	105.41±1.59[a]	95.08±1.22[b]	89.48±1.51[c]	80.19±0.82[c]	+	+	+	+
Stenotrophomonas maltophilia	108.32±1.40[a]	96.46±1.52[b]	93.61±1.29[b]	88.29±0.48[c]	+	+	+	+
Enterobacter sp. (JJG_Zn)	111.37±2.54[a]	100.22±0.92[b]	93.95±0.99[c]	91.70±0.29[c]	+	+	+	+

The values of the experiment are means of three replications ± standard error. Means with the same letter along the row are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a>b>c.

TABLE 6
Germination Percentage (%) of bio-primed fodder sorghum seeds with liquid bacterial inoculants under salinity stress

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	74.61±1.12[a*]	64.93±1.29[a*]	50.56±0.93[a*]	40.62±0.95[a*]
2.	*Burkholderia seminalis*	85.94±0.91[b]	76.83±1.15[b]	65.72±1.38[b]	57.17±0.77[c]
3.	*Stenotrophomonas maltophilia*	89.98±1.12[bc]	82.46±1.22[bc]	67.79±2.10[c]	64.40±1.10[d]
4.	*Enterobacter sp.*(JJG_Zn)	91.84±0.95[c]	84.84±1.14[c]	78.08±1.75[d]	70.05±0.81[e]
5.	Dual inoculation:*B. seminalis +S. maltophilia*	99.66±1.19[d]	92.53±1.08[d]	85.17±1.52[e]	70.25±0.76[e]

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

TABLE 7
Germination speed of bio-primed fodder sorghum seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	2.21±0.01[a*]	2.11±0.01[a*]	1.84±0.03[a*]	0.97±0.01[a*]
2.	*Burkholderia seminalis*	2.32±0.06[a]	2.26±0.03[a]	2.02±0.02[b]	1.29±0.01[c]
3.	*Stenotrophomonas maltophilia*	2.56±0.05[b]	2.40±0.02[b]	2.04±0.02[b]	1.43±0.02[d]
4.	*Enterobacter* sp. (JJG_Zn)	2.71±0.07[b]	2.61±0.02[b]	2.24±0.03[c]	1.62±0.02[e]
5.	Dual inoculation*: B.seminalis +S. maltophilia*	2.91±0.07[c]	2.74±0.04[c]	2.43±0.02[d]	1.65±0.02[e]

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

(11.50cm). However, the shoot length decreased with the increasing NaCl concentrations. All the treatments were statistically significant over the treatment T_1 (Table 9). The elevated shoot lengths might be attributed to the production of phytohormones, which play a vital role in maintaining hormonal balance in plants. Spaepen *et al.,* (2007) also reported that the external supply of auxin by endophytic bacteria assists the plant in coping with stress.

The maximum root length was documented by T_5 under both non-stressed and stressed conditions (Table 10). Salinity reportedly hinders a plant's overall growth and activity. However, the boost in root length even under the increased concentrations of NaCl could be accredited to the production of auxin Indole Acetic Acid (IAA) by the inoculated Plant Growth-Promoting Bacteria (PGPB). These auxins play a critical role in enhancing root morphology in plants. The findings of the present investigation are in line with the experiments of Dehnavi *et al.,* (2020) and Shiade *et al.,* (2020) who reported a constant decline in the seedling growth of *Sorghum bicolor* and *Festuca arundinacea* with the increasing saline concentrations.

Fresh and dry shoot weight

The fresh and dry shoot weight of seedlings germinated from seeds bio-primed with LBIs of PGPBs ameliorated under both unstressed and NaCl-simulated stressed conditions. The highest fresh shoot weight

TABLE 8
Mean Germination Time (days) of bio-primed fodder Sorghum seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	3.69±0.017^{d*}	3.74±0.09^{d*}	4.80±0.083^{e*}	5.98±0.04^{c*}
2.	*Burkholderia seminalis*	3.11±0.017^{c}	3.34±0.02^{c}	4.22±0.067^{c}	4.95±0.06^{b}
3.	*Stenotrophomonas maltophilia*	2.82±0.017^{b}	2.96±0.02^{b}	4.11±0.017^{c}	4.90±0.02^{b}
4.	*Enterobacter* sp. (JJG_Zn)	2.76±0.023^{b}	2.96±0.03^{b}	3.78±0.066^{b}	4.80±0.07^{b}
5.	Dual inoculation*: B. seminalis+S. maltophilia*	2.37±0.088^{a}	2.94±0.03^{a}	3.24±0.026^{a}	4.50±0.112^{a}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard Error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

TABLE 9
Shoot Length (cm) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	11.50±0.028^{a*}	9.30±0.15^{a*}	8.08±0.04^{a*}	7.40±0.20^{a*}
2.	*Burkholderia seminalis*	14.61±0.33^{b}	12.7±0.26^{b}	10.65±0.22^{b}	9.26±0.33^{b}
3.	*Stenotrophomonas maltophilia*	15.04±0.030^{bc}	12.89±0.06^{bc}	11.01±0.72^{c}	9.67±0.18^{b}
4.	*Enterobacter* sp. (JJG_Zn)	15.43±0.222^{d}	13.26±0.08^{c}	11.37±0.08^{c}	10.57±0.15^{c}
5.	Dual inoculation*:B. seminalis +S. maltophilia*	15.52±0.265^{d}	13.55±0.16^{c}	11.57±0.154^{c}	11.09±0.20^{c}

Control*is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c

TABLE 10
Root Length (cm) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	13.00±0.075^{a*}	12.10±0.11^{a*}	10.23±0.06^{a*}	8.23±0.14^{a*}
2.	*Burkholderia seminalis*	14.64±0.324^{c}	12.61±0.13^{c}	10.81±0.16^{b}	8.79±0.037^{bc}
3.	*Stenotrophomonas maltophilia*	15.04±0.096^{cd}	13.05±0.10^{cd}	11.15±0.09^{bc}	9.04±0.20^{cd}
4.	*Enterobacter* sp. (JJG_Zn)	15.26±0.059^{d}	13.13±0.06+^{d}	11.23±0.06^{c}	9.30±0.07^{de}
5.	Dual inoculation*: B. seminalis + S. maltophilia*	16.20±0.144^{e}	13.41±0.11^{e}	11.52±0.16^{c}	9.58±0.10^{e}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

was reported in treatment T_5 i.e dual inoculation with liquid bacterial inoculants (LBI) of *B.seminalis* and *S.maltophilia* followed by treatment T_4 (Tables 11,12).

Furthermore, both parameters declined with the elevating NaCl concentrations. The heightened salinity levels cause the accumulation of sodium (Na+) and potassium (K+) ions, leading to decreased water potential due to increased solute concentration. This reduction in water potential likely contributes to decreased FSW and DSW under higher salt concentrations compared to unstressed conditions. However, bio-primed seeds with potent plant growth-promoting bacteria (PGPB) showed significantly higher FSW and DSW under stressed conditions compared to untreated controls. This positive impact may be attributed to the release of plant growth-promoting substances such as IAA, or osmolytes like proline, which offer protection and support seedling proliferation under stress conditions, thereby enhancing shoot and root biomass. The findings of the present investigation were in harmony with the experiment of Rahnama *et al.,* (2023) who reported the highest increase in biomass fresh and dry weights (above- and below-ground parts and seedlings) in seeds inoculated with *B. cereus, A.chroococcum, P. aeruginosa,* and *A. lipoferm* respectively.

TABLE 11
Fresh Shoot Weight (g) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	0.033±0.003^{a*}	0.030±0.003^{a*}	0.011±0.000^{a*}	0.008±0.00a^{a*}
2.	*Burkholderia seminalis*	0.063±0.003^{c}	0.046±0.003^{c}	0.030±0.000^{bc}	0.013±0.001^{abc}
3.	*Stenotrophomonas maltophilia*	0.066±0.003^{c}	0.056±0.003^{c}	0.031±0.000^{bc}	0.015±0.001^{bc}
4.	*Enterobacter* sp. (JJG_Zn)	0.070±0.002^{cd}	0.060±0.005^{de}	0.040±0.003^{cd}	0.017±0.001^{c}
5.	Dual inoculation*: B. seminalis +S. maltophilia*	0.076±0.005^{d}	0.066±0.003^{e}	0.050±0.001^{d}	0.022±0.001^{d}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c.

TABLE 12
Dry Shoot Weight (g) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	0.006±0.001^{a*}	0.005±0.001^{a*}	0.002±0.001^{a*}	0.001±0.000^{a*}
2.	*Burkholderia seminalis*	0.011±0.001^{ab}	0.008±0.001^{ab}	0.004±0.01^{a}	0.003±0.001^{a}
3.	*Stenotrophomonas maltophilia*	0.014±0.001^{bc}	0.009±0.002^{bc}	0.005±0.002^{a}	0.004±0.000^{a}
4.	*Enterobacter* sp. (JJG_Zn)	0.015±0.002^{c}	0.011-±0.001^{bc}	0.007±0.002^{a}	0.006±0.001^{a}
5.	Dual inoculation *:B. seminalis +S. maltophilia*	0.016±0.001^{c}	0.013±0.001^{c}	0.012±0.001^{a}	0.008±0.000^{a}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c.

Fresh root and dry root weights

In the non-saline conditions, the highest fresh root weight was observed in the treatment T_5 (0.126g) which was at par with the treatment T_4 (0.116g). All the treatments were statistically significant over T_1 (0.060g). At 50mM saline stress, the maximum fresh root weight was observed in the seedling germinated from the seed bio-primed with T_5 followed by the treatment T_4, T_3, and T_2 (Table 13).

Likewise, the maximum dry root weight under unstressed conditions was recorded in the treatment T_5. With the elevating salt concentrations, there was a gradual decline in the dry root weight however, bio-priming with the PGPB increased the biomass over the control (Table 14). Furthermore, the seedlings germinated from bio-primed Sorghum seeds showed improved fresh and dry weight of roots over the control treatment. This might be due to the release of auxins and ACC deaminase by the PGPB that promoted the growth and development of plants thereby resulting in enhanced root biomass. The findings are in line with the experiment of Singh *et al.,* (2015) who reported that inoculation of wheat plants with salinity-resistant

TABLE 13
Fresh root weight (g) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	0.060±0.003^{a*}	0.050±0.008^{a*}	0.040±0.003^{a*}	0.030±0.000^{a*}
2.	*Burkholderia seminalis*	0.093±0.003^{b}	0.060±0.002^{b}	0.070±0.001^{b}	0.070±0.000^{b}
3.	*Stenotrophomonas maltophilia*	0.103±0.003^{b}	0.110±0.02^{b}	0.093±0.003^{c}	0.081±0.001^{c}
4.	*Enterobacter* sp. (JJG_Zn)	0.116±0.003^{c}	0.130±0.04^{c}	0.100±0.005^{d}	0.083±0.003^{c}
5.	Dual inoculation*: B. seminalis +S. maltophilia*	0.126±0.003^{e}	0.132±0.03^{e}	0.110±0.002^{e}	0.086±0.002^{e}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

TABLE 14
Dry root weight (g) of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1	Control	0.008±0.001^{a*}	0.007±0.001^{a*}	0.006±0.001^{a*}	0.004±0.000^{a*}
2	*Burkholderia seminalis*	0.010±0.001^{a}	0.009±0.001^{ab}	0.008±0.01^{ab}	0.006±0.001^{ab}
3	*Stenotrophomonas maltophilia*	0.014±0.001^{b}	0.011±0.002^{bc}	0.010±0.002^{bc}	0.008±0.000^{bc}
4	*Enterobacter* sp. (JJG_Zn)	0.016±0.002^{b}	0.012-±0.001^{cd}	0.011±0.002^{c}	0.009±0.001^{c}
5	*Dual inoculation:B. seminalis +S. maltophilia*	0.018±0.001^{b}	0.014±0.001^{d}	0.012±0.001^{c}	0.010±0.000^{c}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c.

PGPB resulted in better growth of plants at 100mM saline stress conditions as compared to uninoculated ones owing to their ACC deaminase activity of PGPB.

Seed vigor index

Seed vigor encompasses the collective characteristics that govern the vitality and effectiveness of seed batches with satisfactory germination across diverse environmental conditions. High seed vigor ensures good quality seed and enhanced productivity. Seed vigor stands out as a critical factor in seed quality, as it has a direct impact on crop productivity. The seedlings germinated from the bio-primed sorghum seeds showed more vigor as compared to the control even under the increasing salt concentration i.e 50mM,100mM and 150mM (Tables 15 and 16).

The elevated seed vigor index observed in the seedlings derived from bio-primed seeds may be attributed to the synthesis of phytohormones such as IAA, Gibberellic acid, and cytokinins. The current

TABLE 15
Seed vigor index I of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	1827.94±171.74^{a*}	1389.50±132.39^{a*}	0925.75±83.78^{a*}	634.89±55.18^{a*}
2.	*Burkholderia seminalis*	2513.73±229.32^{abc}	1944.56±176.74^{abc}	1410.35±126.81^{bc}	1031.91±93.59^{bc}
3.	*Stenotrophomonas maltophilia*	2706.58±251.75^{bc}	2139.00±201.05^{bc}	1502.22±136.45^{c}	1204.92±112.46^{cd}
4.	*Enterobacter* sp. (JJG_Zn)	2818.56±255.17^{c}	2238.91±202.77^{c}	1764.60±164.16^{cd}	1391.81±133.76^{d}
5.	Dual inoculation*: B. seminalis +S. maltophilia*	3161.20±293.75^{c}	2494.59±228.70^{c}	1966.57±178.19^{d}	1452.06±131.82^{d}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d.

TABLE 16
Seed vigor index II of fodder sorghum seedlings germinated from bio-primed seeds with liquid bacterial inoculants under saline stress conditions

S. No.	Treatments	Non-stressed	50mM	100mM	150mM
1.	Control	1.04±0.096^{a*}	0.77±0.072^{a*}	0.40 ±0.037^{a*}	0.20±0.018^{a*}
2.	*Burkholderia seminalis*	1.80±0.166^{b}	1.30±0.120^{bc}	0.78±0.072^{a}	0.51±0.047^{b}
3.	*Stenotrophomonas maltophilia*	2.51±0.232^{c}	1.64±0.152^{cd}	1.28±0.118^{b}	0.77±0.071^{c}
4.	*Enterobacter sp.* (JJG_Zn)	2.83±0.262^{cd}	1.95±0.180^{d}	1.71±0.158^{c}	1.05±0.096^{d}
5.	Dual inoculation*:B. seminalis +S. maltophilia*	3.38±0.312^{d}	2.49±0.230^{e}	2.12±0.196^{d}	1.26±0.116^{d}

Control* is exposed to experimental treatment without PGPB. The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a<b<c<d<e.

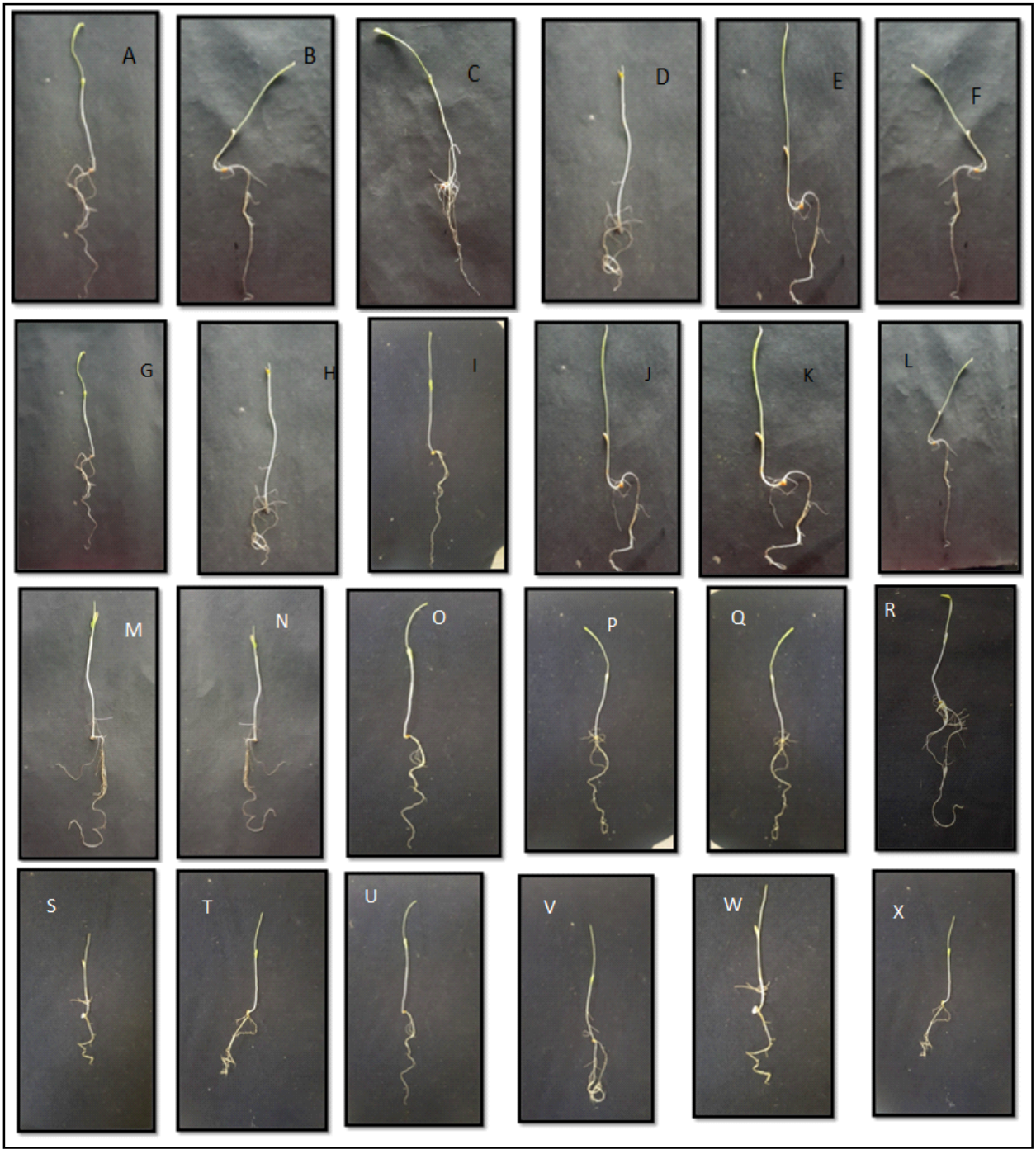

Plate: (A- F) Seedling length of forage Sorghum seeds germinated from bio priming with liquid bacterial inoculants under 0mM saline stress conditions, (G-L) Seedling length of forage Sorghum seeds germinated from bio priming with liquid bacterial inoculants under 50mM saline stress conditions, (M-R) Seedling length of forage Sorghum seeds germinated from bio priming with liquid bacterial inoculants under 100mM saline stress conditions, (S-X) Seedling length of forage Sorghum seeds germinated from bio priming with liquid bacterial inoculants under150mM saline stress conditions.

study's findings align with Rahnama *et al.,* (2023), who observed that the interaction between plant growth-promoting bacteria (PGPB) and water stress led to maximum vigor indices in treatments involving *B. cereus, A. chroococcum, P. aeruginosa*, and *A. lipoferm* compared to untreated seeds across various water stress levels. Similarly, Yaghoubian *et al.,* (2022) demonstrated that employing culture filtrate from a *Bacillus* strain increased the Seedling Vigor Index (SVI) of soybean plants under salt stress conditions.

CONCLUSION

In the present investigation, LBIs of *Stenotrophomonas maltophilia, Burkholderia seminalis,* and *Enterobacter* sp. (JJG_Zn) were evaluated to alleviate the increasing saline stress in forage sorghum under *in vitro* conditions. It was observed that the seedlings germinated from seeds bio-primed with the LBIs of dual inoculation of *Stenotrophomonas maltophilia* and *Burkholderia seminalis* demonstrated promising results in terms of germination indices or growth parameters. Thus, it is believed that the LBIs of plant growth-promoting bacteria (PGPB) can play a crucial role in addressing the challenge of the anticipated rise in soil salinity which is expected to significantly threaten agricultural productivity in the coming years. Consequently, it is suggested that these bacterial cultures should be evaluated under field conditions to further strengthen the research work.

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