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Studies of Plant Growth Promoting Rhizobacterial Inoculants on Sugarcane in Saline Soil

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Abstract

Salinity of soil is an emerging problem of the agriculture that reduces plant growth and yield. Use of plant growth promoting rhizobacteria (PGPR) inoculant in saline soil improves fertility and induces plant growth promotion. These beneficial microorganisms colonize the rhizosphere /endo-rhizosphere of plants and impart saline tolerance. Halo tolerant PGPR cultures were isolated from saline soil fields based on soil's physico-chemical properties from Baramati region. The selected isolates *Azotobacter* spp., *Rhizobium* spp. and *Azospirillum* spp. were characterized on the basis of morphological and biochemical tests. These cultures were salt tolerant up to 2 % NaCl and having nitrogen fixation, alkaline phosphatase, indole acetic acid (IAA) and exopolysaccharide production activity. We assessed PGPR inoculants on sugarcane grown in saline soil by pot assay method. This method carried out by giving treatment to saline soil with halo tolerant PGPR inoculants. Sugarcane plantlets germination rate, shoot length, chlorophyll content of leaf and percent nitrogen content of leaf improved in pots inoculated with of halo tolerant PGPR inoculants. Simultaneously, all halo tolerant PGPR inoculants improved saline soil health in treated pot soil over control, with respect to available nitrogen, phosphorus, potassium and organic carbon also decreasing electrical conductivity, pH and sodium adsorption ratio of saline soil. The present article focuses on evaluation of halo tolerant bacterial strains to stimulate saline tolerance and promote growth of sugarcane in saline soil. It inferred that PGPR inoculants are applicable in promoting plant growth under salt stress.

Significance Statement:

Sugarcane is the major crop of farmers. Salinity of soil affects growth and productivity of sugarcane. This study was conducted in an attempt to isolate and characterize halo tolerant PGPR from saline soil habitat and its efficacy in it.

Keywords

Saline soil, halo tolerant PGPR, sugarcane, pot assay.

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INTRODUCTION:

Salinity of agriculture soil is one of the most common environmental stress factors that adversely affect plant productivity by retarding plant growth and development. The overuse of water and chemical fertilizers has plays significant role in increasing

salinization of soil. One of the major complications in this process is the increase in the concentration of soluble salts in the root zone of soils, which affects the rhizospheric populations thereby affecting plant productivity [1]. Soil salinity limits the lands capability for supporting optimum plant growth therefore growing demands of expanding population for various biomass products have necessitated an exploitation of these soils [2]. A new biological approach of plant microbe interaction to conquer salinity troubles has recently gained a great interest from many workers throughout the world. Use of rhizobacteria is one of the most acceptable approach to reduce the effect of salt stress on plants by mechanisms which either modulate or ameliorate the salt stress [3].Soil organic matter and beneficial soil microbes have been recognized as key factor in maintaining soil quality and crop production. Bioinoculants contain beneficial microbes that enhance plant growth when applied in soil by nutrient solubilization, nitrogen fixation, phytohormones production resulting in available forms of nutrients in soil which improved soil properties and productivity [4,5].To make agriculture sustainable and less dependent on chemical fertilizers it is important to know how to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce IAA that can contribute to improvement of crop growth.

Plant growth promoting rhizobacteria (PGPR) can protect plants from deleterious effects of environmental stresses including drought, salinity, heavy metal and phytopathogens. Many plant growths promoting rhizobacteria (PGPR) facilitate plant growth indirectly by reducing plant pathogens or directly by facilitating the uptake of nutrients from environment. PGPR influence the plant hormonal balance by producing compound such as phytoharmone indole acetic acid. They can mobilize nutrients to plants such as phosphorus by solubilization of soil insoluble phosphates. Some rhizobacteria produce microbial inhibitory compounds such as siderophore Fe chelating molecules that inhibit growth of phytopathogen in soils with low content of this ion promoting indirectly the plant growth. PGPR fixes nitrogen from environment that becomes available to plants [3]. To rescue plant growth in saline conditions, PGPR have been known to play an essential role in the growth and metabolism of plants [6]. Certain varieties of PGPR *Bacillus*, *Burkholderia*, *Acenitobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Flavobacterium*, *Rhizobium* and *Serratia* are now being used worldwide as biofertilizer to enhance crop productivity [5,7]. Strains from *Azospirillum*, *Bacillus*, *Azotobacter* are commercialized as biofertilizers for non-legumes plants. There is no commercial biofertilizer for non-legumes based on *Rhizobium*. But *Rhizobium* has potential as non-legume plant growth promotion by producing IAA, phosphate solubilization, exopolysaccharide production and siderophore production. *Rhizobium* promotes the growth of non-leguminous plants like sunflower, canola, tomato, pepper shown in other reports [8, 9]. So, we selected *Rhizobium* as PGPR inoculants in this study.

Today, much of agriculture land in Maharashtra has become saline due to faulty irrigation practices and overuse of chemical fertilizers. Sugarcane is the major crop of farmers. Salinity of soil effect on growth and productivity of sugarcane. This study was conducted in an attempt to isolate and characterize halo tolerant PGPR from saline soil habitat and to evaluate their ability of improvement in saline soil properties and sugarcane plant growth promotion in saline soil by pot assay method.

MATERIAL AND METHODS:

Sample collection:

Baramati Tehsil region, Maharashtra, India was chosen for sample collection. The locations were Dorlewadi, Zargardwadi, Malegaon,Medad, Shardanagar, Krishi Vigyan Kendra Malegaon, Songaon. A total of 50 saline sites were chosen from the locations mentioned. Soil with pH higher than 8.5 and electrical conductivity above 2.5 dS/m were chosen for the study. From each saline site at least 60cm deep soil was taken. Soil samples were collected from the rhizosphere area of plants. The soil samples were placed in plastic bags and stored at room temp. At selected point in the trial area without bulking sample, because soil is spatially variable. For *Rhizobium* strains roots of leguminous plants were removed. All the samples were taken in different polythene bags and brought to the laboratory [10].

Isolation and identification of PGPR cultures

Enrichment of organism carried out in Ashby's broth and yeast extract mannitol broth. All bacteria were

isolated on yeast extract mannitol agar and Ashby's mannitol agar media. Isolates biochemically characterized by Gram's staining, motility and biochemical tests like catalase, oxidase, sugar utilization, ammonia production, amylase test and citrate utilization tests were performed as per standard methods [11]. All isolates were identified as per the Bergey's Manual of Determinative Bacteriology 9th Edition [12]. Specific medium for *Azotobacter* spp. Ashby's mannitol media, *Rhizobium* spp. yeast extract mannitol media and *Azospirillum* spp. medium for *Azospirillum* used for inoculants production.

Determination of salt tolerance

Isolated cultures were screened for salt tolerance. These cultures were grown in specific medium broth supplemented with NaCl so to give 0.4-2% NaCl concentration. Each tube was then added with actively growing selected PGPR and incubated on rotary shaker at 30 °C. Bacterial growth was determined as OD_{540} to find out NaCl tolerance.

Abbreviations: PGPR=Plant Growth Promoting Rhizobacteria, IAA=Indole Acetic Acid, AN= Available Nitrogen, AP= Available Phosphorus, AK= Available Potassium

Characterization of PGPR for plant growth promotion traits

Production of Indole acetic acid

The isolates were tested for production of growth hormone i.e. auxins (IAA). The bacterial cultures were inoculated in Jenson's broth (0.5g of Tryptophan for 100ml media). Incubation was done at 28^{0}C for 7 day at 100 rpm on orbital shaking incubator. After completion of incubation days the broths were centrifuged at 10,000 rpm for 15min at 4^{0}C. 2 ml supernatant was taken and 2 drops of orthophosphoric acid and 4ml of Salkowasky's reagent was added. Pink color production indicated IAA production. Absorbance was measured at 530nm. The absorbance was compared with standard curve and the concentration of IAA produced was calculated accordingly [13].

Phosphate solubilization in liquid culture (Alkaline phosphatase activity):

Isolates were grown in selective media. One ml of culture supernatant was incubated at room temperature with 1.0 ml of 25 mM q-nitro phenyl phosphate and 4.0 ml modified universal buffer, pH 11, alkaline phosphatase. After 1 hour the reaction was terminated by adding 1.0 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. The assay mixtures were filtered through a Whatman No. 2 filter paper and the yellow color measured at 410 nm. A standard curve drawn from known concentration of q-nitrophenyl phosphate was used to quantify alkaline phosphatase activity present in the culture supernatant.

Nitrogen fixation

PGPR cultures were tested for nitrogen fixation in Ashby's broth nitrogen free medium. Inoculation of PGPR culture in Ashby's broth incubated at 28^{0}C-30^{0}C for 7-8 days then observed it for turbidity formation.

Exopolysaccharide production

PGPR isolates were grown on selective media broth. Cell mass was removed from 30 days old cultures broth by centrifugation (10,000 rpm) for 10 min at 20°C. In 20 ml supernatant, double volume ice cold isopropanol was added and kept overnight at 4°C. The precipitated polysaccharides were separated by centrifugation (10,000 rpm) and dried in pre weighed porcelain dish which were kept in the oven. Extracellular polysaccharide content (mg/ml) was determined from the dry weights of cell extract.

Soil physiochemical analysis

Saline soil samples were analyzed for physicochemical parameters like pH, electrical conductivity, total organic carbon, total nitrogen, phosphorus content and potassium content by standard methods [14].

Pot assay

PGPR liquid inoculants of *Azotobacter* spp., *Rhizobium* spp. and *Azospirullum* spp. were prepared in their specific medium with the cell population adjusted to 1×10^8 - 1×10^9cfu/ml determined by standard plate count method. Efficacy of inoculants was studied by pot assay with sugarcane variety co-86032(*Saccharum officinarum*) as a test crop. Eight treatments in triplicate were used. Three bacterial cultures which are *Azotobacter* spp. (AZT), *Rhizobium* spp. (RZB) and *Azospirullum* spp. (AZSP) were used treatments are AZT+RZB, AZSP+RZB, AZT+AZSP, AZT+RZB+AZSP and control [5]. Saline soil collected from salt affected field

used for pot assay. 5 kg saline soil was added in each earthen pot and saline soil was treated with PGPR inoculants as per the treatment given in the table, 300ml per 5 kg soil or 100 ml of each inoculant for consortia treatment kept it for one day. Sugarcane eye buds surface sterilized with 0.1% $HgCl_2$ and washed with water before using. In each pot sugarcane eye buds sown at 5 cm depth as four buds in each pot. The moisture content maintained by irrigating pots 1-day interval.

Table 1 Details of the treatments for sugarcane pot assay

Sr. No.	Treatment code	Treatment forms	Short Treatments details
1	T1	SS	Saline soil as a control
2	T2	SS+AZT	Saline soil + *Azotobacter* spp.
3	T3	SS+RZB	Saline soil + *Rhizobium* spp.
4	T4	SS+AZSP	Saline soil + *Azospirillum* spp.
5	T5	SS+AZT+RZB	Saline soil + *Azotobacter* spp. + *Rhizobium* spp.
6	T6	SS+AZSP+RZB	Saline soil + *Azospirillum* spp. + *Rhizobium* spp.
7	T7	SS+AZT+AZSP	Saline soil + *Azotobacter* spp. +*Azospirillum* spp.
8	T8	SS+AZT+RZB+AZSP	Saline soil + *Azotobacter* spp. + *Rhizobium* spp. + *Azospirillum* spp.

Sugarcane pot assay

Figure 1: T1

Figure 2: T2

Figure 3: T3

Figure 4: T4