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Role of Indole Acetic Acid (IAA) Producing Rhizobacteria and its Effect on Plant Growth of Mustard Crop under Salt Stress Condition

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A B S T R A C T

Rhizobacterial strains having plant growth promoting hormone can helps in promoting plant growth and mitigates adverse conditions. Keeping in view of their positive impact, present study was undertaken to identify the Salt Tolerant Rhizobacteria (STR) having IAA production activity. Out of 300 bacteria from the mustard rhizospheric soil 25 could able to grow at 10% NaCl medium termed as STR were assessed for their plant growth promoting hormones. Bacterial strains (STR PGP) B1, B14, B17 and B25 identified as genus *Enterobacter* showing IAA production ranges from 1 - 3 µg/ml. The seeds of salt sensitive mustard (var. Rohini) treated with these STRP GP were sown in pots subjected to salt stress (5M) along with untreated control without salt and STRPGP. At flowering stage, plant vigour and nutritional status was assessed that results an increased value of chlorophyll and flavonol in salt stressed plants as compared to control, while anthocynin remained almost same. The role of STRPGP under salt stress along with increased above parameters indicated the improved ability of mustard to sustain normal growth when subjected to stress condition.

Introduction

Salinization is recognized as one of the major constraints threatening natural resources in many countries and affects around one billion hectares of land worldwide (Munns and Tester, 2008). Salt stress is a major abiotic factor that turns agronomically useful lands into unproductive areas. Excessive salinity of soil inhibits crop growth and development, consequently, reducing yield and quality of crops. Mustard crops in India are grown in diverse agroclimatic conditions.

The Indian mustard, contributing nearly 80% of the total *rabi* oil seed production, is a vital component of edible oil sector constituting a total oilseed acreage and production in India

which is 23.7% and 26.0%, respectively (ICAR-CSSRI Vision 2030). The phytohormone auxin is one of other important factors to controls a maximum level of plant growth and development (Grossmann 2010). This phytoharmone play important role in much plant physiological activity like cell division, elongation, fruit development and senescence.

It was also reported by Phillips *et al.,* 2011 that IAA initiates roots, leaves and flowers in plants and helps to promote growth development of plants. A potential strategy to improve mustard plant growth and yield under saline conditions is the commercial application of microbial inoculants in agricultural field.

PGPR are free living bacteria having beneficial effects on plants and they can improve plant growth and yield by indirect and direct mechanisms (Gupta *et al.,* 2013).

Many of the microbes can do production of phytoharmone IAA under salt stress and the use of these kinds of microbes can improve plant growth under salt stress (Lugtenberg *et al.,* 2013a). Application of PGPR in agriculture can enhance plant's resistance under adverse environmental stresses, was reported by many of the researchers like Glick *et al.,* 2007; Dodd and Perez-Alfocea 2012; Berg *et al.,* 2013.

They stimulate plant growth promotion by mobilizing nutrients, producing many plant growth promoting hormones. The present investigation is aimed to identify and characterize potential bacterial strains from soil having salt tolerance along with IAA production as PGPR to counter the adverse effects of salinity and to enhance plant growth promotion to improved yield and sustainability in mustard crop subjected to salinity stress.

Materials and Methods

Soil sampling and screening of Salt Tolerant Rhizobacterial strains

Mustard rhizospheric saline-sodic soil samples were collected from the different places of four district of Purvanchal region *viz*. Mau, Ballia, Gorakhpur and Varanasi, U.P India was mentioned in table 1. From the rhizospheric soil sample, bacteria were isolated in nutrient agar medium followed by serial dilution method. Screening of salt tolerant bacteria was done in 10% NaCl supplemented Nutrient Agar medium plate assay and subsequently 10% NaCl supplemented Nutrient Broth Medium also. Both Nutrient Agar and Nutrient Broth cultures were incubated at 37ºC for 48-72 hrs. On plate assay growth visible bacterial colonies were recorded while bacterial growth in nutrient broth were recorded by taking OD at 600nm were found positive for salt tolerant rhizobacteria.

Phytoharmone IAA production

All the isolated salt tolerant bacterial (STB) strains were proceeds for their IAA production activity

Indole Acetic Acid (IAA) production

IAA production was performed as per description by Brick *et al.,* 1991. A 24-48 hrs old fresh bacterial broth culture was centrifuged at 10,000 rpm for 10 min. From this centrifuged culture 2 ml of supernatant was taken and subsequently 2 drop of orthophosphoric acid and Salkowski reagent (50 ml, 35% of Perchloric acid, 1ml 0.5M $FeCl_3$ solution) is added in a test tube. Further the test tubes were incubated at room temp for 30 mints for the development of the pink colour and then intensity of pink colour was measured in spectrophotometer at 530 nm.

Further concentration of IAA produced was measured with the help of IAA standard graph.

16S sequencing

An authenticated technique I6S rRNA sequence analysis is used to study bacterial isolates at species level (Imran *et al.,* 2010; Alam *et al.,* 2011). All the four amplified PCR product were outsourced for partial 16s sequencing from omega biotech limited (Kanpur (India) Pvt. All bacterial identification was performed using NCBI BLAST searches.

Pot experiment

A pot experiment was conducted using salt susceptible mustard plant var. Rohini in *rabi* season. Soil was autoclaved at 121°C for 1hr for three consecutive days and each pot was filled with 10 kg sterilized soil. A total of 18 pots including one positive control (without salt without bacteria), one negative control (with 5M salt and without bacterial treatment) and four potential bacterial isolates in 3 replications. Mustard seed (*var. Rohini)* were surface sterilized with distilled water then treated with each potential bacterial strains (50 ml i.e. ~ 1×10^7cells ml^{-1}). Negative control represented without bacterial strain treated seeds in 5M NaCl soil and the positive control represented the treatment with potential bacterial strains in normal soil without salt.

One gram of mustard seed were treated with each bacterial strain and incubated at normal temperature for 4 hrs before sowing. Treated Seeds were air dried and then sown in pots filled with soil. The pots were kept under natural conditions and plants were raised. For maintaining uniform salt stress condition throughout the growth period, plants were regularly irrigated with 5M of salt solution after seven days interval.

Results and Discussion

The present study led to characterization of Salt tolerant Rhizobacteria (STR) having IAA production for the plant growth promoting activity on mustard crop subjected to salt stress. Identified 25 bacterial isolates were identified as genus Enterobacter by using using authenticating method of 16s rRNA PCR and NCBI BLAST searches. Four potential bacterial strains B1, B14, B17 and B25 having IAA production activities ranges from 2.2 µg/ml to 3 µg/ml (Fig. 1) were selected for further Pot studies. A pot trial for salt sensitive mustard crop var. rohini was conducted in November month under natural conditions. Seeds of mustard crops were treated with the mentioned above bacterial strains B1, B14, B17 and B25 along with a control without treatment of bacterial strains.

All the pots were filled with the autoclaved soil and 5 M salt stress conditions was given in the pot with autoclaved soil. A comparative result was observed during the flowering stage when the physiological parameters of mustard crops were recorded with SPADMETER. Results shows an elevated value of chlorophyll, NBI (Nitrogen Binding Index) and flavenol in treated plants as compared to control without treatment while the value of anthocynin will remain same in all the conditions (Table 2). PGPR found in rhizospheric region can have beneficial role in plant growth promotion and their sustainability (Glick *et al.,* 1999; Gerhardt *et al.,* 2009). It was reported that the 16S rRNA gene sequencing is one of the most common methods for the housekeeping genes to study bacterial genus/species classification (Imran *et al.,* 2010; Alam *et al.,* 2011). Identification of four bacterial isolates B1, B14, B17 and B25 bacterial species at genus/species level was done by the 16S rRNA gene sequencing method and all four bacterial strains were identified as genus *Enterobacter* (Table 3).

Table.1 Soil sampling sites and GPS locations

District	Villages	GPS location	Electric conductivity of soil (µs/cm)
Gorakhpur	Pipaganj	N-26 44 53.448 E-83 22 50.988 Elevation 56m	689
	Doharighat	N-26 44 53.448 E-83 22 50.988 Elevation 56m	689
	Sahjanva	N-26 44 53.448 E-83 22 50.988 Elevation 56m	1151
	Khajni	N-25 41 20.544 E-69 28 34.068 Elevation 56m	954
Varanasi	Amra Khera	N-25 21 8.28 E-85 58 31.224 Elevation 56m	615
	Chaitipur	N-25 21 8.28 E-82 58 31.224 Elevation 56m	635
	Suswahi road	N-25 15 38.088 E-82 58 51.096 Elevation 56m	645
	Tarna	N-25 21 8.28 E-82 58 31.224 Elevation 56m	526
	Ahirana	N-25 21 8.28 E-82 58 31.224 Elevation 56m	685
Mau	Baharvara	N-25 56.535 E-083 41.059 Elevation 56m	728.58
	Haldharpur	N-25 57.967 E-083 41.609 Elevation 56m	445
	Jagbhanpur	N-25 56.535 E-083 40.915 Elevation 57m	658
	Sahupur	N-25 59.216 E-083 41.411 Elevation 98m	425
	Ghosi	N-26 06 36.00 E-083 32.240	
Ballia	Narla	N-26 06.043 E-083 56.148 Elevation 46m	825
	Nadua	N-26 06.479 E-083 56.093 Elevation 47m	978
	Narayanpur	N-26 06.265 E-083 57.823 Elevation 55m	843
	Rampur	N-26 06.880 E-083 56.306 Elevation 50m	768

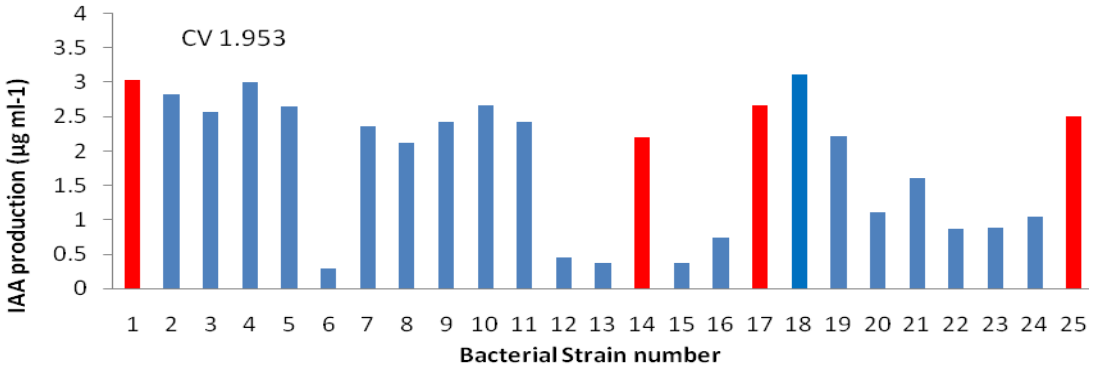
Table.2 Identification of isolated bacterial strains by partial 16s rRNA sequence analysis

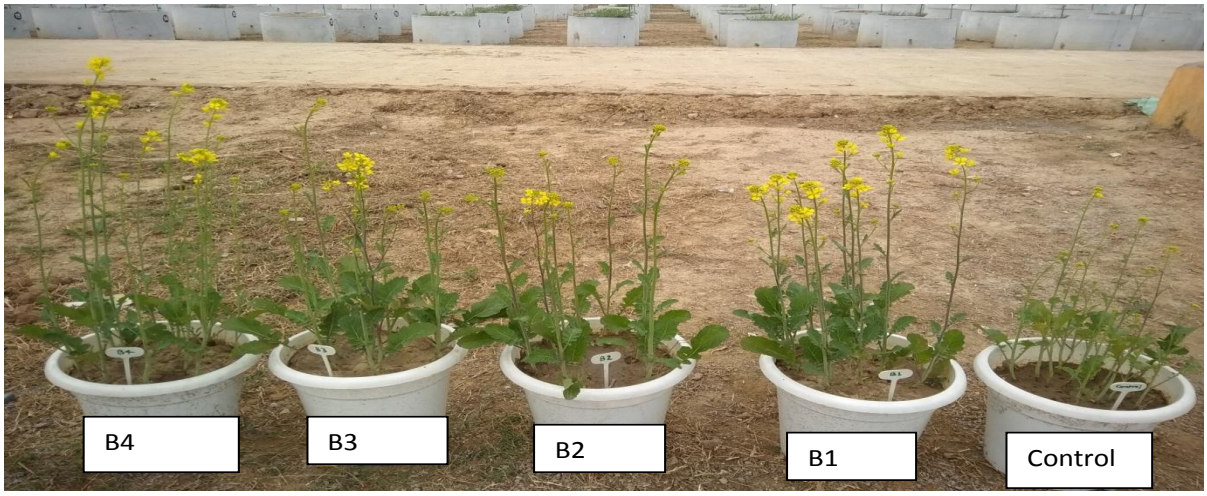
Isolate code	Genus and species	NCBI accession no.	16s r RNA sequencing
B1	Enterobacter	MK446245	Partial sequencing
B14	Enterobacter	MK463952	Partial sequencing
B17	Enterobacter	MK 478371	Partial sequencing
B25	Enterobacter	MK478386	Partial sequencing

Table.3 Identification of isolated bacterial strains by partial 16s rRNA sequence analysis

Leaf (parameter)	Control positive * (without sat and without bacterial strain)	Control Negative** (5M NaCl and no bacterial strains)	B1	B2	B3	B4 (with 5M NaCl)	C.D.	SE (m)	C.V.	F value	Significance level
NBI	24.55	15.83	19.43	18.22	17.0	19.03	2.21	0.71	6.48	17.93	1%
Chl	31.13	19.64	27.8	24.93	25.23	27.3	3.37	1.08	7.22	12.49	1%
Flav	1.27	1.24	1.43	1.37	1.43	1.43	0.08	0.02	3.19	12.57	1%
Anthocynin	0	0.01	0	0.04	0.01	0,03	N/A				

Fig.1 Mean value for IAA production by different bacterial strains





Pot B4, 3, 2 and 1 is bacteria treated plants along with control (without treatment)

Under salt stress conditions plant hormones can reduce water loss and cause a concomitant increase in leaf water potential rate (Aldesuquy and Ibrahim, 2001), so the

problem can be overcome by the PGPR having salt tolerant and IAA production activity. Production of IAA by PGPR is a relatively common trait and believed to counteract salt stress in plants. It was reported that PGPR producing different plant growth promoting phytohormones can helps to promote plant growth development (Azcon and Barea, 1975; Sharma and Kaur, 2014). In-vitro screening for the IAA production showed that all the identified four bacterial isolates have good efficiency to produce IAA. A variations in the IAA production observed by salt tolerant PGPR in the present study is consistent with earlier reports (Mansour *et al.*, 1994; Zahir *et al.*, 2000). Phytohormone Indole Acetic Acid (IAA), by PGPR is believed to support increase plant growth parameters, leading to enhanced uptake of nutrients thereby improving plant health under stress conditions (Egamberdieva and Kucharova, 2009).

It can be concluded that treatment of these salt tolerant bacterial strains with IAA application could alleviate the deleterious effect of salt stress on the growth and yield of mustard crop.

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