due to the continuous use of chemical fertilizer, the environment of soil will become infertile (marginal), (Rainey and Paul,1999).

Therefore, sustainable agriculture is vitally important in today's world because it offers the potential to meet our future agricultural needs, something that conventional agriculture will not be able to do. And so, there has been a great interest in eco-friendly and sustainable agriculture.

Soil biology in agriculture shows , plant growth promoting rhizospheric microorganisms have one or more specific associations with plants that influence plant growth. The rhizosphere microbial community is generally metabolically highly active and associated with specific plant types. Among the many mechanisms associated with plant-rhizosphere microorganism interactions, the production of biologically active metabolites is one of the most important ways that rhizosphere microbiota influence plant growth (Brady and Weil,1996).

Plant growth-promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth to describe soil bacteria that colonize the roots of plants following inoculation onto seed and that enhance plant growth. They are a group of bacteria that actively colonize plant roots and increase plant growth and yield. And are an important group of microorganisms used as biofertilizer. Biofertilization accounts for about 65% of the nitrogen supply to crops worldwide. PGPRs have different relationships with different species of host plants. The two major classes of relationships are rhizospheric and endophytic. Rhizospheric relationships consist of the PGPRs that colonize the surface of the root, or superficial intercellular spaces of the host plant, often forming root nodules. Endophytic relationships involve the PGPRs residing and growing within the host plant in the apoplastic space.

PGPR affect plant growth in two different ways, indirectly or directly. The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example phytohormones, or facilitating the uptake of certain nutrients from the environment (Glick, 2012). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick, 2012). A particular PGPR may affect plant growth and development by using any one, or more, of these mechanisms. PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as by known production of auxin phytohormone ,decrease of plant ethylene levels or nitrogen fixing associated with roots.

Bengal gram *(Cicer arietinum L.),* is one of the important pulses crop, grown throughout the country. The pulse 'Black gram' plays an important role in Indian diet, as it contains vegetable protein and supplement to cereal based diet. It contains about 26% protein, which is almost three times that of cereals and other minerals and vitamins. Besides, it is also used as nutritive fodder, specially for milch animals. Its high-yielding varieties have resulted in an increase in black gram production but requires large amounts of chemical fertilizers, leading to health hazards and environmental pollution. In order to make rice cultivation 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 some substances like indole acetic acid (IAA). that can contribute to the improvement of the plant's growth. And so, the PGPR and their interactions with plants are exploited commercially (Aziz et al.,2012) and hold great promise for sustainable agriculture.

Recently, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops (Thakuria et al.,2004). Therefore, the present study was undertaken to screen the PGPR strains for production of IAA , biological nitrogen fixation and phosphate solublization . We also investigated the effect of PGPR strains on seed germination and growth of Bengal gram (*Cicer arietinum* L.), as well.

Materials and Methods :

Sample collection : The rhizospheric soil samples were collected in sterile polythene bags on 7th August 2019 from a depth of 5.4 cm, at 07:51 a.m., 28! temperature, at 25.5809 latitude and 85.0093 longitudes from Nasri ganj- Chandi- Bihta-Piro road, Khagaul Shivala Par, Bihar 801113, India. The rice crop sample after aseptic collection was kept refrigerated until further analysis was carried out.

Isolation of bacteria from sample : The roots of rice plant were aseptically cut using sterile knife. The roots were then placed in conical flask and washed thoroughly with autoclaved tap water. The final rinsate was collected. For cultivation of Plant growth promoting Rhizobacteria, the dilutions were prepared till 10^{-6} of rinsate and were inoculated on NA media plates by spread plate method and kept at 37! for 24 hours. Then, from spread plates, single colonies were streaked on NA media plates and kept at 37°C for 24 hours in order to obtain pure cultures. The streaked plates were refrigerated at 4°C for further analysis and spot inaulation test was also performed.

***In vitro* PGPR characterization of bacterial isolates**

Biological nitrogen fixation test : The test organisms were inoculated on Burk's medium (N-free agar medium) and incubated at 37°C for 48 hrs. Appearance of growth on Burk's media indicates +ve result.

Phosphate solubilization test : The test organism were inoculated on Pikovskaya's (Pikovskaya, 1948) agar media and incubated at 37°C for 48 hrs .Appearance of zone of clearance around the growth on medium indicates +ve result.

IAA production test : A loopful of the test organism were inoculated in tryptophan broth and incubated at 37°C for 48 hrs. When growth is obtained it is centrifuged at 8000 RPM for 15 min. After, centrifugation 1ml of supernatant was taken in a test tube and 2ml of salkowski reagent was added to it. Appearance of pink color after 15min indicates +ve result, (Khakipoure, et. al.,2008).

Biochemical test : The isolates were further subjected to different biochemical tests for identification.

Starch hydrolysis ,Nitrate reduction ,catalase, ureaseindole productiopn, MP-VP etc.

Seed Germination : To determine the effect of PGPR on germination rate of Bengal gram *(Cicer arietinum L.)seeds,*100 seed were taken in sterile erlenmeyer flask for further treatment. For sterilization, seeds were soaked in 1% Sodium hypochlorite for approximately 15 min. Then, the soaked gram seeds were washed 3 times with sterilized D.W.

In second step, NA broth was prepared in erlenmeyer flask and then inoculated with starter culture (test bacteria) and kept in shaker incubator at 170 rpm for 24 hrs. Then, broth was filtered with filter paper to make the filter paper wholly moistened with the culture. The soaked gram seeds were kept in between two moistened filter papers in a petri dish. After, seed germination of gram seeds, germination rate was calculated at 24 and 48 hrs. and the length of roots was also measured.

In third step, test tubes were taken and were filled 3/4th with autoclaved soil. Then, one of the test tubes soil was left without inoculation with test culture and taken as control while others were inoculated with test cultures. Then in all the test tubes ,germinated gram seeds were sown and left for further growth and development of plant from seed. Growth was checked after every 48hrs and readings were noted till one week of ,sowing the seeds. Further statistical analysis of germination rate ,root and shoot growth was done using ANOVA.

Results and Discussion :

In this study, 5 bacterial strains were isolated from rhizopheric soil of rice plants in Patna district of state Bihar.

Bacterial strains were examined for their plant growth promoting activities .Bacterial strains were selected on the basis of colour , morphological characteristics like colony morphology (shape, margin, elevation, and surface.) and detailed study was done, (Table-1).

The total of (5) isolates were obtained where isolates R 1,R 3 and R 4 were gram positive rods while, isolates R 2 and R 5 were gram negative rods in long chains.

Table 1. The morphological and cultural characterization of isolated strains

Sl. No.	Strain	Grams stain	Shape	Color of colony	Elevation	Surface	Margin	Form	Density
1.	R1	+ve	Long rods	whitish	Convex	Mucoid	Entire	Irregular	Opaque
2.	R2	-ve	Short rods	Off white	Raised	Mucoid	Undulate	Round	Opaque
3.	R3	+ve	Short rods	Creamy white	Flat	Dry	Entire	Irregular	Opaque
4.	R4	+ve	coccus	Creamy white	Slightly raised	Mucoid	Entire	Irregular	Opaque
5.	R5	-ve	Long rods	Off white	Convex	Mucoid	Entire	Irregular	Translucent

These isolates were further subjected to invitro PGPR activity tests like IAA production test, phosphorus solublization test, nitrate reduction test for PGPR properties (Kloepper and Joseph, 1980).

Each isolate was plated on pikovskaya's Agar medium (selective media) for isolating phosphorous solublizing bacterial (PSB) species . Inoculated plates were incubated aerobically at 37°C for 5-6 days. Clear zone appearance indicate positive results. Out of 5 isolates ,only one isolates i.e isolate R5 showed positive result (Table 2).

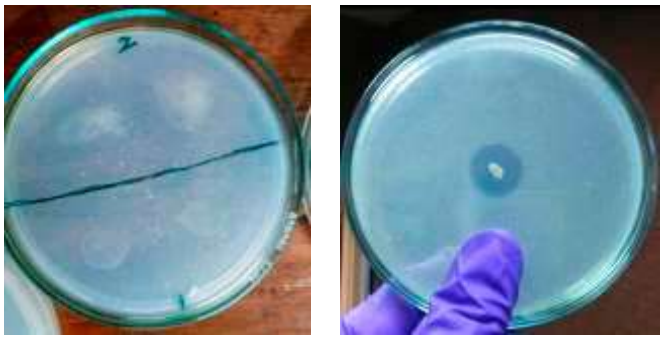
All the 5 isolates were then subjected to IAA production test ,where, Out of 5 isolates 3 gave positive results (pink colour appearance in supernatants of inoculated nutrient broth on adding Salkowski Reagent). The other two strain showed moderate to low results, which is considered as negative .Production of IAA by bacillus is a general characteristics of our test isolates. Higher levels of IAA production by *pseudomonas* was recorded by other researchers. The ability of the bacteria to produce IAA in the Rhizosphere depends on the precursor available and the ability of the plant to uptake microbial IAA.

Isolates were also subjected to Biological Nitrogen Fixation test using Burk's media. This media is recommended for detection of nitrogen fixing organisms from soil. Burk's media contain inorganic salts along with carbohydrate source but lacks nitrogen source .Nitrogen fixing bacteria grows on this medium by fixing nitrogen (Fig. 1).

All the 5 isolates, i.e R1, R2, R3, R4 and R5 grew on this media showing positive results.

Table 2 . Characterization of PGPR traits

Test name	Isolate				
	R1	R2	R3	R4	R5
PO_4solublization	-ve	-ve	-ve	-ve	+ve
IAA Production	+ve	+ve	-ve	-ve	+ve
Nitrogen fixation	+ve	+ve	+ve	+ve	+ve



(a) (b)

Fig. 1. (a) Isolates showing +ve growth on Burk's nitrogen free media, (b) Isolate, R5 showing halo zone formation on Pikovskaya's agar media

And next, biochemical characterization was further done and shown in Table 3.

Table 3. Biochemical characterization of bacterial isolates from rice rhizosphere

Sr. No.	Tests	Isolate				
		R1	R2	R3	R4	R5
1.	Starch hydrolysis	+ve	-ve	+ve	+ve	-ve
2.	Nitrate reduction	+ve	+ve	-ve	+ve	+ve
3.	Catalase	+ve	+ve	+ve	+ve	+ve
4.	Urease	-ve	-ve	-ve	-ve	-ve
5.	H_2S production	-ve	-ve	-ve	-ve	-ve
6.	Indole production	+ve	-ve	-ve	+ve	+ve
7.	MR	-ve	-ve	-ve	-ve	-ve
8.	VP	+ve	+ve	+ve	+ve	+ve
9.	Citrate utilization	+ve	+ve	+ve	+ve	+ve

Further we performed the seed germination test, from the isolated strains on Bengal gram (*Cicer arietinum* L.) seeds. The surface sterilised seeds (by 3% sodium hypochlorite), overnight soaked, were inoculated with culture broth on filter paper placed in petri plates.

After one day the difference in germination rate was observed, in the seeds, which were grown in presence of these isolated strains to the ,seeds which was grown in absence of any isolates (control) and germination percentage was calculated, and shown in Table 4.

A comparative study was also done where, seeds were inoculated with isolated plant growth promoting bacterial strain and the gram seeds without inoculation was taken as control were sown in sterilized soil. After 7-8 days ,significant increase in both the root and shoot was observed. The growth character of different isolates are variable and it was shown maximally by the strain R5 as shown in Fig. 2. Overall, the effect was more pronounced in shoot(in terms of length) than in roots.

The study showed that seed treatment of bengal gram with selected isolates benefitted the plant during the early growth stages by increasing seed germination, and root lengths of seedlings, as well as shoot length of the plant. Increases in early seedling growth due to seed treatment with bacterial strains have also been reported in other grains such as wheat (Shaukat et al., 2006) and rice (Chithrashree et al., 2011). The percentage germination of gram seeds can be improved by up to 7.5-12.7%, which is comparable with the percentage germination of untreated seeds.

Similar results have been reported in earlier studies on pearl millet in which seed germination was enhanced by up to 8% due to seed treatment with two Bacilli strains (Niranjan et al., 2003). Increased vigour improves a seedlings ability to withstand infections by pathogens and survive under harsh environmental conditions (ISTA, 2014).

Seed treatments which increase germination and vigour could be important for carryover seeds that have been stored for a long time, since seed ageing is one of the major aspects influencing seed quality (Powell, 1998).

Table 4. The effect of PGPR on seed germination and growth of Gram seeds

Sr. No.	Isolate	Seed Germination (%)		Avg. Root (radicle) Length (cm)
		Day 1	Day 2	
1.	Control	55	70	2.4 cm
2.	R1	60	74	2.2 cm
3.	R2	70	82	2.9 cm
4.	R3	62	78	2.5 cm
5.	R4	69	74	3.2 cm
6.	R5	75	90	2.1 cm

Where the isolate R2 and isolate R5 showed the longest length of radicle as well as highest germination rate.

Fig. 2. Effect of inoculation on plant growth

Table 5. Effect of PGPR on plant growth at interval of 2 days

Isolate	Shoot growth (cm)			
	Day 2	Day 4	Day 6	Day 8
Control	2 cm	5 cm	9 cm	12 cm
R1	3 cm	5 cm	10 cm	14 cm
R2	4 cm	9 cm	12 cm	18 cm
R3	2 cm	5 cm	11 cm	15 cm
R4	2 cm	6 cm	13 cm	16 cm
R5	5 cm	8 cm	14 cm	20 cm

Significant increase in rate of seed germination was observed as compared to control (Fig. 3).

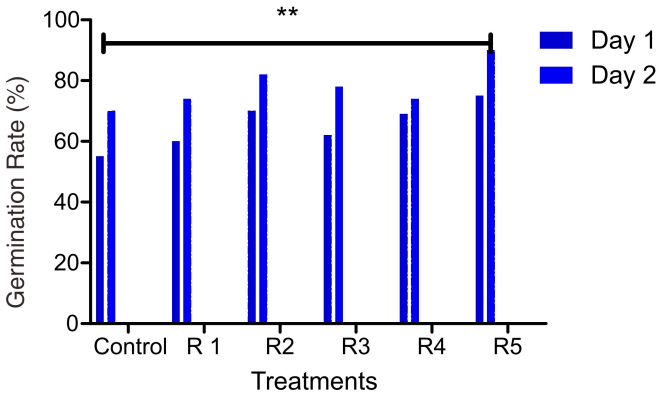


Fig. 3. Graph of statistical analysis of germination rate

Similarly significant difference in increase of root growth and shoot growth was also observed on bacterial inoculationas as shown in Fig.4 and 5.

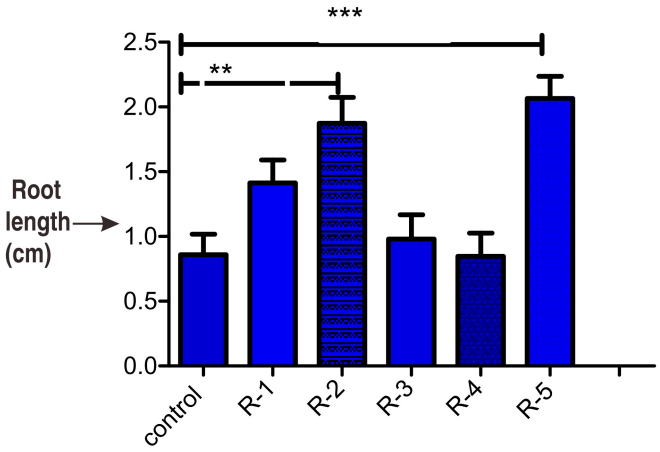


Fig. 4. Graph of statistical analysis of root growth

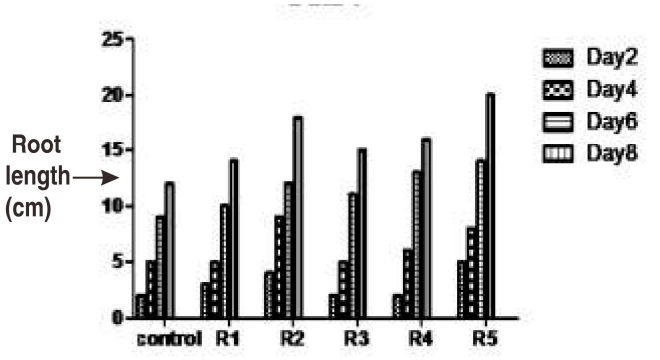


Fig. 5. Graph of statistical analysis of shoot growth

Therefore, these isolated strains have some growth promoting activity and can be used as inoculant biofertilizers, which might be beneficial for crop cultivation as they enhanced growth of black gram (seeds) ,and induced IAA production and phosphorus solubilization and biological nitrogen fixation.

Conclusion:

Thus, on the basis of above research work and biochemical tests result, we can conclude that the isolates R1, R3 and R4 may be of *Bacillus sp.*; isolates R2 and R5 may be of *Pseudomanas sp.* And further species specification can be done by 16sRNA.Thereby, briefly shows the characteristics of the IAA production ,nitrogen fixation and phosphate solubilisation.

Furthermore, these isolates remarkably increased seed germination rate, plant root and shoot growth of bengal gram. Further aspects can be used as the growth promotor in rice as well as other crops and can be used as superior bio fertilizer. Thus, Plant growth promoting rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. As the increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism.

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