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INFLUENCE OF ISOLATED PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON THE GROWTH OF BRASSICA JUNCEA L.

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ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by wide variety of mechanisms which includes direct and indirect mechanisms like nitrogen fixation, phosphate solubilization, phytohormone production, antifungal activity, etc. Eleven bacterial isolates were identified from rhizosphere of *Brassica juncea L.* using morphological, biochemical and molecular analysis. The isolates were designated as AN1, AN2, AN3, AN 4, AN5, AN6, AN7, AN8, AN9, AN10 and AN11. Subsequently to investigate the effects of PGPR isolates alone and in two combinations named consortium (Cons. 3) and consortium (Cons. 4) were made for combination studies in *Brassica juncea L.* along with absolute control. Isolated PGPRs showed significant difference among seed germination percentage (GP) and germination index (GI) over to controls. The treatments with AN4, AN5, AN6, Cons. 3 and Cons. 4 play significant role in enhancement of shoot length in all developmental stages of the plant growth along with fresh weight of the plant. AN4, AN5, Cons. 3 and Cons. 4 showed significant increase in root length, weight and fresh pod weight of Brassica juncea. These results show the potential to use selected PGPRs in order to improve yields in *Brassica* crop.

KEYWORDS: PGPR, *Brassica juncea L.*, rhizosphere, isolates, Consortium.

INTRODUCTION

Rhizobacteria is the vital components of the soil which enriches the soil to be fertile enough to be productive. Roots are the lifeline of a plant, which takes up essential elements viz. air, water, and nutrients from the soil and transporting them up to the leaves and stems, where

they can interact with sunlight to produce sugars, flavors, and energy for the growth and development of the plant. Further, roots also secrete compounds that affect the microorganisms in the soil in general and PGPR in particular and protect the plant from disease and encourage it to absorb nutrients from the soil. There is a whole world of activity underground carried out by roots. The narrow zone of soil directly surrounding the root system is referred as Rhizosphere. It is populated with diverse range of microorganisms and the colonizing bacteria are named rhizobacteria. The rhizosphere, microbiota can contain up to 10^{11} microbial cells per gram of root (Egamberdieva, et al., 2008) and more than 30,000 prokaryotic species (Mendes et al., 2011). A group of biofertilizers contains beneficial, free-living soil-borne bacteria that colonize the rhizosphere, and when applied to seed or crops enhance the growth of plants either by direct or indirect mechanisms, termed as plant growth-promoting rhizobacteria (PGPR) (Kloepper J. W. et al. 1980; Glick B.R., 1995). The utilization of PGPR is steadily enhanced in agriculture as it is an attractive substitute of chemical fertilizers, pesticides, and supplements. Preparations of live microorganisms (bacteria, fungi) utilized for improving plant growth and crop productivity are generally referred to as biofertilizers or microbial inoculants. Rhizobacteria increase the availability of nutrients through the solubilizing unavailable forms of nutrients which aids in the facilitation of nutrient transport. The PGPR gain importance after a large number of bacterial strains have been isolated, screened (Chanway C.P., Holl F.B., 1993; Bertrand H. et al., 2001) and evaluated for plant growth promotion (Lifshtiz R. et al., 1987; Chanway C.P. et al., 1989; Abbas Z., Okon Y., 1993; Glick B. R. et al., 1997; Bashan Y., Holguin G., 1998; Bent E. et al., 2001; Salamone I.E.G., 2000).Indirect stimulation is the ability to reduce the deleterious effects of soil born pathogenesis on crop yield such as suppression of phytopathogens by producing siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens (Glick B.R., 1995; Kloepper J. W. et. al., 1988; Liu L. et. al., 1995; Glick B. R. et al., 1999) while the direct stimulation occurs due to the fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Glick B.R. et. al., 1997; Cartieaux F. P. et. al., 2012). Bertrand H. et al., (2001) and many other researchers identified bacteria belonging to the genera Pseudomonas, *Varivorax Agrobacterium* and *Phyllobacterium* as the most efficient PGPR associated with different plants or crops mineral uptake (James E.K., Olivares F.L., 1997). Modern agriculture

system is substantially increasing the application of rhizospheric microorganisms and aims at mutual relationship between agricultural productivity and conservation of nature. *Brassica juncea* is primarily grown for edible oil which catrers the need of fat and lipids. It is highly used in Indian cooking culture due to its good taste and high smoking point.

In present research work isolated PGPR from *Brassica juncea L* were used to study their effect on the germination and growth of the plant.

MATERIAL AND METHODS

Rhizospheric soil samples had been collected from the fields of Brassica juncea L. grown and cultivated in Meyar village of Bihar. Isolated bacterial strains were identified and tested for their PGPR activities alone and in combination through pot experiments. Identification and characterization of selected isolate were done by using morphological, physiological, biochemical and 16srRNA sequencing methods. AN1 was identified as *Proteus vulgaris*, AN2 as *Escheria coli*, AN3 as *Staphylococcus aureus*, AN4 as Achromobacter xylosoxidans, AN5 as *Lysinibacillus macroides* partial AN6 as *pseudomonas aureginosa*, AN7 as *Bacillus subtilis*, AN8 as *Proteus mirabilis*, AN9 as *Achromobacter sp*. AN10 as *Streptoccus pyogens*, AN11 as *Enterococcus faecalis*.(Sinha T., Jee C., 2018) *Brassica juncea L*. seeds were used as plant materials. Seeds of *Brassica juncea* were sterilized in 3% sodium hypochlorite ($NaOCl_2$) for 5 minutes, for surface sterilization and then, washed with sterilized distilled water at least 5 times to remove traces of toxic $NaOCl_2$. After air drying seeds were subjected to filter paper assay for germination studies. 48h old culture of different bacterial isolates was prepared in nutrient broth. 50 seeds of *Brassica juncea L*. were dipped in 48h old cultures for 6 h (Walia, 2008). Treated seeds were placed between the layers of moist filter paper in glass Petri dish at 27 °C with a cycle of 10 h dark followed by 14 h of light. The Petri dishes were arranged in a Complete Randomized Design (CRD) with three replicates. Starting on the first day of imbibition, counts of germinating seeds were made at 24 h intervals till maximum germination was attained. Germination percentage (GP) and germination index (GI), which indicates the seeds of germination of seeds. These were estimated in accordance with the Association of Offcial Seed Analysis (AOSA, 1990). Germination per cent was calculated as the number of seeds plated and the number of seeds germinated expressed in percentage. The GI is the more comprehensive measurement parameter combining both germination percentage and speed.

GP= no. of seeds germinated / no. of seeds plated X100

$$GI = \frac{\text{no. of germinated seeds}}{\text{Day of first count}} + \ldots\ldots + \frac{\text{no. of germinated seeds}}{\text{Day of last count}}$$

After germination studies green house pot studies were performed. Plastic pots of size 13 X 12 X 5 inches were filled with sieved clay loamy soil (< 2 mm) having pH 7.1. Treatments included isolated PGPR alone as well as in combination of two or more PGPR strains. Two combinations named CONS. 3 and CONS. 4 were made for studies in Brassica juncea L. CONS. 3 consist of AN4 and AN5 and CONS. 4 consists of AN6 and AN9. The study also includes an absolute control with only soil that is without PGPR. The experiment was conducted in three replications. Seeds of Brassica juncea L. were treated with a broad range of fungicide carbendazim at 2 g kg-1 before treatment with PGPR suspension. Seeds were soaked in 1% starch solution containing respective PGPR suspension (~X10^7cfu mL-1) for 1h shade dried for 24 h and sowed @ one seed per pot.

All the pots were applied with major nutrients at rate equivalent to the recommended dose of NPK for Brassica juncea L. which was 60-30-30kg $ha^{-1.}$ The inorganic sources of NPK used were urea, rock phosphate and muriate of potash respectively. Three replications were maintained for each treatment.

Plant characteristics Studies like Root length, Shoot length, fresh weight and weight of pods were observed. The data collected on various parameters under laboratory and green house conditions were subjected to statistical analysis as per standard methods. The statistical analysis was done using OPSTAT (Sheoran, 1998) and expressed as the mean of three independent replications ± standard error (SE) of each mean along with Analysis of Variance (one way ANOVA) at the $p < 0.05$ significance level. Data were analyzed with the help of Critical Difference (CD) and used to compare means of different treatments that have an equal number of replications. Positively significant difference is greater than CD.

RESULTS AND DISCUSSION

Seed germination studies are the first and most common techniques used for studying the effects of PGPR isolates on growth and development of crops. Germination percentage and germination index were calculated and the experimental findings are presented in Table 1 and

table 2 given below. The germination index calculated for the different isolates ranged between 8.58 to 17.08. The lowest and highest value of GI was found to be of the isolate AN3 and AN6 respectively.

Table 1: Germination Observations at 48 h intervals and GI of (Brassica juncea L.)

Treatments	Day 2	Day 4	Day 6	Germination Index (GI)
AN1	0	15	32	9.08
AN2	0	16	31	9.16
AN3	0	15	29	8.58
AN4	0	35	45	16.25
AN5	0	37	46	16.91
AN6	0	39	44	17.08
AN7	0	33	41	15.08
AN8	0	16	30	9.0
AN9	0	29	43	14.41
AN10	0	19	36	10.75
AN11	0	20	38	11.33
Control	0	24	44	13.33
Cons. 3	0	25	46	13.91
Cons. 4	0	25	45	13.75

Table 2: Germination Observations after 6 days and germination percentage Brassica juncea L.) (n= 50 seeds per replication).

Treatments	R1	R2	R3	Mean± S.E	Total no. of germination	% germination
AN1	32	33	30	31± 0.67	95	63.33
AN2	31	31	28	30 ± 1.0	90	60
AN3	29	28	28	28.33±0.33	85	56.66
AN4	45	46	44	45 ± 0.57	135	90
AN5	46	45	47	46 ± 0.57	138	92
AN6	44	41	45	43.33 ± 1.2	130	86.66
AN7	41	40	40	40.33±0.33	121	80.66
AN8	30	32	31	31 ± 0.57	93	62
AN9	43	41	42	42 ±0.57	126	84
AN10	36	39	40	38.33 ±1.2	115	76.66
AN11	38	40	39	39 ±0.57	117	78
Control	44	46	45	45 ± 0.57	135	88
Cons. 3	46	48	47	47 ± 0.57	141	94
Cons. 4	45	47	46	46 ± 0.57	138	92

The observations for the shoot length, measurement was made on 10 DAS (Establishment stage), 30 DAS (young stage), 60 DAS (flowering stage), 90 DAS (fruiting stage) and 120DAS (maturity of the plant). The observation made and the data obtained helped in

understanding the growth behavior of B. juncea at different stages of plant growth arepresented below.

Treatment	10DAS	30DAS	60DAS	90DAS	120DAS
AN1	4.6±0.05	16.26 ± 0.24	79±0.61	139.06 ±0.57	165.86±0.17
AN2	3.86±0.03	14.63 ± 0.08	71.86±0.17	132.60±0.30	161.26±0.14
AN3	3.70±0.05	14.43 ± 0.08	64.53±0.37	128.13±0.17	160.66±0.23
AN4	6.16±0.03	25.56 ± 0.47	94.86±0.17	155.46±0.35	176.46±0.24
AN5	6.83±0.03	26.56 ± 0.21	96.13±0.17	156.80±0.11	177.33±0.24
AN6	5.73±0.03	24.10 ± 0.15	93.2±0.23	153.26±0.34	176.33±0.27
AN7	5.06±0.03	20.96 ± 0.41	87±0.70	150.46±0.23	172.90±0.20
AN8	3.63±0.03	14.96 ± 0.27	74.66±0.63	137.26±0.57	163.73±0.28
AN9	5.30±0.11	22.93 ± 0.29	90.06±0.63	152.40±0.30	174.66±0.35
AN10	4.76±0.06	17.46 ± 0.55	82.33±0.17	143.46±0.23	169.53±0.17
AN11	5.20±0.11	19.40 ± 0.70	85.53±0.48	148.80±0.46	170.63±0.32
Control	6.26±0.14	25.86 ± 0.29	93.06±0.43	155.13±0.23	176.00±0.11
Cons. 3	6.86±0.08	27.20 ± 0.70	98.33±0.17	160.00±0.50	179.06±0.22
Cons. 4	6.70±0.05	27.53 ± 0.69	97.46±0.24	158.93± 0.521	178.80±0.11
CD at 5% level of significance	0.215	1.246	1.232	1.111	0.681
Cv	2.402	3.482	0.849	0.446	0.236

The observation of the shoot length at maturity of Brassica juncea was recorded at 176.00 cm in the control plant. Five treatments (AN4, AN5, AN6, Cons. 3 and Cons.4) recorded better shoot length compared to control, whereas eight treatments (AN1, AN2, AN3, AN7, AN8, AN9, AN10 and AN11) were recorded lesser shoot length than the control. The analysis of the data for shoot length growth at different intervals of Brassica juncea indicated that the treatments with AN4, AN5, AN6, Cons 3 and Cons 4 play significant role in enhancement of shoot length in all developmental stages of the plant growth.

Table 4: Crop growth parameters studies in pots under glass house conditions for Root Length (cm) with Brassica juncea crop at maturity stage (120 das).

Treatment	R1	R2	R3	Mean
AN1	17.4	17.2	17	17.20±0.11
AN2	16.4	16.2	15.8	16.13±0.17
AN3	15.6	15.4	14.8	15.26±0.24
AN4	20.8	20	20.2	20.33±0.24
AN5	21	20.8	20.6	20.80±0.11
AN6	20.2	19.8	19.6	19.86±0.18
AN7	18.6	18	18.2	18.26±0.17
AN8	17.2	16.8	16.6	16.86±0.18
AN9	19.6	18.8	18.8	19.06±0.26
AN10	14.8	14.6	14.2	14.53±0.17

AN11	18.2	17.8	17.6	17.86±0.17
Control	19	18.8	18.6	18.80±0.11
Cons. 3	22.2	22	21.8	22±0.11
Cons. 4	21.7	21.8	21.5	21.67±0.88
CD at 5% level of significance				0.513
CV				1.651

The root length in average in Brassica juncea experiment was recorded at 18.80 cm in control. However, five treatments (AN4, AN5, AN9, Cons. 3 and Cons. 4) recorded higher root length than the control. However, lower than the control in root length were found in eight treatments (AN1, AN2, AN3, AN6, AN7, AN8 AN10 and AN11).The maximum root length was recorded was 22 cm in Cons. 3 and the minimum in 14.53 cm in AN10. The comparative study of the two growth parameters i.e. root length and shoot length showed similar trends, whereas in treatments AN4, AN5, AN6, AN9, Cons. 3 and Cons.4 showed significantly better performance for both the parameters for plant growth.

Beside, shoot and root length the general growth and development of the plant also play significant role in overall production. Therefore, under this project the behavior of the fresh root, shoot and pod weight was observed in all the treatments in order to access the comparative role of various treatments in qualitative and quantitative development of the total plant morphology. Table no. 25 presents the mean value of three replicates for Shoot fresh weight, Root fresh weight, Pod weight and Total plant weight in gram for Brassica juncea.

Table 5: Mean data for Shoot, Root and Pod Weight for Brassica juncea, 2017-18.

Treatment	Shoot Fresh weight(g/ plant)	Root Fresh weight g/ plant)	Total plant weight	Pod weight g/ plant)
AN1	318.33	26.50	344.83	111.7
AN2	263.00	22.13	288.03	113.8
AN3	256.33	23.03	278.46	133.6
AN4	461.33	39.33	500.66	202.3
AN5	462.33	39.70	502.03	200.9
AN6	459.00	37.33	497.66	197.9
AN7	427.33	35.73	463.06	184.2
AN8	283.00	25.43	308.43	99.2
AN9	457.33	37.80	495.13	195.3
AN10	364.00	28.46	392.46	155.6
AN11	407.33	29.50	436.83	187.6
Control	458.66	38.20	496.86	199.7
Cons. 3	467.66	42.40	510.06	207.5
Cons. 4	465.00	42.73	507.73	205.3

The maximum and minimum fresh shoot weight was recorded in Cons. 3 and AN3 respectively. The fresh shoot weight for the control was found to be 458.66 g. Five treatments recorded higher than the control in fresh shoot weight i.e. (AN4, AN5, AN6, Cons. 3 and Cons. 4) while eight treatments recorded lower than the control in fresh shoot weight i.e. (AN1, AN2, AN3, AN7, AN8, AN9, AN10 and AN11), indicating that seed treatment with AN4, AN5, Cons. 3 and Cons. 4 have significant positive effect in enhancing the fresh shoot weight of the plants.

Similarly, in respect of fresh root weight significant difference was observed in Brassica juncea in different treatments. The fresh root weight recorded ranged between 22.13 g to 42.73 g. The maximum and minimum fresh root weight was recorded in Cons. 4 and AN2 respectively. The fresh root weight for the control was found to be 38.20 g. four treatments recorded higher than the control in fresh root weight i.e. AN4 (39.33 g), AN5(39.70 g), Cons. 3 (42.40 g) and Cons 4(42.73 g). Nine treatments (AN1, AN2, AN3, AN6, AN7, AN8, AN9, AN10 and AN11) recorded lower than the control in fresh root weight.

Further the total fresh plant weight was also recorded. In fresh plant weight, significant difference was observed in Brassica juncea with different treatments. The total fresh weight of plant recorded ranged between 278.46 to 510.06 g. The maximum and minimum plant weight was recorded in Cons. 3 and AN3 respectively. The fresh weight for the control was found to be 496.86 g. Five treatments recorded over than the control in fresh weight i.e. AN4 (500.66 g), AN5 (502.03 g), AN6 (497.66 g), Cons. 3 (510.06 g) and Cons. 4. (507.73). Eight treatments (AN1, AN2, AN3, AN7, AN8, AN9, AN10 and AN11) recorded below than the control in fresh weight, emphasizing that seed treatment with AN4, AN5, AN6, Cons. 3, Cons. 4 have significant positive effect in enhancing the fresh weight of the plants.

The analysis of the data presented in above table showed a significance difference in pod weight of Brassica juncea in different treatments. The fresh pod weight recorded, varied between 111.7 g to 207.5 g. The highest and lowest fresh pod weight was recorded in Cons. 3 and AN1 respectively. The fresh weight for pod in the control was found to be 199.7g. Four treatments recorded higher than the control i.e. AN4, AN5, Cons. 3, Cons. 4 Nine treatments recorded lower than the control i.e. AN1, AN2, AN3, AN6, AN7, AN8, AN9, AN10 and AN11 suggesting that seed treatment with AN4, AN5, Cons. 3, Cons. 4 have significant positive effect in enhancing the fresh pod weight of the plants.

The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial (Kloepper J.W. et. al., 1989). Many researchers (Lifshtiz R. et al., 1987; Chanway C. P. et. al., 1989; Abbas Z., Okon Y., 1993; Glick B. R. et al., 1997; Bashan Y., Holguin G., 1998; Bent E. et al., 2001) records the ability of microbial inoculants, to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease. This present investigation confirms the earlier works. It revealed that use of PGPRs with seed treatment improve seed germination, seedling emergence, seedling vigor and seedling stand over the control.

5. CONCLUSION

In conclusion, it was found that all the bacterial isolates were significantly affecting root and shoot length and biomass production of Brassica juncea. Further, the result also indicated that co-inoculation of PGPR in form of Consortium significantly enhances all the parameters of germination and growth in both the crops in comparison to control and mono inoculation. These novel information generated through in depth research investigations have added our knowledge on effects of PGPR on development of the significant oil crop *Brassica juncea* popularly and extensively cultivated in Indogangetic plains. It may go long way in be replacing chemical fertilizers thereby maintaining healthy environment and ensuring agricultural sustainability. This needs investigations, including efficiency test under field conditions, are needed to clarify the role of PGPR as bio fertilizers that exert beneficial effects on plant growth and development. The extended research work may include biochemical characterization and role of the secondary metabolites derived from the PGPR strains, Identify different mechanisms of action which facilitate the combination of strains, device formulation of bioinnoculants and development of transgenic plants that having multiple mechanisms of action of PGPR strains.

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