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PLANT GROWTH PROMOTING RHIZOBACTERIA AND THEIR ACTIVITY AGAINST EARLY BLIGHT OF TOMATO

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ABSTRACT

Early blight caused by *Alternaria solani* has been known to cause severe yield losses in tomato. Hence, attempts were made to develop an effective ecofriendly strategy to manage the disease using plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria (PGPR) are a group of beneficial bacteria that can be found in the rhizosphere, in association with root which can enhance the growth of plant directly or indirectly by several mechanisms. In-vitro and field experiments were conducted to evaluate the efficacy of different PGPR i.e., TR22, TR25, TR21, TR15, TR18 and TR24 against Early blight of tomato. These bacterial isolates were screened in-vitro for different plant growth promotion activities i.e. phosphate solubilization, IAA production, siderophore production. Five bacterial isolates were positive for IAA production and siderophore production and one (TR 25) isolate was found to be positive for phosphate solubilization. Most of the isolates were grown best under the temperature of 20°C & 28°C when compared to 10°C & 37°C and none of them grew at 45°C, Furthermore, most of the PGPR isolates shown antifungal activity against A. s1olani. The highest degree of mycelium inhibition was observed with TR22 which inhibited 53.28 % colony growth of A. solani, followed by TR 25 (36.25%). Minimum % inhibition was found in TR24 (19.94 %). In field evaluation TR25 was found most effective showing least per cent disease index of 7.33 per cent as against 21.33 percent in control, followed by TR22 and TR21 in 2011-12. Similar data was also found in 2012-13. Highest yield (252.67 q/ha) was recorded in TR25 followed by TR22 and TR21 in 2011-12 while it was reduced a little bit in 2012-13.

KEYWORDS : Tomato, PGPR, Siderophore production, Phosphorous solubilization, Rhizosphere

Alternaria solani (Ellis and Martin) Sorauer is an important pathogen causing early blight disease in tomato. This disease, which in severe cases can lead to complete defoliation, is most damaging on tomato [*Solanum lycopersicum* L.(Peralta et al., 2005,syn. Lycopersicon esculentum Mill.)] in regions with heavy rainfall, high humidity, and fairly high temperatures (24°29°C). Yield losses up to 79% from early blight damage have been reported from Canada, India, the United States and Nigeria (Datar and Mayee, 1981).

Over the last decades, world agriculture experienced high increase in crop yield. This was achieved through high input of inorganic fertilizers and pesticides, and mechanization driven by fossil fuel. Over the years this led to serious environmental problems such as depletion of soil quality and health, ocean and ground water pollution, and emergence of resistant pathogens. It is a big challenge to feed the increasing world population on decreasing farmland areas without damaging environment. The utilization of potential microflora may help to develop an ecofriendly control strategy for disease management. It is well known that rhizosphere and soil microorganisms (PGPR) play an important role in maintaining crop and soil health through versatile mechanisms: nutrient cycling and uptake, suppression of plant pathogens, induction of resistance in plant host, direct stimulation of plant growth (Kloepper et al., 2004) Plant Growth Promoting Rhizobacteria (PGPR), especially Pseudomonas fluorescens (Pf1, Py15 and Fp7) strains have been developed commercially as a talc based formulation and tested against several crop diseases (Kavino et al.,2007)

Several approaches have been tried for the sustainable management of early blight in tomato. However, no attempts have been made for the management of early blight disease with PGPR strains. Therefore, the present study was designed to evaluate protective effect of PGPR strains (Pseudomonas spp.) against tomato early blight disease caused by *A. solani*.

MATERIALS AND METHODS

Isolation of PGPR from Tomato Rhizosphere

Soil sample were collected from the rhizosphere of tomato plant in the areas of Indian Institute of Vegetable Research, Varanasi. The rhizosphere was dugout with interact root system. The sample were placed in plastic bags and stored at 4°C in refrigerator. Ten grams of rhizosphere

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soil were taken in a 250 ml of conical flask and added 90 ml of sterile distilled water to it. The flask was shaken for 10 min on rotatory shaker. One milliliter of suspension was added to 10 ml vial and shaken for 2 min. Serial dilution technique was performed up to 10-7 dilution. An aliquot (0.1) of this suspension was spread on the plates of Luria Bertany (LB) agar medium. Plates were incubated for 3 days at 28°C for observing the colonies of bacteria. Typical bacterial colonies were observed over the streak well isolated single colony was picked up and re-streaked to fresh LB agar plates and incubated similarly.

Growth of PGPR Under Different Temperature Conditions

The culture of 6 isolates was streaked on LB agar plates and incubated at 10°C, 28°C, 37°C and 45°C the change in growth and color was observed and recorded after 3days of incubation.

In-*vitro* Screening of Different Isolates for PGPR

Phosphate solublization

The cultures of six isolates were spot inoculated on Pikovskaya's agar plates for phosphate solubilization under aseptic conditions and incubated for 3 days at 30°C. The appearance of clear halo zone on Pikovskaya's agar plates showed positive phosphate solubilization ability as described by Gaur (1990).

lAA Production

Indole acetic acids (IAA) production was carried out as described by Brick et al. (1991). The culture of 6 isolates was incubated in the nutrient broth enriched with 50 mg/ml of L-tryptophan at 30°C for 48 h. Fully grown cultures were centrifuged at 8000 rpm for 10 min. The supernatant (2ml) was mixed with two drops of orthophosphoric acid and added 4 ml of the Solkowski reagent (50 ml. 35% of perchloric acids, 1 ml 0.5 M $FeCl_3$ solution). The tubes were kept at room temperature for 20 minutes. Development of pink color indicates IAA indicates IAA production.

Siderophore Production

Siderophore production was tested qualitatively using chromo azurol S medium (CAS- medium The culture of 6 isolates were streak on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by orange halo around the colonies after the incubation and these was done in three replications.

Antagonism Activity Against *A. solani*

All the Six isolates were assayed for antifungal activities against Alternaria solani by using potato Dextrose Agar (PDA) medium. The isolates (PGPR) were streaked on PDA medium 3 cm in distance opposite to pathogenic fungi inoculated in the medium. The barrier between isolates and fungi indicated antagonistic interaction between them. Antagonist activity was investigated 7 days after incubation at room temperature. The value of inhibition zone was measured using the following formula.

The percent inhibition was calculated using the formula

$$= \frac{(R-r)}{R} \times 100$$

Where, PI = Percent inhibition

R = Radial growth of pathogen in control plate

r = Radial growth of the fungal colony opposite the bacterial colony

Preparation of Selected PGPR Bioformulation

A loopful of selected bacterial isolates were inoculated in to the sterilized KB and Nutrient broth respectively and incubated in a at shaker at 150 rpm for 48 h of incubation, the broth containing 9×108cfu / ml was used for the preparation of talc-based formulation. 400 ml of bacterial suspension added 1 kg of talc powder (sterilized at 12h), calcium carbonate 15 g (to adjust the PH to neutral) and Carboxyl Methyl Cellulose (CMC) 10 gm as Co adhesive) were mixed under sterile conditions, After shade drying overnight, it was packed in polypropylene bag and sealed, at the time of application the population of bacteria in talc formulation was not less than 2.5 - 3×108 cfu/gram.

Effect of Different PGPR on the % Disease Index of *A. Solani* Under Field Conditions

Susceptible genotype (DVRT-1) of tomato seedlings were raised in nursery beds and 30 days old seedlings were transplanted into the main field. The talc based product was dissolved in water (10 g/l) and allowed to settle for 1 hour, filtered through with muslin cloth. The

roots of 30 days old tomato seedlings were washed several times with sterile distilled water and dipped in cell suspension of different isolates of PGPR individually for five minutes. Samples with roots dipped in sterile distilled water were used as controls. Distance between row to row and plant to plant was 60 and 50 cm in 3m ×2 m plot size. One week after transplanting, plants of tomato were challenged with the spore suspension (5×106 spores/ml) of *A. solani*. The investigation was carried out at the experimental farm of Division of Vegetable Protection, Indian Institute of Vegetable Research, Varanasi, during the main cropping seasons of (Rabi) 2011-12 and 2012-13.

Ten days of inoculation, observation on development of early blight symptoms were maintained for each treatment; each replication consisted of 3 plots and in each plot carried twelve plants, were maintained in Randomized Block Design under field's conditions in 2011-12 and 2012-13. Twenty plants were selected randomly in each field and observation on disease severity recorded individually using 0-5 rating scale based on leaf area, stem and fruit covered by blight symptoms following the rating scale described by Pandey et al. (2003). Disease incidence was calculated on the basis of per cent of infected leaves and stem. Percentage disease index (PDI) was calculated as follows:

PDI = Sum of all rating × 100 / Total no. of observations × Maximum rating grade.

RESULTS

Six bacterial isolates were successfully isolated from the rhizosphere soils of tomato field from different areas in Indian Institute of Vegetable Research in Varanasi district. They were designated as TR22, TR25 TR21, TR15, TR18 and TR24.

Microscopic Observation of PGPR isolates

Microscopic observations were performed to investigate the some characteristics of PGPR isolates such as shape, gram reaction and motility (Table-1) each isolates were rod shaped while two TR25, TR21 showed ellipsoidal shape. All the isolates were found motile and gram negative in reaction.

Effect of Different Temperature on PGPR

Most of the isolates were grown best under the temperature of 20°C & 28°C when compared to 10°C & 37°C and none of them grew at 45°C. Isolates TR28 and TR15 were grown at all temperature except 45°C (Table-2).

Table 1 : Morphological and Gram Reaction of PGPR Isolates

S.No.	Isolates	Cell shape	Motility	Gram reaction
1	TR22	Rod	Motile	Gram$^-$
2	TR25	Rod	Motile	Gram$^-$
3	TR21	Ellipsoidal	Motile	Gram$^-$
4	TR15	Rod	Motile	Gram$^-$
5	TR18	Ellipsoidal	Motile	Gram$^-$
6	TR24	Rod	Motile	Gram$^-$

Table 2 : Growth of PGPR Isolates at Different Temperature

S.No.	Isolates	Temperature				
		10°C	20°C	28°C	37 °C	45°C
1	TR22	+	++	++	++	-
2	TR25	+	+ +	++	++	-
3	TR21	+	++	++	+	-
4	TR15	+	++	++	++	-
5	TR18	++	++	++	+	-
6	TR24	++	++	++	++	-

+Good , ++ Excellent, - Not growth

Table 3 : Effect of Different PGPR on IAA, Siderophore Production and Solubilization of Phosphorous

S. No.	PGPR isolates	IAA production	Phosphorous solubilization	Siderophore production
1.	TR22	+	Not solubilizing	Positive
2.	TR25	++	solubilizing	Positive
3.	TR21	++	Not solubilizing	Positive
4.	TR15	+	Not solubilizing	Negative
5.	TR18	-	Not solubilizing	Positive
6.	TR24	+	Not solubilizing	Positive

+Good , ++ Excellent, - Not growth

Table 4 : Dual Culture Experiment With Different Isolates of Selected PGPR on *A. solani*

S.No.	PGPR + Pathogen (*A. solani*)	% Inhibition in mycelial growth
1.	TR22+ *A. solani*	53.28*
2.	TR25+ *A. solani*	36.25
3.	TR21+ *A. solani*	34.56
4.	TR15+ *A. solani*	24.01
5.	**TR18+** *A. solani*	24.00
6.	TR24+ *A. solani*	19.90
	CD .(0.05%)	16.80

*Value is Arsine Transformed

Table 5 : Effect of PGPR Strains on Percent Disease Index and Yield of Tomato in Field Conditions

S. N .	Treatment	PDI		Yield (Q/H)	
		2011 -12	2012 -13	2011 -12	2012 -13
1.	TR - 22	8.00*	9.00	250.00	219.29
2.	TR - 25	7.33	11.00	252.67	225.43
3.	TR - 21	8.67	11.00	237.67	206.43
4.	TR - 15	10.67	12.00	223.33	190.00
5.	TR - 18	8.00	9.00	242.00	202.86
6.	TR - 24	8.00	12.00	240.00	213.29
7.	Control	21.33	22.00	210.00	181.43
	CD (0.05 %)	**3.9**	**3.5**	**8.1**	**15.24**

*Values were arc sine transformed before the analysis, PDI- Percent Disease Index

Production of IAA and Solubilization of Phosphorous

Investigation was conducted for IAA production and phosphorous solubilization of PGPR isolates. As shown in table 3. Out of 6 isolates (Table-3), 5 isolates TR25, TR21, TR24, TR15, TR22 induced the production of IAA. Isolates TR25, TR21 were found to be good producers of IAA. On the other hand only TR25 isolates had ability to make soluble the phosphorous.

Siderophore Production

Out of six isolates 5 isolates TR25, TR21, TR22, TR18 and TR24 were able to produce siderophore and it is confirmed by the development of orange halo surrounding to those colonies(Table 3).

Antagonistic assay against *A. solani*

Opposition assay was used to determine the isolates that inhibit the growth of Alternaria solani. In this study among of the six PGPR isolates that significant promoted plant growth of tomato seedling. All isolates (TR25, TR21, TR22, TR24, TR15, TR23) that inhibited the radial growth of the *A. solani*. Most effective PGPR was TR22 which inhibited 53.28 % colony growth of *A. solani*,

followed by TR 25 (36.25%). Minimum (Table 4) % inhibition was found in TR24 (19.94 %)

Efficacy of PGPR Strains on Percent Disease Index and Yield in Tomato Under Field Conditions

In the field evaluation, the minimum PDI value was recorded (7.33 %) in treatment TR25 in 2012-13 followed by TR-24 while in 2013-14 minimum value was recorded (9.00; 9.00) in TR-22 & 18. Maximum PDI was recorded in control (21.33) in the year 2012-13 same trend of PDI was recorded in 2013-14. TR-25(Table 5) was found most effective showing least percentage disease index of 7.33 % as against 21.33 % in control, followed by TR-22 and TR-23. TR-25 was recorded highest yield (252.62; 225.43 q/ha), followed by TR-22 (250.00; 219.29 q/ha) in the crop seasons 2011-12 and 2012-13 respectively.

DISCUSSION

PGPR play a vital role in management of various fungal and bacterial diseases. The results obtained from in-vitro and in-vivo experiment demonstrated significant suppression of early blight of tomato. IAA, a member of the group of phytohormones, is generally considered to be the most important native of auxin. IAA may function as important signal molecule in the regulation of plant development. Out of six isolates, five isolates were found for IAA production. It has been reported that IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza, 2001). Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan, 2006). The ability of bacteria to solubilize mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth. In our experiments, 5 isolates of PGPR were able to solubilize phosphate in the rhizosphere soil.

The results of the present study provide evidence that the P. fluorescens strains (TR22, TR25 TR21, TR15, TR18 and TR24) compatible and effectively inhibited the growth of A. solani. Earlier it has been reported that the bio control agents such as Trichoderma viride and P. fluorescens significantly reduced the mycelial growth, spore germination, spore production and germ tube formation of *A. solani* and *A. alternata* (Veerasamy,1997). Silva et al. (2004) evaluated five rhizobacterial strains for biological control of multiple pathogens causing foliar diseases in tomato plants including P. syringae and A. solani and observed reduced disease intensity.

Result from the present study clearly indicates maximum reduction in mycelial growth due to the biocontrol strain in reducing the mycelial growth of the pathogen result in reducing the disease severity. Results from the present study clearly indicated maximum reduction in mycelia growth of *A.solani*.

CONCLUSION

The present study, therefore, suggest that the use of PGPR isolates TR25, TR22 and TR21 as inoculants might be beneficial for tomato cultivation as they enhanced growth of tomato due to production of Indole acetic acids, phosphate solubilization and having antifungal activity against *A. solani*.

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