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Field evaluation of Fluorescent *Pseudomonas* isolates (PGPR) for yield enhancement and spot blotch (*Bipolaris sorokiniana*) suppression of wheat (*Triticum aestivum L.*)

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Wheat cultivators in India's North Eastern plain region have long contended with spot blotch disease. Yet, few investigations have explored PGPR for its management. The present research investigates the efficacy of 47 Fluorescent*Pseudomonas* isolates – sourced from Southern and Northeastern India (Latitudes 9° to 27°North), along with untreated control against grain yield andspot blotch disease of wheat. Field experiment conducted during the years (2023-24 & 2024-25)*rabi* seasonresulted in obtaining a best performing isolate. The average grain yield recorded was 4.6 to 4.8 tonnes hectare^{-1} during the two years. Among all the treatments, seed treatment with UBPF-30 strain recorded maximum yield in two years; nearly 5.6 tonnes hectare^{-1}, accounting for 34% more yield when compared to control. Seed treatment with fluorescentpseudomonads besides increasing the yield, also reduced the severity of spot blotch disease of wheat under field conditions. From the pooled data, the lowest AUDPC was recorded with UBPF -30 isolate with 368.6 and 63.6% reduction in disease, followed by UBPF-17 strain with 50 to 60% reduction in disease when compared to control.PGPR characters like nitrogen fixation, phosphate solubilization, production of HCN, IAA and siderophore were studied *invitro* for UBPF-30 isolate and was molecularly sequenced to be *Metapseudomonas lalkuanensis*.

Keywords : *Bipolarissorokiniana*,fluorescent *Pseudomonas*, *Metapseudomonas lalkuanensis,* PGPR, wheat

INTRODUCTION

Wheat (*Triticum aestivum L.*)is a significant cereal cultivated globally and forms one ofthe staple foods for approximately 2.5 billion people worldwide (Ramadas*et al.* 2019). Wheat production in India is nearly 113.29 million metric tonnes during the year 2024 and contributed 14% of Global Production share (FAS-USDA, 2024).Wheat farming sustains the livelihood of numerous farmers and promotes agricultural economies around the world (Sharma and Sharma, 2025).

Wheat cultivation is affected by numerous biotic stresses, especially diseases are major constraint for production throughout the world. Among more than 200 wheat diseases reported, approximately 50diseases cause substantial economical crop losses and are prevalent across the globe(Sharma *et al.* 2017).Among the wheat diseases, the most damaging is spot blotch disease caused by fungus *Bipolarissorokiniana*. It is prevalent in all major wheat growing areas and is almost as destructive as leaf rust (Joshi and Chand, 2011). 15-25% yield losses were observed in hot and humid areas (Gupta *et al.* 2018) signifying its impact on wheat production. In India, the frequency of occurrence is more in Nort Eastern Plain Zone (NEPZ) due to its warmer climate (Singh *et al.*2002). Use of chemical fungicides adversely affect the environment and non-target microbial-communities, biological control provides a sustainable and environment friendly alternative for reducing plant diseases (Koure*t al.*2019).

Many Plant-growth promoting rhizobacteria (PGPR) have been identified across diverse

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genera such as *Arthrobacter, Azospirillum, Azotobacter, Bacillus, Clostridium, Enterobacter, Pseudomonas, Gluconoacetobacter, Rhizobium* and *Serratia* (Kour *et al.*2019). Among these, fluorescent pseudomonads are widely recognized for their effective root colonising ability and production of metabolites that stimulate plant growth and yield(Rasouli Sadaghiani, 2007).

Further, *Pseudomonas* spp. resides in rhizosphere region and strengthens plant's defence mechanisms against phytopathogens (Choudhary *et al.*2016).The primary mechanisms through which PGPR promotes plant growth include nutrient mobilization to plant (Nitrogen and Phosphorous), Iron chelation by siderophore synthesis, phyto-hormone production (Auxins) (Verma *et al.*2013). The indirect mechanisms by which PGPR supresses disease development includes competition for nutrients, biosynthesis of antibiotics, secretion of lytic enzymes, elucidation of induced defense response in plants (Olanrewaju *et al.* 2017).

Therefore, an experiment was conducted for screening of the most effective Fluorescent *Pseudomonas* (PGPR) against spot blotch disease and enhanced yield under field conditions.

MATERIALS AND METHODS

Isolation and purification of root endophyte Fluorescent Pseudomonas

The root endophyte fluorescent *Pseudomonas* isolates where collected from different regions of India. Healthy root samples were collected from wild and cultivated plants at random, placed in sterile polythene bags and brought to laboratory for isolation. Source of Samples have been given in Table 1. Samples are washed, surface sterilized with 75% ethanol followed by 2% sodium hypochlorite solution and subsequently rinsed with distilled water for more than 6 times (Saini *et al.* 2016) and plated on Kings B media (King, 1954) for isolation. The final washed aliquot was also cultured on Kings B media for testing effective surface sterilisation (Tanvir *et al.* 2013) and incubated at 28^0 Cfor 2-3 days. Colonies exhibiting distinct morphological size, pigmentation and fluorescence activity were selected. Talc formulations with different fluorescent *Pseudomonas* strains were prepared with 2-3 x 10^9 CFU/ml for seed treatment.

Meteorological observations

The experimental site has subtropical humid climate, where winters are usually dry. The temperature typically declines from December, drop to minimum in January and rise from mid-February, reaching peaks in late March and April. Relative humidity remained elevated year- round. The climate generally supported optimal crop growth. The meteorological data during the cropping season was shown in Fig. 1.

Experimental layout

Field screening of 47 fluorescent *Pseudomonas* isolates was carried out during *rabi* 2023 & 2024 at experimental site of Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal. Randomized Block Design (RBD) was followed with 47treatments,1 Control and 3 replications in 2x1m plots. Wheat variety HD-2967 was used and followed by boarder cropping with highly susceptible variety Sonalika. Different fluorescent *Pseudomonas* talc formulations were seed treated @ 5-10g per kg of seed and sown in the field. Standard agronomic practices were followed throughout the cropping season.

Pure culture of *Bipolaris sorokiniana* isolate was obtained from Department of Plant Pathology, UBKV and tested for pathogenicity test. Sterilized wheat grains were used for mass production of spores. Spore suspension was checked in haemocytometer and adjusted to $1x10^5$ per ml (Dulliverand Garcia, 2000). At heading stage spore suspension of *Bipolarissorokiniana* was artificially inoculated in main field.

Grain Yield

At maturity stage, crop was harvested, threshed and grain yield was measured on plot basis and later converted to hectare equivalent. Percent increase in grain yield was calculated from the normalized yield data and calculated by formula according to formula given by (Kumar, 2021). % Increase in yield = (Yield of Treatment – Yield of Control/ Yield of Control) X 100

AUDPC

Disease severity was assessed by following Saari & Prescot scale (Saari & Prescot, 1975). Disease data was recorded from appearance of the first lesion on the leaf from heading to crop maturity stage at 7-days frequency. The double-digit scores were converted to a percent disease index using the formula {(D1/9 x D2/9) x 100)} (Duveiller*et al.* 2005).

The amount of disease and disease progress was measured by Area Under Disease Progress Curve (AUDPC). Through trapezoidal integration method, the AUDPC was calculated by formula given by Sharma *et al.* (2004).

$$AUDPC = \sum_{i=1}^{n-1} [((Xi + Xi + 1)/ 2] ((ti + 1)-ti)]$$

Where, Xi = Spot blotch disease severity at date i, ti = i^{th} day and n = number of scoring days.

The percent reduction in disease is calculated in relative to untreated control (Kumar, 2020) for knowing the efficacy of the applied seed treated fluorescent *Pseudomonas*.

PGPR characterization

For validating the nitrifying ability, the best screened fluorescent *Pseudomonas* was cultured on Jensen's media (Jensen, 1942). Phosphate solubilization was confirmed by growing in Pikovskaya's Agar (Pikovskayas, 1948). Formation of halo around the culture indicates the phosphorus solubilization. Siderophore production was confirmed by culturing isolate in 35mL Chrome azurol S (CAS) reagent with 100mL King's B media (Bagg &Neilands, 1987). Formation of orange halo around the culture indicates the siderophore production by that particular strain. HCN production was validated by growing isolate in King's B media with 0.44% glycine (Bakker & Schippers,1987). IAA assay was done by Salkowski reagent (Glickmann& Dessaux,1995). Change in colour to pink indicates the production of IAA.

Molecular Characterization by NCBI - BLAST

The 16S rRNA sequencing was carried out by Barcode Biosciences Pvt. Ltd., Bangalore. The 27 F (5'AGAGTTTGATCCTGGCTCAG3') forward primer and 1492 R (5'TACGGTTAC CTTGTTACGACTT3') reverse primer was used for PCR amplification (Narde *et al.* 2004). The PCR product was sequenced for 16S rRNA. The obtained nucleotide sequences were aligned using NCBI –BLAST **(http://www.ncbi.nlm.nih.gov)** program for bacterial strain identification and sequence was submitted for getting accession number. Based on percent identity and query coverage, the phylogenetic tree was made using Neighbour Joining Method in NCBI-BLAST.

RESULTS AND DISCUSSION

Evaluation of Fluorescent Pseudomonas isolates on grain yield of wheat

The data regarding grain yield and percent increase in yield during 2023 and 2024 cropping seasons, are shown in (Table2). During 2023, the average grain yield is 4.75 tonnes per hectare and highest recorded with UBPF-30 strain (5.74 t. ha^{-1}) followed by UBPF-29 strain (5.57 t. ha^{-1}). In 2024, the average grain yield is 4.68 t. ha^{-1} and UBPF -30 isolate recorded highest with 5.67 t. ha^{-1}, followed by UBPF-17 strain (5.51 t. ha^{-1}). The results showed that UBPF -30 isolate performed well under the field conditions during both the seasons with highest grain yield among all isolates. However, it was observed to be significantly at par with UBPF -17 and UBPF -29 strain performance. The percent increase in yield ranged from 4.3 to 34.3% during the year 2023, whereas in 2024, varied between 3.9 to 34.5% when compared to untreated control. Here also the result showed consistent performance of UBPF -30 isolate during both years.

To validate the performance of different Fluorescent *Pseudomonas* isolates, the data of both seasons were pooled (Fig. 3- Fig.2). The pooled analysis of data across both cropping seasons revealed that all other Fluorescent *Pseudomonas* isolates significantly enhanced grain yield under field conditions when compared

Table 1. Collection of Endophytic Fluorescent *Pseudomonas* Isolates from different locations of India

Sample No.	Place	Crop	Latitude (°N)	Longitude (°E)
UBPF 01	Pundibari, Coochbehar (West Bengal)	Wheat	26.5243	89.1075
UBPF 03	Khagrabari, Coochbehar (West Bengal)	Mungbean	26.3665	89.4092
UBPF 04	Madhupur, Coochbehar (West Bengal)	Potato	26.3641	89.3779
UBPF 05	Pundibari, Coochbehar (West Bengal)	Tea	26.5243	89.1075
UBPF 06	Pundibari, Coochbehar (West Bengal)	Ginger	26.5243	89.1075
UBPF 07	Dinhata, CoochBehar (West Bengal)	Brinjal	26.1291	89.4695
UBPF 08	Pundibari, Coochbehar (West Bengal)	Rice	26.5243	89.1075
UBPF 09	Pundibari, Coochbehar (West Bengal)	Okra	26.5243	89.1075
UBPF 10	Pundibari, Coochbehar (West Bengal)	Mustard	26.5243	89.1075
UBPF 11	Pundibari, Coochbehar (West Bengal)	Wheat	26.5243	89.1075
UBPF 12	Pundibari, Coochbehar (West Bengal)	Lentil	26.5243	89.1075
UBPF 13	Satmile, Coochbehar (West Bengal)	Citrus	26.3301	89.2201
UBPF 14	Cooch Behar (West Bengal)	Chaenopodium (weed)	26.3452	89.4482
UBPF 15	Alipurduar (West Bengal)	Kalanchoe	26.4918	89.5271
UBPF 17	Pakyong (Sikkim)	Weed	27.3516	88.3239
UBPF 18	Kaliganj, Cooch Behar (West Bengal)	Coriander	26.3452	89.4482
UBPF 19	Kalimpong (West Bengal)	Ginger	27.0594	88.4695
UBPF 20	Kalimpong (West Bengal)	Ginger	27.0594	88.4695
UBPF 23	Idukki (Kerala)	Black Pepper	9.8279	76.9296
UBPF 24	Edavannapara (Kerala)	Chilli	11.2454	75.9767
UBPF 25	Edavannapara (Kerala)	Wild Amaranthus	11.2454	75.9767
UBPF 26	Hoskote (Karnataka)	Turmeric	13.0690	77.7762
UBPF 27	Rudrasamudram (Andhra Pradesh)	Chilli	15.8512	79.3971
UBPF 28	Makavarapalem (Andhra Pradesh)	Casuarina	17.6175	82.7158
UBPF 29	Anantagiri (Andhra Pradesh)	Coffee (Black Soil)	18.1727	83.0441
UBPF 30	Minumuluru (Andhra Pradesh)	Coffee (Red Soil)	18.0249	82.7088
UBPF 31	Balasore (Orissa)	Ginger	21.4894	86.9192
UBPF 32	Midnapore (West Bengal)	Bitter Gourd	22.4307	87.3186
UBPF 33	Sildiri (Jharkhand)	Periwinkle	23.4805	85.4608
UBPF 34	Sildiri (Jharkhand)	Red-gram	23.4805	85.4608
UBPF 35	Purulia (West Bengal)	Croton	23.3307	86.3624
UBPF 36	Udaipur (Tripura)	Brinjal	23.5299	91.4841
UBPF 37	Imphal (Manipur)	Marigold	24.8062	93.9364

(Contd. Table 1)

UBPF 38	Sabour (Bihar)	Brinjal	25.2442	87.0452
UBPF 39	Maralong (Manipur)	Marigold	25.2703	94.0225
UBPF 40	Maralong (Manipur)	Leafy Mustard	25.2703	94.0225
UBPF 41	**Pundibari (West Bengal)**	**Tea**	**26.4035**	**89.3825**
UBPF 42	Falakata (West Bengal)	Tea	26.5000	89.1500
UBPF 43	Mungpoo– Darjelling (West Bengal)	Cowpea	26.9753	88.3697
UBPF 44	Mungpoo– Darjelling (West Bengal)	Radish	26.9753	88.3697
UBPF 45	Kalimpong (West Bengal)	Pumpkin	27.0620	88.4772
UBPF 46	Kalimpong (West Bengal)	Okra	27.0620	88.4772
UBPF 47	Sakyong – Kalimpong (West Bengal)	Garlic	27.0620	88.4772
UBPF 48	Central Pandem (Sikkim)	Dalley Chilli	27.2053	88.5534
UBPF 49	Itahar, Uttar Dinajpur (West Bengal)	Datura	25.4486	88.1629
UBPF 50	Alipur (West Bengal)	*Ageratum conyzoides*	22.5184	88.3282
UBPF 51	Raiganj, Uttar Dinajpur (West Bengal)	Wild Amaranthus	25.6118	88.0936

to control. However, UBPF -30 recorded highest grain yield (5.67 t. ha^{-1}) and highest percent in increase in yield (34.4%). Moreover, it is significantly at par with UBPF -17 strain. It yielded 5.51 t. ha^{-1} and 30.6% increase in yield.

Evaluation of fluorescent Pseudomonas isolates on wheat spot blotch disease

The disease data was recorded from heading stage of crop and continued till 28 days after flowering. During 2023, the AUDPC ranged from 348.9 to 844.8 and reduction in disease percentage ranged from 4 to 63.4%. In 2024, the AUDPC ranged from 388.3 to 1076.7 and percent decrease in disease varied from 8.5 to 63.9 percent. The results showed that during both cropping seasons, UBPF-30 strain performed better with low AUDPC (Table 3). Moreover, it showed to be significantly at par with UBPF – 29 & UBPF – 17 strains.

Pooled analysis of both seasons showed that all fluorescent *Pseudomonas* isolates were significantly effective in managing the spot blotch disease. However, the lowest AUDPC was recorded with UBPF -30 isolate with 368.6 and 63.6% reduction in disease. UBPF -30 isolate is significantly at par with UBPF -17 isolate with 431.6 AUDPC and 55.7% in percent decrease in disease (Fig.3).

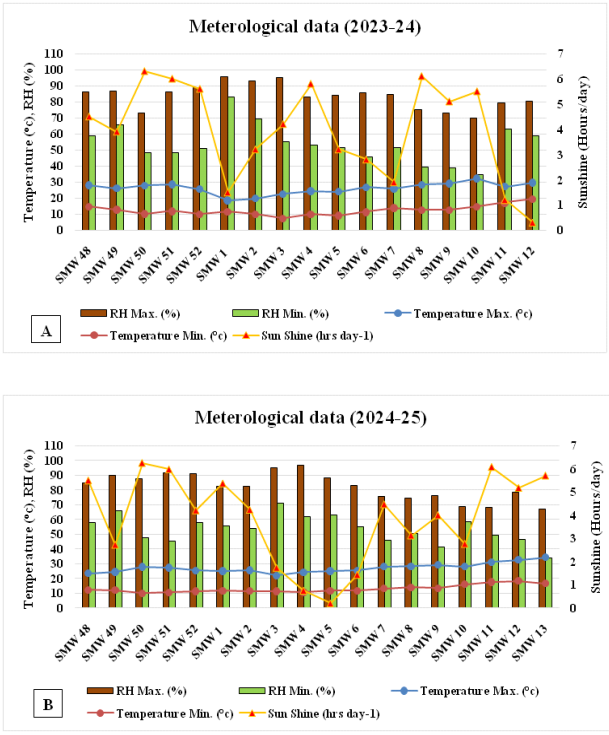

Fig 1: Meteorologicalparameters for (SMW) standard meteorological weeks from (A) 2023-24 and (B) 2024-25; RH-Relative Humidity observed during experimentation period.

Table 2 : Grain yield of wheat seed treatment of different fluorescent *Pseudomonas* isolates.

	Grain Yield (t. ha-1)		Percent Increase in Yield		Pooled
	2023-24	2024-25	2023-24	2024-25	
Control	4.28	4.16	-	-	-
PF 01	4.46	4.33	4.3	3.9	4.1
PF 03	4.61	4.46	7.9	7.3	7.6
PF 04	4.51	4.70	5.6	12.9	9.2
PF 05	5.50	5.36	28.6	28.8	28.7
PF 06	4.67	4.27	9.2	2.6	5.9
PF 07	4.60	4.36	7.5	4.8	6.2
PF 08	5.16	4.69	20.7	12.7	16.7
PF 09	4.66	4.75	9.0	14.0	11.5
PF 10	4.91	4.74	14.9	13.9	14.4
PF 11	4.83	4.72	13.0	13.5	13.3
PF 12	4.78	4.64	11.8	11.4	11.6
PF 13	4.50	4.74	5.2	13.8	9.5
PF 14	4.57	4.53	7.0	8.8	7.9
PF 15	4.75	4.69	11.0	12.7	11.8
PF 17	5.51	5.51	28.8	32.3	30.6
PF 18	4.66	4.56	9.0	9.6	9.3
PF 19	4.67	4.69	9.1	12.6	10.9
PF 20	4.66	4.69	9.0	12.6	10.8
PF 23	4.64	4.78	8.4	14.8	11.6
PF 24	4.55	4.55	6.5	9.2	7.9
PF 25	4.68	4.95	9.5	18.9	14.2
PF 26	4.90	4.42	14.6	6.1	10.3
PF 27	4.63	4.59	8.4	10.2	9.3
PF 28	4.65	4.64	8.7	11.5	10.1
PF 29	5.57	5.28	30.4	26.9	28.6
PF 30	5.74	5.60	34.3	34.5	34.4
PF 31	4.58	4.46	7.1	7.1	7.1
PF 32	4.57	4.72	6.9	13.3	10.1
PF 33	4.70	4.94	9.9	18.7	14.3

(Contd. Part Table 2)

PF 34	4.78	4.62	11.8	11.0	11.4
PF 35	4.74	4.45	10.8	6.8	8.8
PF 36	4.62	4.65	8.0	11.7	9.9
PF 37	4.67	4.72	9.2	13.5	11.4
PF 38	4.55	4.55	6.4	9.4	7.9
PF 39	4.74	4.66	10.9	12.0	11.5
PF 40	4.56	4.75	6.6	14.1	10.4
PF 41	4.65	4.73	8.6	13.6	11.1
PF 42	4.67	4.72	9.2	13.4	11.3
PF 43	4.62	4.56	8.1	9.6	8.9
PF 44	4.71	4.79	10.1	15.0	12.6
PF 45	4.60	4.53	7.5	8.9	8.2
PF 46	5.43	4.68	26.9	12.4	19.7
PF 47	4.88	4.43	14.2	6.4	10.3
PF 48	4.70	4.41	9.9	5.8	7.9
PF 49	4.56	4.65	6.7	11.7	9.2
PF 50	4.67	4.73	9.2	13.5	11.4
PF 51	4.65	4.71	8.7	13.2	11.0
SeM (±)	2.5191	3.0596			
CD (0.05)	7.6409	9.2803			

PGPR Characterization

From both the cropping seasons 2023 & 2024, the UBPF -30 isolate performed better with low spot blotch disease and high grain yield. The UBPF -30 isolate was further analysed for plant growth promoting characters.

Growth of selected isolates (UBPF-17, UBPF-29 & UBPF-30) on Jensen's media indicated its Nitrifying ability. Presence of halo around the culture in Pikovaskaya's agar media showed its phosphate solubilization capacity. Presence of orange yellow halo surrounding the culture of selected PF isolates in King's B media with CAS reagent showed it ability to produce siderophore. Growth and presence of orange halo around culture in King's B media with 0.44% Glycine

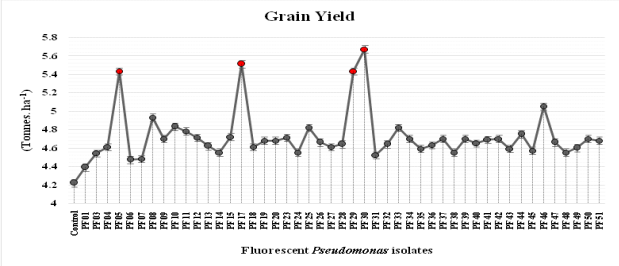

Fig 2 : Grain yield of wheat seed treatment of fluorescent *Pseudomonas* isolates (Pooled data – 2023&2024*Rabi* seasons).

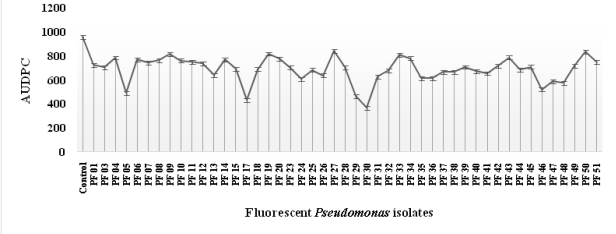

Fig 3 : AUDPC of seed treated Fluorescent Pseudomonas isolates (Pooled data – 2023&2024*Rabi* seasons).

Table 3 : AUDPC of seed treatment of different fluorescent *Pseudomonas* isolates on spot blotch disease of wheat

Treatments	AUDPC		Percent Decrease in AUDPC		Pooled
	2023-24	2024-25	2023-24	2024-25	
Control	844.8	1076.7	-	-	-
PF 01	647.9	800.3	23.8	25.9	24.9
PF 03	704.7	704.3	17.2	34.5	25.8
PF 04	772.9	798.8	5.5	25.7	15.6
PF 05	436.1	545.9	51.5	49.1	50.3
PF 06	790.7	752.2	9.8	30.1	20.0
PF 07	721.5	769.9	15.4	28.1	21.8
PF 08	763.3	762.3	11.2	29.0	20.1
PF 09	731.7	902.5	15.5	16.3	15.9
PF 10	676.9	846.7	19.6	21.2	20.4
PF 11	603.9	895.8	27.3	17.1	22.2
PF 12	681.6	792.8	21.0	26.3	23.6
PF 13	650.5	629.6	21.7	41.5	31.6
PF 14	656.9	884.4	22.9	17.8	20.3
PF 15	580.8	807.6	32.4	25.0	28.7
PF 17	425.5	437.8	52.0	59.3	55.7
PF 18	660.8	717.9	22.5	33.2	27.9
PF 19	720.3	916.7	15.4	14.7	15.0
PF 20	664.0	891.1	22.4	17.5	19.9
PF 23	583.1	820.9	26.7	23.6	25.1
PF 24	647.3	565.6	21.5	47.5	34.5
PF 25	627.7	739.6	30.5	31.3	30.9
PF 26	506.7	767.4	43.5	28.6	36.0
PF 27	714.7	969.1	11.0	10.0	10.5
PF 28	552.6	856.8	37.1	20.1	28.6
PF 29	397.4	534.9	55.0	50.3	52.6
PF 30	348.9	388.3	63.2	63.9	63.6
PF 31	639.0	614.8	27.4	42.9	35.1
PF 32	556.1	800.6	38.0	25.7	31.8
PF 33	687.8	931.4	18.7	13.4	16.0
PF 34	577.4	982.5	28.3	8.5	18.4

(Contd. Part Table 3)

PF 35	604.9	622.1	31.7	42.1	36.9
PF 36	441.1	787.2	49.9	26.7	38.3
PF 37	572.2	757.2	29.5	29.5	29.5
PF 38	484.3	849.3	44.7	21.1	32.9
PF 39	556.5	859.5	34.1	20.3	27.2
PF 40	626.7	721.6	27.8	33.1	30.4
PF 41	496.0	815.8	41.8	23.9	32.8
PF 42	614.5	820.0	30.3	24.4	27.3
PF 43	662.9	911.5	25.8	15.1	20.5
PF 44	603.5	770.7	32.0	28.4	30.2
PF 45	699.7	718.5	14.2	33.0	23.6
PF 46	426.2	612.9	51.7	43.2	47.5
PF 47	585.6	588.7	34.2	45.3	39.7
PF 48	493.7	653.9	40.8	39.2	40.0
PF 49	626.3	811.1	26.5	24.5	25.5
PF 50	816.4	854.1	4.0	20.6	12.3
PF 51	676.5	826.5	20.0	23.0	21.5
SeM (±)	8.8142	13.4473			
CD (0.05)	26.7351	40.7882			

Table 4 : PGPR characters of selected PF isolates

PGPR Characters	PF Isolates		
	UBPF-17	UBPF-29	UBPF-30
Nitrogen fixation	+	+	+
Phosphate solubilization	+	+	+
Siderophore production	+	+	+
HCN Production	+	+	+
IAA production	+	+	+

(**+** indicates positive reaction)

Table 5 : Molecular characterization of PF isolates

Isolate	Bacterial Name Assigned	Accession Number	Similarity %	Nearest Neighbour
UBPF-30	*Metapseudomonas lalkuanensis*	PX548784	99.86	*Metapseudomonas lalkuanenis* (PE08 strain)
UBPF-29	*Metapseudomonas lalkuanensis*	PX548785	99.71	*Metapseudomonas lalkuanenis* (PE08 strain)
UBPF-17	*Pseudomonas protegens*	PP025826	99.81	*Pseudomonas protegens* (CHA0 Strain)

indicated its HCN production. Cultures of selected PF isolates strain in King's B broth is centrifuged and 1mL supernatant is taken. By adding of 1mL Salkowski reagent and kept under dark conditions for some time resulted in reddish pink colour formation indicated its IAA production (Table 4, Fig.4).

The nucleotide sequence of 16S rRNA obtained was deposited in GenBank under accession number PX548784 for UBPF-30 strain. BLAST results showed that UBPF-30 isolate has 99.86% similarity to *Pseudomonas lalkuanensis* PE08 strain (Table 5). The phylogenetic relation of UBPF -30 strain is presented in (Fig.5). The molecular analyses results were consistent with PGPR characteristics of the isolate.

In the present research, all the fluorescent *Pseudomonas* formulations were significantly effective in enhancing grain yield and management of spot blotch disease in wheat. Out of 47 formulations, UBPF-30 was observed to be most effective in enhancing grain yield and managing spot blotch disease. However, it was significantly at apar with UBPF-17 and UBPF -29 strains.

The present findings align with the previous research (Singh *et al.* 2024; Mousa *et al.* 2024) who have recommended that bio-formulations with *P. lalkuanensis* provided highest disease management efficiency.

Microbial consortium comprising *Trichoderma afroharzianum* 1F, *Arthrobacter aneurinilyticus* 16B, *Pseudomonas lalkuanensis* 31B and *Bacillus licheniformis* 223B treatment in cumin plants against *Alternaria burnsii* showed 32.47 percent disease severity index and highest percent in reduction of disease (64.87%). Besides that, it recorded highest seed yield of 2.45 g. plant^{-1}. *P. lalkuanensis* showed PGPR characters like Phosphorous solubilization, siderophore production and IAA production (Singh *et al.* 2024). As a PGPR, *P. lalkuanesis* exhibits strong potential for promotion of plant growth and disease suppression. Application of Phage P-PSG11 in combination with *P. lalkuanensis* significantly decreased the incidence of *Ralstonia solanacearum* wilt by 88.2%, while *P. lalkuanensis* alone reduced disease incidence by 81.2% when compared to untreated control in potato (Mousa *et al.* 2024). Li *et al.* (2020) reported that, application of microbial inoculants led to 34.38% enhanced yield compared to untreated control. Potential mechanism underlying the improved plant performance from biocontrol treatments involves elevated production of plant growth hormone Indole-3 acetic acid (IAA). IAA stimulates development of roots and enhances overall plant vigour (Ali *et al.* 2020). Fluorescent Pseudomonads functions as biocontrol agents, that supress plant diseases through direct antagonism via production of anti-microbial metabolites targeting the pathogen or by inducing host plant defense mechanisms (Ongena*et al.* 2005). *P. lalkuanensis* produces HCN, a potent volatile compound, that inhibits pathogen proliferation and confers plant protection against pathogen invasion (Sehrawat*et al.* 2022). Fluorescent Pseudomonads along with HCN, they produce other secondary metabolites like phenazines, pyoluteorin, pyrrolnitrin, phloroglucinols and lipopeptides that supress the pathogen (Haas and Keel, 2003). Seed treatment

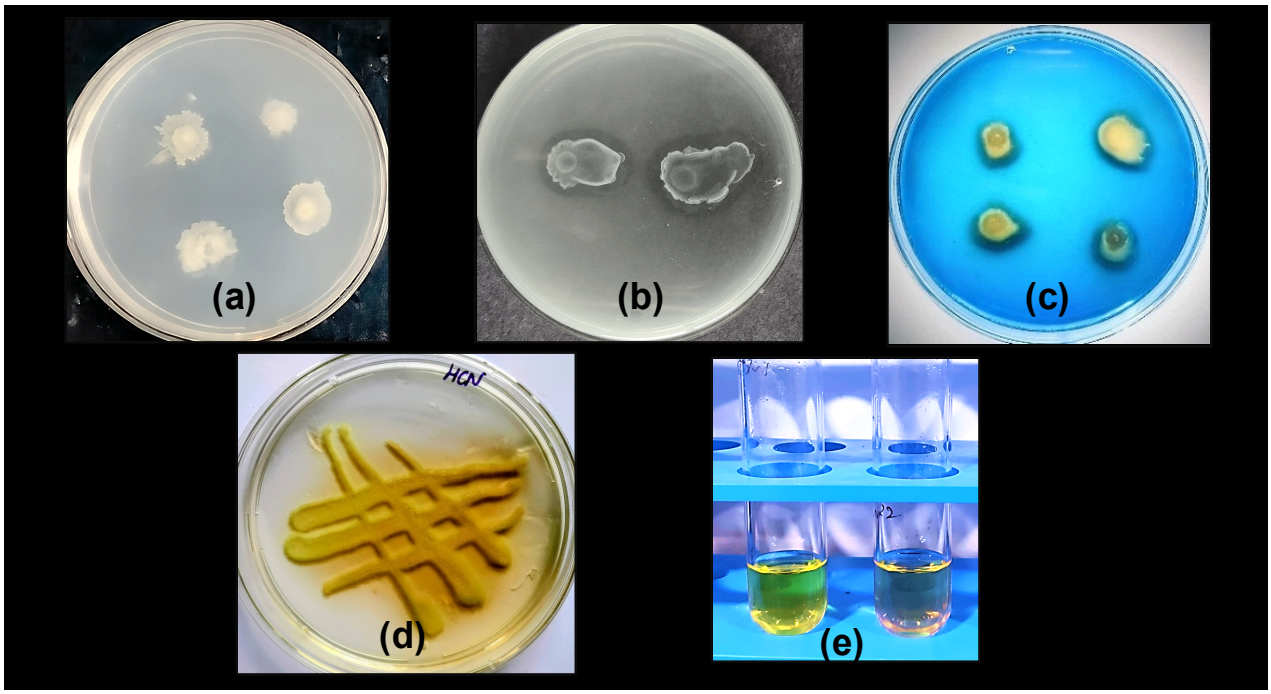

Fig.4: Culture of UBPF – 30 isolate on (a) Jensen's media – Nitrogen fixation, (b) Pikovaskaya's media (Phosphate solubilization), (c) CAS reagent with King's B media (Siderophore production), (d) 0.44% Glycine with King's B media (HCN Production) and (e) Salkowski reagent with King's B broth (IAA production).

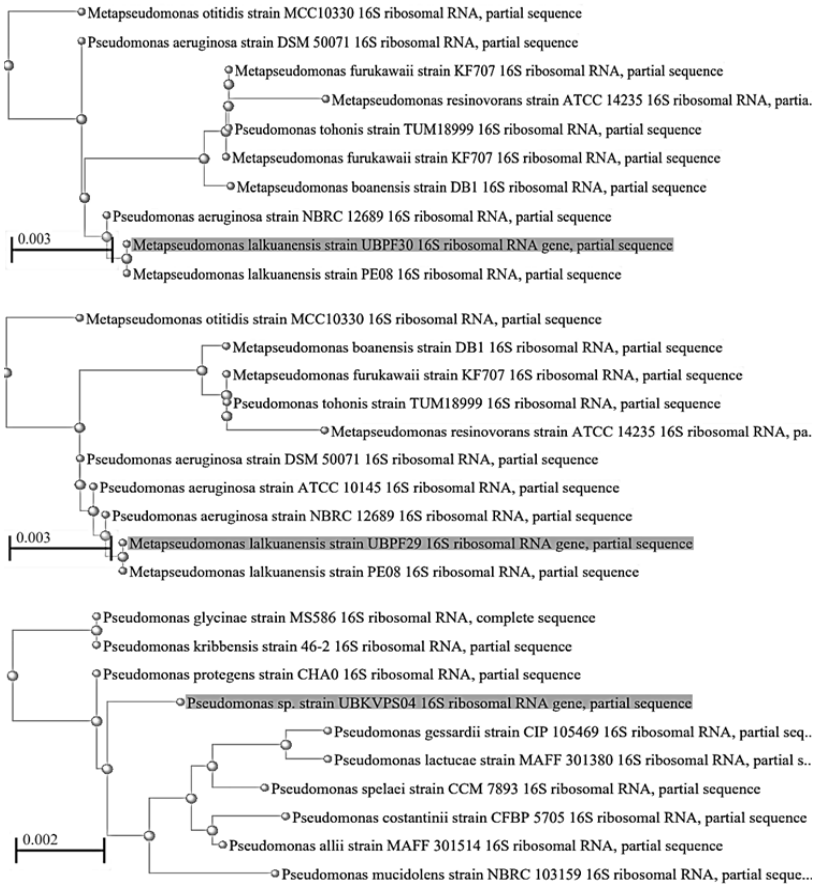

Fig 5: Phylogenetic tree showing the position of PF isolates. Phylogenetic tree constructed using Neighbour Joining method in MEGA software.

of wheat seeds with fluorescent *Pseudomonas* spp. effectively supressed take all disease in both greenhouse and field conditions during winter and spring seasons (Weller *et al.* 2002).

CONCLUSION

Field screening enabled a detailed study of Fluorescent *Pseudomonas* seed treatment activity on yield enhancement and spot blotch suppression. The isolate UBPF-30 collected from Andhra Pradesh state (coffee root endophyte) performed better with regard to increased yield and low AUDPC during 2023 & 2024 *rabi* seasons. *Metapseudomonas lalkuanensis* (UBPF-30) exhibited the plant growth promoting characters nitrogen fixation, phosphate solubilization, production of siderophore, IAA & HCN that enhanced yield and supressed spot blotch disease.

Based on the overall efficacy of fluorescent *Pseudomonas* formulations against spot blotch disease and grain yield of wheat, *Metapseudomonas lalkuanensis* (UBPF-30 strain) will be highly recommended for management of spot blotch disease with minimized yield losses.

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DECLARATION

Authors declare no conflict of interest.

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