https://doi.org/10.56093/ijas.v90i9.106585

Research Articles

Indian Journal of Agricultural Sciences **90** (9): 1646–52, September 2020/Article

Impact of endophytic bacteria against yellow rust (*Puccinia striiformis* f. sp. *tritici*) of wheat

DALJEET SINGH BUTTAR[1], NARINDER SINGH[2], PAARUL KAUR SALH[3] and A K CHOUDHARY[4]

Punjab Agricultural University, Ludhiana 141 004, India

Received: 19 April 2019; Accepted: 18 July 2020

ABSTRACT

Wheat is a dominant cereal crop worldwide and very important as a staple food resource. A number of pathogens cause damage to wheat crop, of which yellow rust caused by *Puccinia striiformis* f. sp. *tritici* is one of the major threats to wheat production in India. Chemical control leads to resistance development against the pathogen. Biological control, the use of natural antagonists to combat plant diseases has emerged as a promising alternative to chemical pesticides. Keeping in view the importance of the disease the present investigation was undertaken with the aim to observe the impact of endophytic bacteria *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* as biocontrol agents against yellow rust of wheat. Talc based bioformulations of two bacterial biocontrol agents, viz. *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* were used at different concentrations to observe their effectiveness against yellow rust. In addition to this, the activity of various defense related enzymes Peroxidase (PO), Polyphenol oxidase (PPO), Phenylalanine Ammonia Lyase (PAL) along with Phenols and Proteins was also observed. Our findings indicated that the yellow rust severity in *P. fluorescens* (T_{14}) fermented liquid (FL) (PF) @15 g/l of water as foiler spray exhibited 43.71 per cent and it gave 50.07 per cent disease control in rabi 2015. Whereas *B. amyloliquefaciens* (T_3) fermented liquid bacterial cell (FLBC) (FDK21) @15 g/kg of seed showed 53.25% disease severity along with 39.20% disease control in *rabi* 2015 and enhanced the grain yield. Both the treatments were significantly better than the untreated control. But they were less superior than the chemical control. Similar trend has been observed in *rabi* 2016 season. The activity of the defense related enzymes that is PO, PPO and PAL as well as phenol and protein content was observed to be elevated in *P. fluorescens* T_4(54.35 Units min^{-1} g^{-1} F W), T_{10}(24.39 Units min^{-1} g^{-1} F W), T_{14}(103.36 µg t-cinnamic acid formed hr^{-1} g^{-1}), T_{10} (6.89 mg/g F W) and T_{14}(111.56 mg/g F W) and *B. amyloliquefaciens* T_3(29.01 Units min^{-1} g^{-1} F W), T_3(19.54 Units min^{-1} g^{-1} F W), T_3(97.98 µg t-cinnamic acid formed hr^{-1} g^{-1}), T_3(46.89 mg/g F W), T_3 (98.08 Units min^{-1} g^{-1} F W)) treated wheat plants as compared to chemically treated (T_2 19.77 Units min^{-1} g^{-1} F W, 7.41 Units min^{-1} g^{-1} F W, 97.94 µg t-cinnamic acid formed hr^{-1} g^{-1}, 2.93 mg/g F W, 72.40 mg/g F W) and untreated control plants (T_1 13.61 Units min^{-1} g^{-1} F W, 1.90 Units min^{-1} g^{-1} F W, 88.73 µg t-cinnamic acid formed hr^{-1} g^{-1} , 2.74 mg/g F W, 44.90 mg/g F W). Since several strains of *Pseudomonas* and *Bacillus* can act as BCA through an induced systemic resistance response in the plant against pathogens. So these biocontrol agents played major role through an induced systemic resistance response in the plant against pathogens and also help the plant in accelerating their defense response against the pathogens. Therefore it is suggested that the novel use of biocontrol agents *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens*.

Key words: *Bacillus amyloliquefaciens, Pseudomonas fluorescens, Puccinia striiformis* f. sp. *tritici*, Wheat, Yellow rust.

Wheat is a dominant cereal crop worldwide and very important as a staple food resource. Multiple diseases can attack the crop, of which the disease yellow rust caused by *Puccinia striiformis* f. sp. *tritici* is seen as one of the major threats to wheat production (Reiss and Jørgensen 2017). Currently, 88% of the world's wheat production is effected by wheat yellow rust, lead to global losses of over 5 million tons of wheat with an estimated market value of $USD 1 billion annually (Beddow *et al.* 2015). Yellow rust caused 100% yield losses if infection occured very early and the disease continued developed afterwards in the whole wheat growing season. This disease is a major biotic factor for wheat production in India and has been known as an endemic disease in the north-western plains zone (NWPZ), north hills zone, and south hills zone, which is 40% of the total area under wheat cultivation in India (Prashar *et al.*

[1]Senior Plant Pathologist (e-mail: pau_daljeet_2@pau.edu), [2]Senior Plant Pathologist-cum-Head (e-mail: narindermutti@pau.edu), [3]Research Fellow (e-mail: paarulsalh@gmail.com), [4]Research Fellow (e-mail: choudhary.ajay11@gmail.com), Department of Plant Pathology, Punjab Agricultural University, Ludhiana (Punjab).

2007). Chemical control relies on few modes of action, which may increase selection pressure and eventually leads to resistance development. This calls for investigating alternative control measures including biological control, which are also generally seen as more environmentally sound solutions. PGPR bacteria act as biofertilizer and biocontrol agent against several pathogens. Their effects can occur through induction of systemic resistance against pathogens throughout the entire plant (Beneduzi *et al.* 2012). Endophytic bacteria *Pseudomonas* spp. and *Bacillus* spp. are well known for their antagonistic effects and their ability to trigger ISR against plant pathogens (Podile and Kishore 2006). The PGPR endophytes also have the ability to induce plant mediated resistance in the host plant (Ongena *et al.* 2007). These beneficial microorganisms can improve plant performance by inducing systemic defense responses that confer broad-spectrum resistance to plant pathogens (Van Wees *et al.* 2008).

Considering the importance of the malady the present investigation has been undertaken with the objective to observe the impact of *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* FDK21 isolates against yellow rust pathogen. The study was designed to: (i) Determine the impact of biocontrol agent, on yellow rust of wheat severity and (ii) Induced defense response towards yellow rust in different treatment using biochemical estimations.

MATERIALS AND METHODS

The experiment was carried out at experimental area of department of plant pathology during *rabi* season 2015-16 and 2016-17 at Punjab Agricultural University, Ludhiana in randomized block design with three replications using yellow rust susceptible wheat cultivar PBW 550. The crop was sown in plot size (2.5×2 m^2) with row to row distance of 15 cm. All the recommended practices were followed to raise the crop. There were total 12 treatments of talc based bioformulation of *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* FDK21 as Fermented Liquid (FL) and Fermented Liquid Bacterial Cell (FLBC) at different concentrations.

Mainly @15 g/kg as seed treatment and 10 g/l of water as foliar spray. Two checks, i.e chemical (Propiconazole 0.1%) and untreated control were also maintained for comparison. After comparison data on yellow rust severity was recorded at end of the spray from each treatment in all the replication according to the modified cobbs scale (Peterson *et al.* 1948) and the final data of disease severity percentage and grain yield (q/ha) were analysed according to analysis of variance procedure. For the extraction of PO and PAL, was done, using 100 mg of sample tissue which was homogenised in 2 ml of ice-cold potassium phosphate buffer (0.1 M, pH 7.5) containing 10 mM mercaptoethanol, 1% PVP and 1 mM EDTA. The homogenate was centrifuged at $10000 \times g$ for 20 min at 4°C. The supernatant thus obtained was used for the assay of enzymes. While PPO was extracted by homogenizing 100 mg of sample tissue in 2 ml of ice-cold sodium phosphate buffer (0.1 M, pH 7). The homogenate was then centrifuged at 10 000x g for 20 min at 4°C. The supernatant obtained was used for the assay of oxidative enzymes. All enzymes were extracted at 4°C and assayed at 25°C. Phenylalanine ammonia lyase (PAL) activity was measured following the method of Burrell and Rees (1974) and was expressed as mg of t-cinnamic acid formed hr^{-1} g^{-1}. Peroxidase activity on the other hand was determined by using the method of Kaur *et al.* (2014). The activity was expressed as change in absorbance min^{-1} g^{-1} of FW. Polyphenol oxidase activity was determined as per the procedure of Archana *et al.* (2011). The enzyme activity expressed as change in absorbance at 495 nm min^{-1} g^{-1} fresh weight of tissue. The dried tissue sample (400 mg) was refluxed with 5ml of 80% aq. methanol for 1 hr. The refluxed material was then filtered and diluted with hot 80% methanol to a volume of 10 ml. Extract so obtained was used for the estimation of total phenols by the method of Swain and Hills (1959). Proteins from tissue (50-300 mg) were extracted with 2-4 ml of 20 mM Tris-HCl buffer containing 0.5% NaCl (pH 7.5). The extract was centrifuged at 8000 g for 20 min. and the pellet was discarded. The supernatant was used for protein estimation by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Role of Pseudomonas fluorescens and Bacillus amyloliquefaciens FDK21 as BCA against yellow rust pathogen

The yellow rust was statistically significantly less in biocontrol agents treated wheat cultivar PBW550 as compared to untreated wheat plants. In *rabi* 2015-16 and 2016-17, the disease severity was observed to be minimum in chemical treatment T_2 (Propiconazole 0.1%) treated wheat plants (20.07% and 19.06%) and it gave disease control (77.10% in 2015 and 78.77% in 2016) respectively followed by fermented liquid *Pseudomonas fluorescens* treatment as foliar spray T_{14} (FL (Pf) @15g/l of water) which showed (43.71% in 2015 and 39.51% in 2016) disease severity along with disease control of 50.07% in 2015 and 55.97% in 2016 seasons. The fermented liquid bacterial cell *Bacillus amyloliquefaciens* T_3 FLBC (FDK21) @ 15 g/kg of seed as treatment showed disease severity (53.25% in 2015 and 43.58% in 2016) and exhibited (39.20% in 2015 and 52.20% in 2016) disease control. This was further reflected with respect to the yield data. Maximum wheat grain yield was observed in chemically treated T_2 (Propiconazole 0.1%) (40.58 q/ha in 2015 and 46.50 q/ha in 2016) followed by the fermented liquid *Pseudomonas fluorescens* T_{14}(FL (Pf) @15g/l of water) which showed (32.57 q/ha in 2015 and 35.33 q/ha in 2016) followed by fermented liquid bacterial cell *Bacillus amyloliquefaciens* T_3 FLBC (FDK21) @15g/kg of seed (26.50 q/ha in 2015 and 25.67 q/ha in 2016). The minimum yield was observed in untreated control T_1 (19.23 q/ha in 2015 and 17.67 q/ha in 2016). Severity of yellow rust in bacterial antagonist treated plants was also found

Table 1 Effect of *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* FDK21 against yellow rust of wheat in cultivar PBW 550 plants during 2015 and 2016

	Treatment	Percent disease severity 2015	Percent disease control 2015	Yield (q/ha) 2015	Percent disease severity 2015	Percent disease control 2016	Yield (q/ha) 2016
T_1	Control	87.59±1.15a	-	19.23±1.17f	89.73±1.42a	-	17.67±0.58e
T_2	Chemical (Propiconazole 0.1%)	20.07±1.53i	77.10±1.57a	40.58±0.98a	19.06±2.85f	78.77±2.98a	46.50±2.00a
T_3	FLBC (FDK 21)15g/kg of seed	53.25±1.02g	39.20±1.83c	26.50±2.42cd	43.02±1.72e	52.03±2.42b	25.67±2.25c
T_4	FLBC (Pf)15g/kg of seed	45.30±1.78h	48.29±1.42b	27.27±1.10cd	42.67±10.52e	52.48±11.38b	29.67±2.52b
T_5	FL (FDK 21)15g/kg of seed	61.89±2.27c	28.32±3.19ef	23.23±2.93de	63.78±2.15cd	28.91±2.79cd	22.00±2.00de
T_6	FL (Pf)15g/kg of seed	54.96±0.56fg	37.24±1.43cd	25.63±2.40cde	57.42±0.44d	35.99±1.37c	24.00±2.00cd
T_7	FLBC (FDK 21)10g/l of water	59.63±0.64cd	31.90±1.61ef	24.23±2.80de	60.99±1.30cd	32.02±2.13c	22.67±2.31cde
T_8	FLBC (Pf)10g/l of water	58.18±0.71de	33.56±1.34e	24.67±1.19de	60.24±0.41cd	32.85±1.51c	23.10±2.76cde
T_9	FL (FDK 21)10g/l of water	59.83±1.79cd	31.67±2.89ef	24.10±2.46de	65.35±3.32bc	27.15±4.14cd	21.90±0.95de
T_{10}	FL (Pf)10g/l of water	44.95±1.60h	48.69±1.15b	29.33±2.31bc	41.52±0.39e	53.72±1.05b	31.30±1.25b
T_{11}	FLBC (FDK 21)15g/l of water	67.89±1.12b	22.47±2.29g	22.00±2.00ef	71.05±3.29b	20.79±4.17d	20.63±1.52de
T_{12}	FLBC (Pf)10g/l of water	56.93±1.40ef	35.00±1.63de	25.33±2.08cde	58.20±0.44cd	35.12±1.42c	23.33±1.46cde
T_{13}	FL (FDK 21)15g/l of water	62.18±1.89c	29.01±2.15f	23.33±1.53de	61.21±6.38cd	31.75±7.45c	22.13±1.83cde
T_{14}	FL (Pf)15g/l of water	43.71±2.45h	50.07±3.32b	32.57±2.60ab	39.51±6.67e	55.97±7.31b	35.33±2.08a

Values are mean ±SD of three replicates; values with same letter(s) are not significantly different at $P \leq 0.05$ (Duncan post-hoc test).

statistically lower when compared to untreated control plants. This indicated the defensive role against the yellow rust pathogen played by endophytic bacterial antagonists under field conditions (Table 1).

Similar results were observed by Chandrasekaran *et al.* (2016) who reported that *P.fluorescens* and *B.amyloliquefaciens* played a vital role in the management of *Ralstonia solanacearum* in potato and they considered on effectivness of biocontrol and significantly enhanced the yield. Both bacterial antagonists, *viz. P. fluorescens* and *B. amyloliquefaciens* considered as an effective means of biocontrol. Kulimushi *et al.* (2017) used a particular strain of the well-known *B. amyloliquefaciens* species as a biological control agent for reducing the impact of fungal infection of maize in the field. Also Sun *et al.* (2017) reported a 4 day delay in the symptoms of bacterial wilt of tomato when *Pseudomonas putida* stain was applied. In comparison with the chemical streptomycin, *P. putida* strain showed more effective control of tomato bacterial wilt. Reiss and jorgensen (2017) also reported that Serenade®ASO (*Bacillus subtilis* strain QST713) control the yellow rust up to 60% under moderate disease pressure but under high disease pressure it is reduced to 30%. Li *et al.* (2013) used *Bacillus subtilis* strain E1R-j as bacterial cell suspension (BCS) and fermentation liquid with and without bacterial cells (FLBC and FL) against wheat yellow rust and it reduced the disease in treated plots as compared to untreated plots.

Biochemical study in suppression of rust pathogen

Peroxidase Activity (PO): Flag leaves of most of the treated wheat plants invaded by yellow rust showed significantly higher activity of peroxidase as compared to untreated control (Fig 1). POD activity of T_4 treated wheat plants (FLBC (Pf) 15 g/kg of seed) (54.35 Units min^{-1} g^{-1} F W) was found maximum followed by T_{14} (FL (Pf) 15g/l of water) (48.52 Units min^{-1} g^{-1} F W) and T_{10} (FL (Pf) 10g/l of water) (43.14 Units min^{-1} g^{-1} F W). Additionally, T_3 (FLBC (FDK 21) 15 g/kg of seed) (29.01 Units min^{-1} g^{-1} F W) and T6 (FL (Pf) 15g/kg of seed) (28.49 Units min^{-1} g^{-1} F W) treated wheat plants also revealed significantly better POD activity than the untreated control (T1). 4-fold higher activity of POD was observed in T_4 when compared to that of the untreated control (T_1). Chemically treated wheat plants (T_2) (19.77 Units min^{-1} g^{-1} F W) demonstrated POD activity higher than that of the control (T_1) (1.5- fold), however it was not found to be significantly different. On comparison, *Pseudomonas fluorescens* treated wheat plants exhibited significantly higher peroxidase activity than that of the other treatments.

Polyphenol Oxidase (PPO): Just affected wheat plants revealed higher PPO activity when treated with various biocontrol agents (Fig 2). On an average 5.1-fold increase in PPO activity was observed in the leaves of treated wheat plants as compared to untreated control (T_1). Maximum PPO activity was observed in T_{10} (FL (Pf) 10g/l of water)

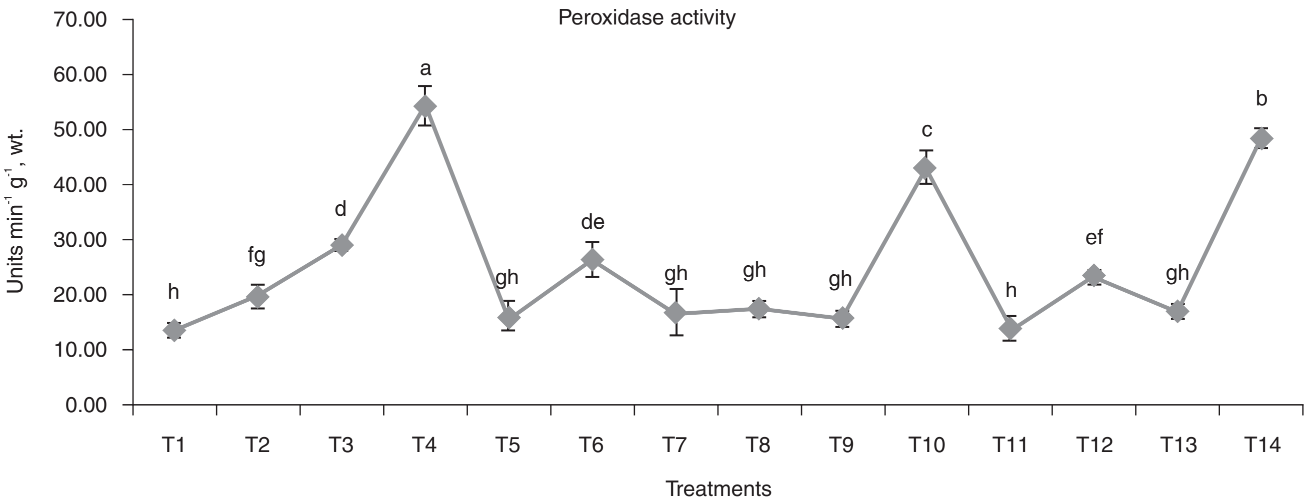

Fig 1 Change in Peroxidase activity in flag leaves of wheat cultivar PBW 550 under different treatments. Error bars denote ±SD of three replicates; bars with same letter(s) are not significantly different at P ≤ 0.05 (Duncan post-hoc test).

(24.39 Units min^{-1} g^{-1} F W) followed by T_{14} (FL (Pf) 15g/l of water) (23.50 Units min^{-1} g^{-1} F W) and T_4 (FLBC (Pf) 15g/kg of seed) (15.05 Units min^{-1} g^{-1} F W). Higher PPO activity was also observed in chemically treated wheat plants (T_2) (7.41 Units min^{-1} g^{-1} F W) than that of the untreated control (T_1) (1.90 Units min^{-1} g^{-1} F W) (6.7 fold). Within treatments, T_4, T_3, T_6 and T_{12} exhibited significantly better activity than the other treatments.

*Phenylalanin Ammonia Lyase (PAL)***:** Significant difference was observed in PAL activity between the leaves of treated wheat plants and the untreated once after the invasion of yellow rust (0.9-fold) (Fig 3). PAL activity of T_{14} treated wheat plants (FL (Pf)15g/l of water) (103.36 µg t-cinnamic acid formed hr^{-1} g^{-1}) was found to be maximum followed by T_{10} (FL (Pf)10g/l of water) (102.05 µg t-cinnamic acid formed hr^{-1} g^{-1}), T_4 (FLBC (Pf)15 g/kg of seed) (99.18 µg t-cinnamic acid formed hr^{-1} g^{-1}) and T_3 (FLBC (FDK 21)15 g/kg of seed) (97.98 µg t-cinnamic acid formed hr^{-1} g^{-1}). 1.1-fold higher activity of PAL was observed in T_{14} when compared to that of the untreated control (T_1) (88.73 µg t-cinnamic acid formed hr^{-1} g^{-1}). Significant difference was observed in the PAL activity of chemically treated wheat plants (T_2) (97.94 µg t-cinnamic acid formed hr^{-1} g^{-1}) and untreated control (T_1) (88.73 µg t-cinnamic acid formed hr^{-1} g^{-1}). As a whole, *Pseudomonas fluorescens* treated wheat plants (T_{14}, T_{10}, T_4) exhibited significantly higher peroxidase activity than that of the other treatments including chemically treated wheat plants.

Phenol content: Biocontrol agents were found to be effective in increasing the phenol content in the flag leaves of yellow rust invaded wheat plants (Fig 4). Comparison with the respective untreated control (T_1) revealed a significant increase in the phenol content in the differencially treated wheat plants (1.5-fold). Phenol content of T_{10} treated wheat plants (FL (Pf) 10 g/l of water) (6.89 mg/g F W) was found to be maximum follwed by T_{14} (FL (Pf) 15 g/l of water)

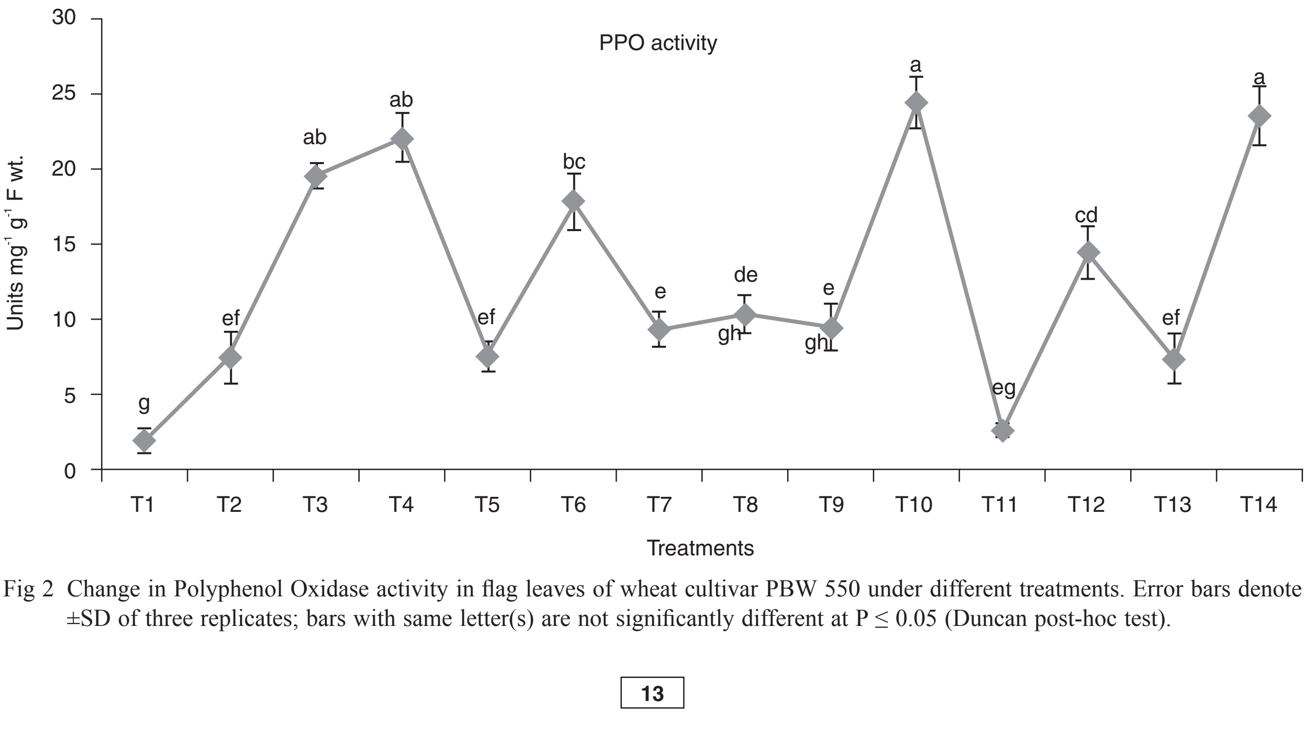

Fig 2 Change in Polyphenol Oxidase activity in flag leaves of wheat cultivar PBW 550 under different treatments. Error bars denote ±SD of three replicates; bars with same letter(s) are not significantly different at P ≤ 0.05 (Duncan post-hoc test).

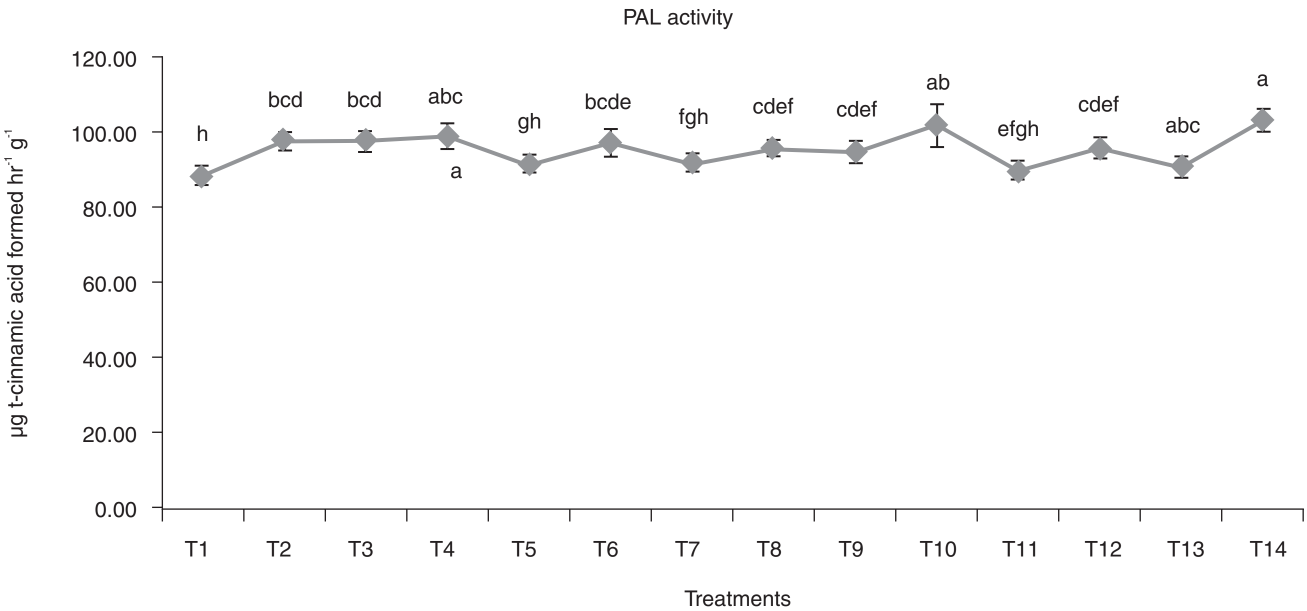

Fig 3 Change in Phenylalanine ammonia lyase activity in flag leaves of wheat cultivar PBW 550 under different treatments. Error bars denote ±SD of three replicates; bars with same letter(s) are not significantly different at P ≤ 0.05 (Duncan post-hoc test).

(6.60 mg/g F W) and T_4 (FLBC (Pf) 15 g/kg of seed) (5.10 mg/g F W). Chemically treated wheat plants (T_2) (2.93 mg/g F W) demonstrated phenol content higher than that of the control (T_1) (2.74 mg/g F W) (1.1- fold) however it was not found to be significantly different. Within treatments, T_{10}, T_{14}, T_3 and T_4 exhibited significantly better activity than the other treatments.

Protein content: Statistically significant difference in the protein content of treated and untreated flag leaves of wheat plants was also observed (1.6-fold) (Fig 5). Protein content of T_{14} treated wheat plants (FLBC (Pf) 15g/kg of seed) (111.56 mg/g FW) was found to be maximum followed by T_{10} (FL (Pf) 10g/l of water) (105.27) and T_4 (FLBC (Pf) 15 g/kg of seed) (100.45 mg/g FW). Moreover, T_3 (FLBC (FDK 21) 15 g/kg of seed) (98.08 mg/g FW) and T_6 (FL (Pf) 15 g/kg of seed) (98.07 mg/g FW) treated wheat plants also revealed significantly better protein content than the untreated control (T_1) (44.90 mg/g FW). Chemically treated wheat plants (T_2) (72.40 mg/g FW) demonstrated protein content higher than that of the control (T_1) (44.90 mg/g FW) (1.6-fold) however it was not found to be significantly different.

As our finding suggested significantly higher activity of peroxidase (PO), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) after the application of *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* against yellow rust of wheat. Similar results were observed by Meena *et al.* (2000). Peroxidase (PO) catalyzes the last step in the biosynthesis of lignin and other oxidative phenols, and it is associated with disease resistance in

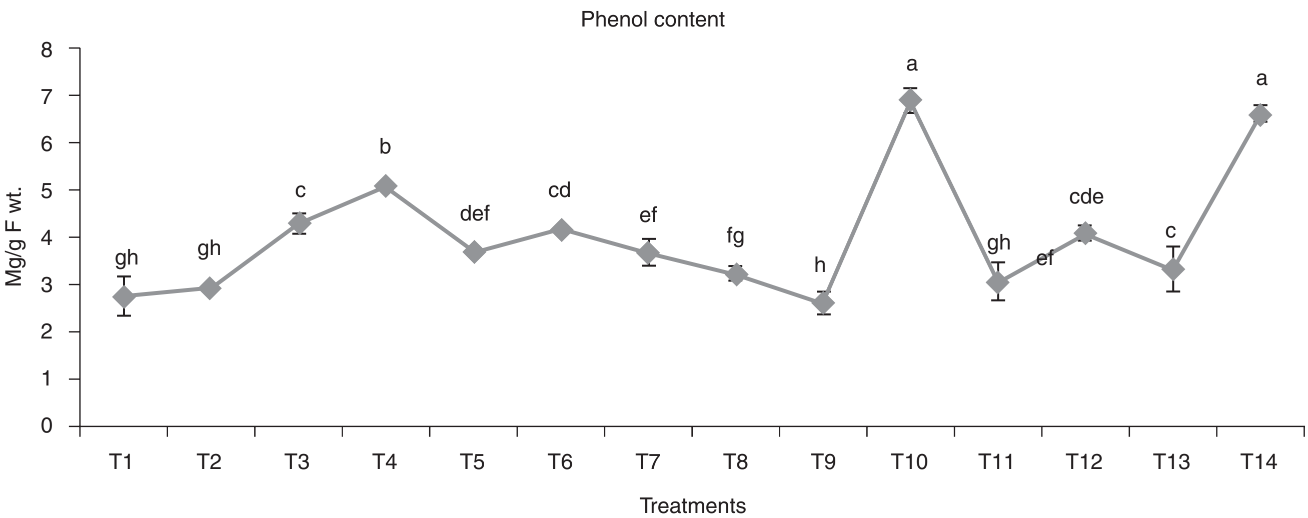

Fig 4 Change in Phenol content in flag leaves of wheat cultivar PBW 550 under different treatments. Error bars denote ±SD of three replicates; bars with same letter(s) are not significantly different at P ≤ 0.05 (Duncan post-hoc test).

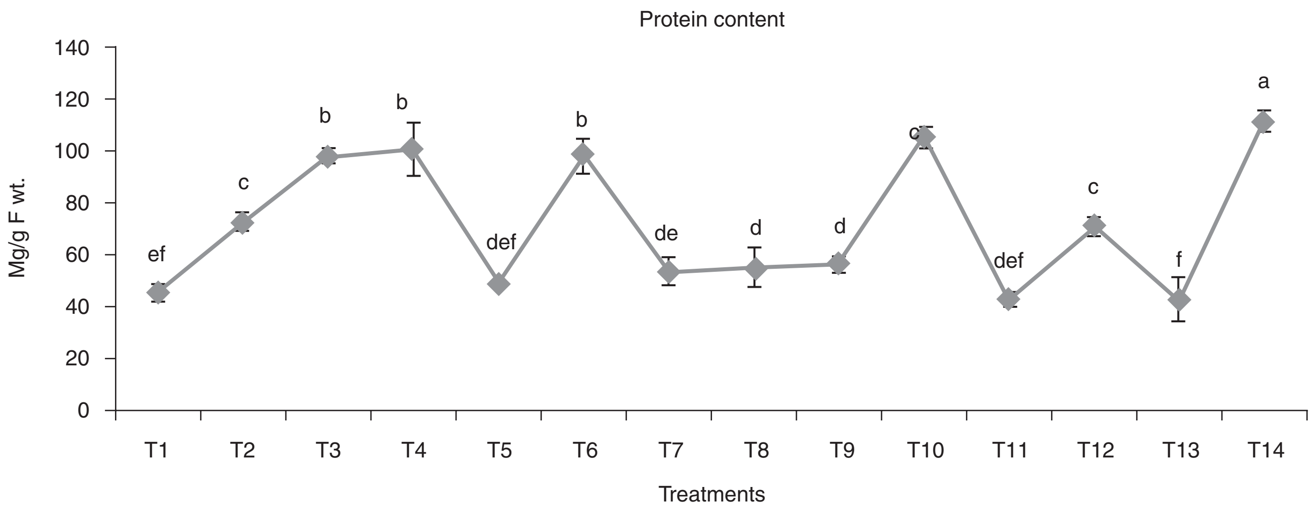

Fig 5 Change in Protein content in flag leaves of wheat cultivar PBW 550 under different treatments. Error bars denote ±SD of three replicates; bars with same letter(s) are not significantly different at P ≤ 0.05 (Duncan post-hoc test).

plants. In groundnut, increased activity of PO was observed due to application of *P. fluorescens*, and PO isoforms were expressed at higher levels. Phenylalanine ammonia lyase (PAL) is the first enzyme involved in phenyl propanoid pathway and plays a key role in the biosynthesis of phenolics and phytoalexins. When cucumber roots were treated with *P. corrugata* or *P. aureofaciens*, PAL activity was stimulated in root tissues in 2 days, and this activated accumulation lasted for 16 days after bacterization (Chen *et al.* 2000). Induction of higher PPO activity was noticed in tomato and hot pepper pretreated with fluorescent pseudomonas strain against *Pythium* diseases (Ramamoorthy *et al.* 2002). Ramamoorthy *et al.* (2002) while working on invasion of *F. oxysporum* f. sp. *lycopersici* in tomato roots suggested induction of defense enzymes involved in phenyl propanoid pathway and accumulation of phenolics and PR-proteins might have contributed to restriction. Phenolics are fungi toxic in nature and increase the physical and mechanical strength of the host cell wall. M'Piga *et al.* (1997) reported that application of *P. fluorescens* strain brought about cell wall thickening, deposition of phenolic compounds and formation of callose resulting in restricted growth of *F. oxysporum* f. sp. *radicis-lycopersici*. Moreover, Park *et al.* (2004) found that *Pseudomonas syringae* pv. *tomato* (Pst) induces a hypersensitive response (HR). HR is effective mechanism deployed by plants to protect themselves against various pathogens (Lam *et al.* 2001). Colonization of cucumber plants and bean roots with *Pseudomonas corrugata* increased the levels of phenylalanine ammonia lyase (PAL) Chen *et al.* (2000) found induced peroxidase (PO) activity in cucumber, which aids in the synthesis of lignin and oxidative phenols, thereby conferring resistance to plant pathogens.

Bacillus spp. activate plant's defence mechanisms by enhancing the levels of defence related enzymes like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and phenolic compounds and makes the plant resistant to pathogens (Hamedo and Makhlouf 2016). The number of *Bacillus* spp., reported as ISR inducers has grown rapidly over the last decade and include *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii, B. cereus, B. pumilus, B. mycoides,* and *B. sphaericus*. These species can elicit significant reductions in disease severity for a broad range of pathogens in a diversity of hosts (Wang *et al.* 2016). Abbasi *et al.* (2014) illustrated the capability of *Bacillus* spp. in accumulating phenolic compounds and enhancing defence enzymatic activities of peroxidase, phenyl alanine lyase, catalase, ascorbate peroxidise and declining super oxide dismutase. *B. amyloliquefaciens* induced systemic resistance against bacterial pustule pathogen.

Our results indicated that the bacterial endophytes, *P. fluorescens* and *B. amyloliquefaciens* were effectively protected the wheat plants against yellow rust pathogen. The ability of *P. fluorescens* and *B. amyloliquefaciens* to successfully manage the interaction between the host plant and pathogen has been well documented. They have also been demonstrated to enhance the defence responses in plants. Thus, as an effective biocontrol agent the use of *P. fluorescens* and *B. amyloliquefaciens* will certainly ensure sustainable disease management of yellow rust of wheat which ultimately enhanced the wheat production of the state.

Therefore the present study laid the basis of developing a biocontrol approach and showed that the bacterial endophytes *P. fluorescens* and *B. amyloliquefaciens* FDK21 played role as promising biocontrol agent to be used for the management of yellow rust of wheat.

ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Plant Pathology, PAU, Ludhiana for providing the required research facilities.

REFERENCES

Abbasi M W, Ahmed N, Zaki M J, Shuakat S S and Khan D.2014. Potential of *Bacillus* species against *Meloidogyne javanica* parasitizing eggplant (*Solanum melongena* L.) and induced biochemical changes. *Plant and Soil* **375**:159-73.

Archana S, Prabakar K, Raguchander T, Hubballi M, Valarmathi P and Prakasam V.2011. Defence responses of grapevine to *Plasmopara viticola* induced by *Azoxystrobin* and *Pseudomonas fluorescens. International Journal of Agricultural Sustainability* **3**: 30-38.

Bakker P A H M, Pieterse C M J and Van Loon L C.2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* **97**: 239–43.

Beddow J M, Pardey P G, Chai Y, Hurley T M, Kriticos D J and Braun J C.2015. Research investment implications of shifts in the global geography of wheat yellowe rust. *Nature Plants* **1:** 15132.

Beneduzi A, Ambrosini A, and Luciane M P Passaglia.2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* **35**: 1044-51.

Burrell M M and Rees T A.1974. Metabolism of phenylalanine and tyrosine in rice leaves infected by *Pyricularia oryzae*. *Physiological Plant Pathology* **4**: 497-508.

Chandrasekaran M, Subramanian D, Ee Yoon1 , Taehoon Kwon T and Se-Chul Chun S C.2016. Meta-analysis reveals that the genus Pseudomonas can be a better choice of biological control agent against bacterial wilt disease caused by *Ralstonia solanacearum*. *Plant Pathology Journal* **32**: 216-27.

Chen C, Belanger R R, Benhameu N and Paulitz T C.2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria. *Physiological and Molecular Plant Pathology* **56**: 13–23.

Hamedo H A and Makhlouf A H.2016. Biological defence of some bacteria against tomato wilt disease caused by *Ralstonia solanacearum. El Minia Science Bulletin Botany Section* **27**: 26-40.

Kaur R, Gupta A K, and Taggar A K.2014. Role of catalase, H_2O_2 and phenolics in resistance of pigeonpea towards *Helicoverpa armigera* (Hubner). *Acta Physiologiae Plantarum* **36**:1513-27.

Kulimushi P Z, Arias A A, Franzil L, Steels S and Ongena M. 2017. Stimulation of fengycin-type antifungal lipopeptides in *Bacillus amyloliquefaciens* in the presence of the maize fungal pathogen *Rhizomucor variabilis. Frontiers in microbiology* **8**:1-12.

Lam E, Kato, N and Lawton M.2001. Programmed cell death, mitochondria and the plant hypersensitive response. *Nature* **411**: 848-53.

Li H, Zhao J, Feng H, Huang L and Kang Z.2013. Biological control of wheat yellowe rust by an endophytic *Bacillus subt*ilis strain E1R-j in greenhouse and field trials. *Crop Protection* 43: 201-6.

Lowry O H, Rosebrough N T, Farr A L and Randall R J.1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-75.

M'Piga P, Belanger R R, Paulitz T C and Benhamou N.1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63–28. *Physiological and Molecular Plant Pathology* **50**:301–20.

Meena B, Radhajeyalakshmi R, Marimuthu T, Vidhyasekaran P, Doraiswamy S and Velazhahan R.2000. Induction of pathogenesis-related proteins, phenolics and phenylalanine ammonia-lyase in groundnut by *Pseudomonas fluorescens*. *Journal of Plant Diseases and Protection* **107**: 514–27.

Ongena M, Adam A, Jourdan E, Adam A, Paquot M, Brans A, Joris B, Arpigny J L and Thonart P. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology* **9**: 1084-10.

Park Y J, Lee B M, Ho-Hahn J, Lee G B and Park D S.2004. Sensitive and specific detection of *Xanthomonas campestris* pv. *campestris* by PCR using species-specific primers based on hrpF gene sequences. *Microbiological Research* **159**: 419–23.

Peterson R F, Campbell A B and Hannah A E.1948. A diagrammatic scale for estimating rust intensity on leaves and stem of cereals. *Canadian Journal of Research* 26: 496-500.

Podile A R and Kishore G K.2006. Plant growth-promoting rhizobacteria. (In) Gnanamanickam S S (ed). *Plant-Associated Bacteria*. Springer, Netherlands, pp 195-230.

Prashar M, Bhardwaj S C, Jain S.K and Datta D.2007. Pathotypic evolution in *Puccinia striiformis* in India during 1995–2004. *Australian Journal of Agricultural Research* **58**: 602–604.

Ramamoorthy V, Raguchander T and Samiyappan R.2002. Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with fluorescent pseudomonads. *European Journal of Plant Pathology* **108**: 429–41.

Reiss A and Jørgensen L N.2017. Biological control of yellow rust of wheat (*Puccinia striiformis*) with Serenade®ASO (*Bacillus subtilis* strain QST713). *Crop Protection* **93**: 1-8.

Sun D, Tao Zhuo, Xun Hu, Xiaojing F and Huasong Z.2017. Identification of a *Pseudomonas putida* as biocontrol agent for tomato bacterial wilt disease. *Biological Control* **114**: 45-50.

Swain T and Hills E.1959. The phenolic constituents of Prunus domestica The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture* **10**: 3-8.

Van Wees S C M, Van Der E N T S and Pieterse C M J.2008. Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology* **11**: 443–48.

Wang J, Liu J, Chen H and Yao J 2007. Characterization of Fusarium graminearum inhibitory lipopeptide from *Bacillus subtilis* IB. *Applied Microbiology and Biotechnology* **76**: 889–94.