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Full Length Article

Evaluating the Antagonistic Role of Fungal Endophytes against Leaf Rust of Wheat Caused by *Puccinia recondita*

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Abstract

Excessive use of pesticides has caused agricultural and environmental hazards. Microbial inoculation is an alternate to pesticides for confronting pathogens and is an environmental friendly approach. In this study, potential of fungal endophytes to control leaf rust in wheat (*Triticum aestivum* L.) was evaluated. *In vitro* efficacy of the fungal endophytes isolated from different desert plants was evaluated and the best four namely *Piriformospora indica*, *Trichoderma viride*, *Acremonium lolii* and *Colletotrichum lindemuthianum* were selected. Seeds of two rust susceptible wheat genotypes namely Faisalabad-85 and Aas-02 were inoculated by dipping in four endophytic spore suspensions and were sown using randomized complete block design under factorial arrangement. Data regarding final disease severity percentage, area under disease progress curve and coefficient of infection were recorded. Results showed that endophytic inoculated susceptible wheat genotypes exhibited the tolerance against the *Puccinia recondita*. The endophyte *P. indica* showed significant decrease in final disease severity and area under disease progress curve, resulting in 17.5% increase in grain yield gain in Faisalabad-85 and Aas-02 followed by the endophytes *T. viride*, *A. lolii* and *C. lindemuthianum* with the grain yield gain of 13.7, 08.2 and 07.1%, respectively. The present study concludes that fungal endophytes are valuable microbes which can be exploited to develop tolerance against *P. recondita* for better and sustainable wheat production.

Keywords: Disease severity; Grain yield; *Piriformospora indica*; *Trichoderma viride*; Tolerance

Introduction

Wheat serves as staple food for over 1 billion people worldwide. Wheat yield is badly affected by many biotic and abiotic factors (Jellis, 2009). Reduced yield imposes food security challenges as a result of increasing demands for wheat consumption from the same land or even decreasing area to feed the ever increasing population of the world (Anonymous, 2016-17). Rust diseases cause heavy qualitative and quantitative losses in wheat produce (Cao *et al.*, 2017). Resistant wheat varieties are compromised by the continuously evolving races of rust pathogens (Falk *et al.,* 2006). *Puccinia recondite f. spp. tritici* causes leaf rust of wheat and appears to be the most damaging pathogen that threatens global food security by inducing yield reductions in wheat (Hovmøller *et al.,* 2011). Diseased symptoms are prevalent on leaf blades, leaf sheaths and glumes along with decreased number of grains per spike and grains weight (Huerta-Espino *et al.,* 2011). Pathogenic attack in the early crop stages may result in increased yield losses up to 30% (Kolmer *et al*., 2005). Yield losses may reach up to 70% due to susceptible genotypes, early infections, high inoculum density and accelerated multiplication of pathogen (Chen, 2005).

To minimize the yield losses in wheat caused by different pathogens and abiotic stresses, there is need to find sustainable and environment friendly approaches to minimize the use of pesticides in cereals. Among the other approaches, control of pathogens through biological means with the use of endophytes is a cost effective and environment safe approach. Endophytes are metabolically active microbes (fungi, bacteria or virus) that colonize healthy plant tissue intra and intercellular without causing any apparent disease symptoms (Reinhold-Hurek and Hurek, 2011; Hardoim *et al.*, 2015). The beneficial effects of endophytes on plants against diseases have increased the interest of researchers and farmers for enhancing agricultural production. Endophytes induce defence mechanisms in host plants against pathogen attack by producing bioactive organic compounds, secondary and antimicrobial metabolites that resist pathogens (Redman *et al.,* 2011; Ambrose and Belanger, 2012; Gond *et al.,* 2015).

Endophytes also play role in their hosts for better adaptability and systemic resistance, augmenting nutrient uptake, stress tolerance and pathogenic tolerance or resistance (Hamilton *et al*., 2010; Kavamura *et al*., 2013).

There is little information available on the fungal endophytes potential regarding confronting pathogen and developing disease tolerance in wheat. The present study aimed at evaluating the potential of fungal endophytes to confront the leaf rust pathogen *P. recondita* by inducing tolerance in wheat. This study was designed to isolate a variety of fungal endophytes from desert plants and their antagonistic capacities were assessed by applying efficient of them to susceptible wheat genotypes in artificially inoculated diseased conditions.

Materials and Methods

Experimental Site and Sowing Conditions

Fifty local wheat genotypes were sown by hand drill for screening against leaf rust in research area of Department of Plant Pathology, University of Agriculture Faisalabad during 1^{st} week of December, 2014. Each genotype was planted in plot size of 1.2 m × 2.5 m and the experimental plots were surrounded by planting three rows of highly susceptible genotype Morocco. Inoculation was done artificially by means of various methods like dusting with talcum powder, rubbing, spraying with distilled water and needle injection methods on Morocco twice in a week at tillering and heading stage for the development of a heavy rust infection pressure (Hussain *et al.,* 2015).

Data Recording of Leaf Rust

Disease severity of leaf rust in percentage and host response was recorded by modified Cobb's scale described by Peterson *et al*. (1948). Disease severity was recorded four times with 10 days interval when Morocco showed 40-50% rust severity. Rating of the final disease severity (FDS) was when Morocco showed 90-100% disease severity. The values of coefficient of infection (CI) were calculated by the equation described by Pathan and Park (2006). Area under disease progress curve (AUDPC) was estimated for each genotype by Pandey *et al.* (1989).

$$AUDPC = d\,[1/2\,(y_1 + y_k) + (y_2 + y_3 + - - - - - + y_{k-1})]$$

Where, d= days between two consecutive records (time intervals).

$y_1 + y_k$ = Sum of the first and last disease records

$y_2 + y_3 + - - - - - + y_{k-1}$=Sum of all in between disease scores.

Isolation of Fungal Endophytes

Samples of naturally occurring healthy leaves, roots and stems were randomly taken from the desert plants from 3 to 5 plants per site from various locations of Cholistan, Thar and Rohi Deserts. Samples were shifted to the lab through ice bucket, stored in refrigerator and were used for isolation of endophytes within 72 h. Samples were sterilized in 1% (v/v) sodium hypochlorite solution and with distilled water for 3 times. By means of aseptic technique, 2-3 cm pieces placed on 10% PDA in Petri plates and incubated at 28°C for 6-8 days to let the emergence of endophytic fungi. Pure culture was obtained by the sub culturing of isolated fungi. Fungal identification methods were based on the morphological characteristics of their colonies (Najjar, 2007). The shape and size of conidia and phailides were calculated and also compared the micro and macro morphological features to the identification key (Hanlin, 1990; Barnett and Hunter, 1998; Pitt and Hocking, 2009).

Optimization of Efficient and Compatible Fungal Endophytes

Spores of many endophytic fungi were harvested in distilled water by rubbing the surface of a sporulating pure culture with a sterile bent glass rod and maintained the spore suspension of $1{\times}10^6$ mL by dilution method. Germinating wheat seed were kept in test tubes containing 0.3% agar concentration in distilled water with fungal spore suspension of $1{\times}10^6$ mL and were incubated. After suitable intervals, root and shoot length of wheat seedlings were measured for investigating the efficacy of fungal endophytes. Consequently four best endophytes were selected for further experimentation.

In-Vivo Potential of Fungal Endophytes

Seeds of two selected leaf rust susceptible genotypes of wheat were soaked separately for 24 h in spore suspensions of four selected (from lab experiments) efficient and compatible endophytes. During last week of November, 2015, these seeds were sown under randomized complete block design through factorial arrangement repeated thrice and untreated as control. Inoculation was done artificially as performed in screening experiment. The FDS (%), AUDPC value, 1000-grain weight (g), Grain yield (g^{-2}) and yield increased (%) were measured for assessing the potential of fungal endophytes against leaf rust pathogen *P. recondita* as well as their symbiotic response for rust susceptible genotypes in disease vulnerable conditions. The endophytes were re-isolated and identified from the inoculated plants to confirm the colonization of the fungal endophytes in plant tissues.

Statistical Analysis

Data were analysed using analysis of variance (ANOVA) and Dunkun's New Multiple Range Test (DNMRT), Tukey's test at 5% probability level in screening experiment and Least Significant Difference (LSD) test for other experiment (Steel *et al*., 1997).

Results

Leaf Rust Susceptibility of Wheat Genotypes

The final disease severity of wheat leaf rust on 50 local wheat genotypes ranged from 30-80% (Table 1). The genotypes of Punjab-11, Faisalabad-85 and Aas-02 showed the highest final disease severity of 80% followed by Sehar-06 and Wafaq-01 (Table 1). In the same way, the highest values of area under disease progress curve (AUDPC) and coefficient of infection (CI) were recorded in genotypes of Punjab-11 (1600, 76.0), Aas-02 (1550, 77.6), Faisalabad-85 (1350, 77.6) and Sehar-06 (1300, 63.6) respectively and were designated as susceptible (Table 1), whereas Shafaq-06, Kohistan-97 and Gomal-08 depicted the coefficient of infection (CI) values of 12.0,14.6 and 14.6 and also the minimum area under disease progress curve (AUDPC) value of 300, thus, were resistant to the pathogen of the leaf rust (*Puccinia recondita*). The rest of the genotypes had the range of values from 450 to 850 were ranked as moderately resistant to susceptible.

Changes in Disease Tolerance with Fungal Endophytes

From a variety of endophytes, the four endophytes namely *Piriformospora indica*, *Colletotrichum lindemuthianum*, *Trichoderma viride* and *Acremonium lolii* were selected as efficient for antagonistic role against pathogens which were derived from *in-vitro* evaluation. The effect of these endophytes on leaf rust susceptible genotypes Faisalabad-85 and Aas-02 in artificially provided disease conditions during 2015-16. These susceptible genotypes showed significant results with fungal endophytes.

Endophytes application at sowing time enhanced 1000-grains weight and grain yield by reducing the final disease severity (FDS) and area under disease progress curve (AUDPC) values of rust susceptible wheat genotypes Faisalabad-85 and Aas-02 under disease conditions as compare to control. Fungal endophytes effectively reduced disease severity as 45% FDS was observed in *P. indica* and *T. viride* followed by *A. lolii* (55%) and *C. lindemuthianum* (60%), respectively (Table 2). Among the tested wheat genotypes, Aas-02 showed least final disease severity (FDS) (56%) than that of Faisalabad-85 (58%) (Table 2). In the same manner minimum area under disease progress curve (AUDPC) value (525.0) was observed in *P. indica* followed by *T. viride* (738.3), *A. lolii* (875.0) and *C. lindemuthianum* (908.3), compared with control (no endophyte application) to the leaf rust susceptible genotypes (Table 2).

Symbiotic effects of fungal endophytes with susceptible genotypes and antagonistic effect for leaf rust fungal pathogen *P. recondita* were contributed significantly for stabilizing wheat plant against leaf rust through confronting the disease attack by decreasing disease severity resultantly improved thousand grain weight and greater grain yield (Table 2). *P. indica* showed significant performance by enhancing 17.5% final grain yield comparing to control followed by *T. viride* 13.7%, *A. lolii* 8.2% and *C. lindemuthianum* 7.1% in leaf rust conditions. Both grain weight and yield were effectively increased by the association of *P. indica* and *T. viride* while the *A. lolii* and *C. lindemuthianum* showed moderate performances in causing tolerance against leaf rust pathogen.

Discussion

Although some of the reports confirm wheat growth enhancement by the exogenous use of *P. indica* and validated that its inoculation augmented the defence mechanisms in wheat, conferred disease tolerance and increased wheat yield and productivity (Shahabivand *et al.*, 2012; Yaghoubian *et al.*, 2014), but none is available in previous studies where *P. indica* and other fungal endophytes confronted wheat leaf rust. In this study, use of fungal endophytes improved the wheat growth and grain yield in disease conditions. Fungal endophytes application proved highly effective in enhancing the yield of susceptible genotypes (Faisalabad-85 and Aas-02). *P. indica* showed best beneficial results followed by *T. viride*, *A. lolii* and *C. lindemuthianum* for inducing tolerance against *P. recondita*. Results of this study confirmed better grains weight and yield linked predominantly to the reduced disease severity of the fungal endophytes inoculated wheat plants. Increased photosynthetic area and net assimilation efficiency of plant implied the prime antagonistic role of fungal endophytes. Reduced disease severity reasons of greater surface area for producing and partitioning of photoassimilates towards reproductive growth resultantly improved grains weight and yield.

The antagonistic role of fungal endophytes against leaf rust pathogen *P. recondita* contributed appreciably for alleviating susceptible wheat plant under disease conditions through inducing disease tolerance. Likewise, Rodriguez *et al.* (2009) and Suryanarayanan *et al.* (2009) reported antagonistic effects of *C. lindemuthianum* in tomato plants with improved disease tolerance and enhanced growth and biomass as observe in this study. Many studies reported the antagonistic effects of *T. viride* as well as other *Trichoderma spp.* for conferring beneficial effects to host plants and managing different diseases (Mastouri *et al.*, 2010; Montero-Barrientos *et al.*, 2010; Shoresh *et al.*, 2010).

According to Rabiey and Shaw (2016) application of *P. indica* reduced 70% disease severity of Fusarium head blight, increased 1000-grains weight and grain yield in wheat. The average increase of 1000-grains weight and grain yield were reported 24.2 and 17.3%. In another study, use of *P. indica* at sowing time reduced the disease severities of yellow rust, powdery mildew and septoria leaf blotch by 29, 63 and 65%, respectively. Consequently, it also increased wheat grain yield by 25, 48 and 27%,

Table 1: Impacts of wheat leaf rust on final disease severity, area under disease progress curve and coefficient of infection in field conditions

Genotypes	FDS (%)	AUDPC	CI	IR
Pb-11	80	1600	76	S
Fsd-85	80	1350	78	S
Aas-02	80	1550	78	S
Wafaq-01	70	875	63	S
Saher-06	70	1300	64	S
Parsab-08	60	825	51	S
Fsd-83	60	725	50	S
Fsd-08	60	950	50	S

FDS= Final disease severity, AUDPS= Area under disease progress curve, CI= Coefficient of infection, IR= Infection response

Table 2: Antagonistic effects of fungal endophytes confronting wheat leaf rust pathogens *P. recondita* in field conditions

Fungal endophytes	FDS (%)			AUDPC			TGW (g)			GY(gm^{-2})			YI (%)		
	Fsd-85	Aas-02	Mean	Fsd-85	Aas-02	Mean	Fsd-85	Aas-02	Mean	Fsd-85	Aas-02	Mean	Fsd-85	Aas-02	Mean
Control	80.0	80.0	80.0a	1350.0	1450.0	1400.0a	34.7	36.8	35.7c	236.6	242.6	239.6d	0.0	0.0	0.0d
C. lindemuthianum	60.0	60.0	60.0b	925.0	891.7	908.3b	37.2	38.8	38.0b	257.6	258.3	258.0c	8.1	5.9	7.1c
A. lolii	60.0	50.0	55.0bc	900.0	850.0	875.0b	37.0	38.8	37.9b	259.6	262.3	261.0c	8.8	7.4	8.2c
T. viride	50.0	50.0	50.0c	733.3	743.3	738.3b	38.8	40.5	39.7a	269.0	287.3	278.1b	12.0	15.5	13.7b
P. indica	40.0	40.0	40.0d	525.0	525.0	525.0c	39.5	41.2	40.3a	280.3	301.3	290.8a	15.5	19.4	17.5a
Mean	58.0a	56.0a		886.6a	892.0a		39.2a	37.4b		260.6b	270.4a		11.1a	12.1a	

FDS= Final disease severity, AUDPS= Area under disease progress curve, TGW= Thousand grains weight, GY= Grain yield, YI= Yield increased

respectively (Rabiey, 2015). Thus, considering alternative of chemical pesticides, use of fungal endophytes is beneficial in achieving better and sustainable wheat yield from leaf rust vulnerable areas.

Conclusion

Fungal endophytes can protect wheat from damage caused by *P. recondita* by reducing the disease severity and consequently enhance the grain yield under field conditions. The findings of this study suggested that *P. indica*, *T. viride*, *C. lindemuthianum* and *A. lolii* inoculation could induce tolerance against leaf rust in wheat plants.

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