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Inducing systemic resistance in cotton plants against charcoal root rot pathogen using indigenous rhizospheric bacterial strains and chemical elicitors

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ABSTRACT

This study was carried out to screen some indigenous rhizospheric bacterial strains and chemical elicitors for their capacity to antagonize and induce systemic resistance to control charcoal root rot of cotton under both greenhouse and field conditions. Twenty rhizospheric bacterial strains and five chemical elicitors were used for resistance induction under greenhouse evaluations. Two *Bacillus* strains viz., *B. megaterium* ZMR-4 and *B. subtilis* IAGS-174 and one chemical elicitor Benzothiadiazole provided highest control over charcoal rot under greenhouse conditions. Increase in quantities of defense related biochemicals as total phenolics and enzymes including peroxidases polyphenoloxidases and phenylalanine ammonia lyase confirmed induced systemic resistance (ISR) phenomenon in cotton plants treated with selected bacterial strains and chemical inducers. Talc based formulations of these two strains were prepared to assess their effectiveness under field conditions. These not only provided protection against charcoal root rot, but also markedly enhanced growth and fruit yield of plants under field conditions. The study clearly indicated the significance of *B. subtilis* IAGS-174 and *B. megaterium* ZMR-4 along with Benzothiadiazole for inhibition of charcoal root rot and growth promotion of cotton in our cultivation system.

1. Introduction

Scientists have described over fifty species of the genus Gossypium and all of these have the ability to grow in a wide range of arid and semi-arid regions of the world's tropical and sub-tropical areas. Cotton (*Gossypium hirsutum* L.) is a very important cash crop that is cultivated all over the world. According to the United States Department of Agriculture, 117.1million bales were produced during the year 2013–2014 (USDA, 2014). Pakistan is a country with an agriculture based economy and cotton plays a significant role in it. *Gossypium hirsutum* contributes 1.5% to the GDP and 7% in value added goods in Pakistan. Cotton is grown on 2.879 million hectares that produces 13.02 million bales (GoP, 2013). To boost the economy of Pakistan there is a need to improve the health of cotton plants and its yield as well. According to an evaluation, one million bales of cotton production will increase 0.5% in country's GDP (GoP, 2013).

All living organisms including plants face infections and diseases caused by different pathogens. Yield losses in crops due to pathogens infections range between 20% and 40% (Savary et al., 2012). *Macrophomina phaseolina* (Tassi) Goid is the cause of charcoal root rot disease and has a wide host range. This pathogen is responsible for causing diseases in more than 500 cultivated and wild plant species (Indera et al., 1986). In Pakistan 67 economic hosts for *M. phaseolina* including cotton, rice, maize, cucurbits, okra and wheat have been reported (Mirza and Qureshi, 1978; Shehzad et al., 1988).

Macrophomina phaseolina has been reported on cotton in the southeastern United States of America, Oklahoma, and Texas (Watkins, 1981), but is of little economic significance there compared with the Indian subcontinent, East and Central Africa, and elsewhere in the tropics and sub-tropics. The severity of this disease in Pakistan, India, Sudan, and Central Africa may be related to soil moisture deficit and hot weather (Watkins, 1981).

The sclerotia of *M. phaseolina* survives in the soil, crop residues and on seeds. Numerous disease management approaches are available such as resistant varieties, crop rotation, cultural practices, soil solarization and minimum supply of soil moisture to diminish the disease incidence. However, these approaches require highly proficient accuracy in measurements as well as long time. Crop rotation is not considered efficient,

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since the fungus has competitive saprophytic ability (Almeida et al., 2001). In the absence of resistant germplasm against virulent strains, utilization of systemic fungicides is the only potential approach to diminish the inoculum density of the pathogen (Bashir, 2018). While public concerns about synthetic chemicals are growing there is a dire need to explore some other ecofriendly strategies to control these problematic pathogens. Induction of systemic resistance in crop plants can be such a safe option.

Plant defense mechanism can be initiated by external agents before infection (Pieterse and Van Loon, 1999; Stadnik, 2000) and is known as induced systemic resistance (ISR). Both biotic and abiotic factors have been used effectively for inducing ISR in plants against different plant pathogens (Akram and Anjum, 2011). ISR has been well documented by earlier workers for plant protection under greenhouse and field conditions by producing antimicrobial compounds and pathogenesis-related proteins (Cachinero et al., 2002; Shoresh et al., 2005). Biocontrol of soil and seed borne diseases is considered as an efficient strategy for their management (Thakore, 2006; Kavino et al., 2007). Plants in presence of plant growth promoting rhizospheric bacteria have not only shown better growth but suppression of various diseases in comparison to the plants grown in absence of these beneficial microbes (Asghar et al., 2002; Vessey, 2003; Gray and Smith, 2005; Silva et al., 2006; Figueiredo et al., 2008; Sharma et al., 2018). Different bacterial genera e.g., *Bacillus, Streptomyces, Pseudomonas* and *Agrobacterium* have been reported as biological control agents against many plant diseases (Lucy et al., 2004; Jangir et al., 2018). These bacteria control diseases through mechanisms like producing antibiotics and siderophores that leads to activation of systemic resistance (Egamberdiyeva, 2005; Mhlongo et al., 2018).

Many synthetic chemical elicitors have also been effectively used to control different diseases and to increase plant growth. Dann et al. (1998) showed that application of 1,2,3 Benzothiadiazole can control white mold caused by *Sclerotinia sclerotiorum.* Many researchers have been demonstrated that treatment with Isonecotinic acid and 1,2,3 Benzothiadiazole can induce resistance against fungal, bacterial and viral pathogens as these chemicals enhanced the accumulation of mRNAs for pathogenesis-related proteins (Ward et al., 1991; Uknes et al., 1992; Lawton et al., 1996; Friedrich et al., 1996). Similarly in 1996, Gorlach et al. successfully used Benzothiadiazole for treating wheat diseases caused by *Erysiphe graminis f. sp. tritici, Puccinia recondita* and *Septoria* sp. Keeping this in view the intention of this study was to screen different rhizospheric bacterial strains and chemical elicitors for their ability to induce systemic resistance in cotton against charcoal root rot disease and elucidation of the mechanism behind the induced resistance.

2. Materials and methods

2.1. Isolation of pathogen and bacteria

Twenty rhizospheric bacterial strains (Table 1) belonging to eight different species were procured from First Fungal Culture Bank (FCBP) of Pakistan and Bacterial Conservatory of Institute of Microbiology and Molecular Genetics, University of the Punjab Lahore. Strains FBL-01 to FBL-12 were isolated from soil of cotton growing fields of various agro-ecological regions of Punjab, Pakistan and identified to species in the Fungal Biotechnology laboratory; Institute of Agricultural Sciences, Punjab University. Bacterial inoculum was prepared by growing in Luria Broth (Merck, Germany). Media containing bacterial growth was centrifuged and the pellet was resuspended in sterile distilled water to obtain the final bacterial concentration of 104 cfu/mL with an OD of 0.8 at 600 nm. A virulent strain of *M. phaseolina* was isolated from infected cotton plants (MNH-886) collected from fields. Pathogen inoculum was prepared by harvesting both micro- and macro-conidia from seven days old cultures grown on sterile potato dextrose agar (Merck, Germany) at concentration of 1×10^3 conidia/mL, by haemocytometer.

Table 1
Assay to evaluate potential of rhizospheric bacterial strains to antagonize *Macrophomina phaseolina* in vitro.

Rhizospheric bacteria	Strains	% inhibition
Pseudomonas putida FBL-04	RB1	48.13 ± 05.14^{F}
Pseudomonas putida FBL-05	RB2	38.37 ± 03.03^{F_I}
Pseudomonas putida FBL-01	RB3	74.10 ± 06.51^{AB}
Bacillus megaterium MRC-8	RB4	73.37 ± 07.21^{AB}
Bacillus fortis IAGS-324	RB5	31.61 ± 02.77^{LJ}
Bacillus thuringiensis IAGS-199	RB6	41.82 ± 04.13^{F_H}
Bacillus megaterium ZMR-4	RB7	78.77 ± 07.45^{A}
Pseudomonas aeruginosa FBL-03	RB8	57.55 ± 07.51^{C-E}
Pseudomonas aeruginosa PM-12	RB9	32.72 ± 03.20^{LJ}
Bacillus fortis IAGS-162	RB10	77.85 ± 07.32^{A}
Pseudomonas fluorescens FBL-02	RB11	61.91 ± 04.32^{CD}
Pseudomonas fluorescens FBL-07	RB12	44.51 ± 03.88^{FG}
Pseudomonas fluorescens FBL-08	RB13	28.44 ± 02.97^{I_K}
Pseudomonas fluorescens FBL-09	RB14	57.36 ± 05.18^{C-E}
Pseudomonas fluorescens FBL-10	RB15	27.05 ± 02.77^{I-L}
Bacillus subtilis IAGS-174	RB16	80.02 ± 06.18^{A}
Bacillus subtilis IAGS-170	RB17	64.81 ± 05.61^{B-D}
Bacillus amyloliquefaciens FBL-11	RB18	63.28 ± 06.31^{B-D}
Rhizobium etli FBL-06	RB19	67.60 ± 05.07^{BC}
Pseudomonas aeruginosa FBL-12	RB20	70.51 ± 06.43^{BC}

Table represents mean value analyzed statistically using DNMRT, values with the same letter showed non-significant difference at P ≥ 0.05.

2.2. Screening of rhizospheric bacterial strains for their potential to antagonize charcoal root rot pathogen

This experiment was performed to check the antagonistic activity of isolated rhizospheric bacteria against *M. phaseolina*. For this purpose dual culture assay was performed. After one week of incubation at 26 ± 1 °C, percentage growth of inhibition (PGI) caused by the antagonistic rhizospheric bacteria was calculated by using the following formula:

$$PGI\ (\%) = [(KR\text{-}R1)/KR] \times 100$$

Where KR represents the distance (mm) from the inoculation point to the colony margin on the control dishes, and R1 is the distance of fungal growth from the point of inoculation to the colony margin on the treated dishes in the direction of the antagonist.

2.3. Integrated management of charcoal root rot of cotton by using biological and chemical inducers under greenhouse conditions

This experiment was conducted in a 20 × 22 ft greenhouse in September 2015. Pathogen inoculum (50 mL) was given to each pot before seed sowing to establish the pathogen. For the management of charcoal root rot, three seeds of commonly grown cotton varieties (FH-142 and MNH-886) were sown in each plastic pot containing sterilized sandy loam soil. The bacterial inoculum was also provided @ 50 mL per pot at the time of sowing.

A second experiment was conducted on cotton seedlings with five selected chemical inducers (Dichloroisonicotinic acid; Benzothiadiazole; Beta amino butyric acid; Isonicotinic acid; Chitosan) in concentrations of 0.5, 1, 2.5 and 5 mM to determine the best performing chemical inducer against the pathogen. 50 mL of each inducer was sprayed on cotton seedlings of each pot at the 3-leaf stage. The temperature conditions were kept as 30/25 ± 2 °C (day/night) with 70% relative humidity during the study period, while the photoperiod was maintained at a 16/8 h light/dark regime during the experiment by exposing the plants to sunlight and artificial light. All 140 pots were watered on alternate days as required. The trial was based on randomized complete block design with five replications for each treatment. Data recording was done after one month of incubation under greenhouse conditions. A scale developed by Rothrock (1987) was used to rate root