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ORIGINAL ARTICLE

Higher induction of defense enzymes and cell wall reinforcement in maize by root associated bacteria for better protection against *Aspergillus niger*

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Abstract

Root associated bacteria were isolated from *Suaeda nudiflora* and two isolates were selected for this study: rhizospheric *Bacillus megaterium* and endophytic *Pseudomonas aeruginosa*. These isolates were inoculated into maize variety Narmada Moti during its germination. TTC (2, 3, 5-triphenyl tetrazolium chloride) staining was used to confirm the association of the isolates with the maize root. The effects of these root associated bacteria were tested alone and in combinations for cell wall reinforcement and the induction of defense enzymes such as phenylalanine ammonia lyase (PAL) and β-1,3-glucanase in the presence of fungal pathogen *Aspergillus niger* in maize. The results indicated that the rhizospheric bacteria had a greater fight response to fungal infection than the endophhytic bacteria due to cell wall lignification as well as the rapid induction of higher concentrations of defense related enzymes.

Keywords: *Aspergillus niger*, β-1,3-glucanase, biotic factor, phenylalanine ammonia lyase, RAPD analysis, rhizosphere, root associated bacteria

Introduction

The narrow zone of soil around plant roots, specifically influenced by plant root systems, is known as the rhizosphere. It is rich in nutrients due to the accumulation of a variety of plant exudates which have various sugars and amino acids that are sources of nutrients and energy for bacteria and other microorganisms (Gray and Smith 2005). The quality and quantity of plant nutrients determine the number of bacteria around the roots of plants, and is generally 10 to 100 times higher than the bulk soil. A diverse range of microorganisms and bacteria colonizing in the plant rhizosphere is generally known as root associated bacteria. Such bacteria have diverse effects on plants and are accordingly classified into beneficial, deleterious and neutral groups. The growth and development of plants are affected by numerous biotic and abiotic factors (Vacheron *et al.* 2013).

Such factors include the deleterious effects of pathogenic microorganisms which are a serious menace to plant growth and yield. Agricultural production has become more intensified to fulfill the requirements of the world's growing population. Farmers have intensified the use of agrochemicals as a relatively reliable method to protect crops. Nevertheless, enhanced use of chemicals leads to indiscriminate resistance, environmental pollution and impacts several other non-targeted organisms. It also results in high levels of chemicals in food chains, which have harmful effects on economically valuable vegetation as well as on human health (Damalas *et al*. 2011).

For self-protection, different defense mechanisms are induced by plants against pathogen attacks. But some root colonizing nonpathogenic bacteria also activate the defense mechanism of plants known as

induced systemic resistance (ISR) (van Loon 2007). Induced systemic resistance starts from roots and extends up to the shoots of a plant and is a plant mediated mechanism. It has been found in several plant species like bean, maize, radish, tomato and cucumber, etc. Such resistance is effective against a wide variety of plant pathogens, ranging from bacteria, fungi, viruses and also to insects (Pieterse *et al.* 2014). By modifying the mechanical strength of the cell wall, modulating various biochemicals and physiological interactions of the plant and stimulating the production of defense chemicals against the pathogen, root associated bacteria provide overall protection of the plant under biotic stress by acting as strong elicitors of plant defense reactions. Such a multilayer protection strategy induced by root associated bacteria which have potential defensive capacity is known as "priming" (Nie *et al.* 2017). Such priming is an economical mechanism induced by root associated bacteria to activate plant defense prior to pathogen infection in a more efficient way upon pathogen attack (Pastor *et al.* 2013). ISR induced by root associated bacteria improves the sensitivity of a target molecule rather than its biosynthesis. The priming reaction due to such bacteria does not directly modify the production of phytohormones or induce resistance-related genes like phenylalanine ammonialyase (PAL) and β-1,3-glucanases in the plant host.

Phenylalanine ammonialyase (PAL) is the first enzyme of the general phenylpropanoid pathway catalyzing the non-oxidative elimination of ammonia from L-phenylalanine to give *trans*-cinnamate. The biosynthesis of phenolic compounds in plants occurs via the concerted action of the shikimate and phenylpropanoid pathways. Phenolic metabolism in plants plays important roles in providing aromatic amino acids, defense-related compounds, chemical attractants or repellents. β-1,3-glucanases are abundant in plants and play key roles in cell division, trafficking of materials through plasmodesmata, in withstanding abiotic stresses and are involved from flower formation through to seed maturation. They also defend plants against fungal pathogens either alone or in association with chitinases and other antifungal proteins. They are grouped into the PR-2 family of pathogenesis-related (PR) proteins (Jha 2018).

Beneficial root associated bacteria activate ISR of a plant by preparing the plant for possible initiation of different cellular protective responses, such as cell wall reinforcement, accumulation of defense-related enzymes, oxidative burst and production of secondary metabolites (Rahman *et al.* 2015).

Therefore, biocontrol is an effective mechanism to reduce the application of chemicals in agriculture and efficiently use root associated bacteria as a biological agent. It is also the best option for enhancing plant growth, managing soil and improving plant health. The biocontrol arbitrated by root associated bacteria is due to competition for an ecological niche or induction of ISR or production of inhibitory allelochemicals in the host plant. The use of root associated bacteria for biological control of diseases in crop plants has not yet been reported. However, it is gaining interest because it resulted in the reduction of disease frequency and an elevation in plant growth and yield without the use of agrochemicals (Jha and Subramanian 2016). The aim of this work was to study the efficiency of two root associated bacteria as biocontrol agents by analyzing their effects on cell wall reinforcement, and defense-related enzyme accumulation in maize plants infected with *Aspergillus niger*.

Materials and Methods

Isolation and molecular identification of bacteria

The root and rhizosphere soil of the plant *Suaeda nudiflora* wild mosque were used for the isolation of bacteria, according to our previously published method (Jha 2017). The molecular identification of the bacterial isolates was done by 16s rDNA analysis, using 16S rDNA primers F: 5'AGAGTTTGATCCTGGCTCAG3' and R: 5'AGGTTACCTTGTTACGACTT3' for PCR amplification, followed by sequencing (Bangalore, GeNei). The obtained sequences were compared with nucleotide database and were aligned with the CLUSTAL-W program. Using the neighbor-joining method and the maximum likelihood method in PHYLIP package, phylogenetic trees were constructed.

Maize inoculation with root associated bacteria

Seeds of maize variety Narmada Moti were obtained from the Main Maize Research Station, Godhra, Gujarat, India and inoculated with bacterial isolates as per our published methods with some modification (Jha and Subramanian 2014). Contamination of seeds with any other organism was checked by placing them on Tryptone glucose yeast agar plates for germination and observed for the effect of the isolated bacteria on the physiological and biochemical parameters. Four-day-old germinated seedlings devoid of any contamination were selected and transferred to culture tubes containing 400 µl Hoagland's nutrient medium, 400 µl micronutrients and 1% agar in 40 ml distilled water. Before the transfer, bacterial inoculums of the isolated bacteria were added to the medium at a concentration of 6×10^8 cfu · ml^{-1}. The mixtures of both bacterial cultures were obtained by mixing equal volumes of both cultures at a concentration of 6×10^8 cfu · ml^{-1}.

Association of bacteria in the maize root

The association of the bacterial strain with the maize root was established during germination and observed by TTC staining (2, 3, 5-triphenyl tetrazolium chloride). For staining, the roots were surface sterilized with sodium hypochlorite and incubated overnight in the TTC stain. The visualization of isolates in roots was done by cross sectioning of roots and examining them under an image analyzer microscope (Carl Zeiss).

Compatibility between bacterial strain and fungus

The compatibility between the bacterial strains was tested by parallel streaking of each other on Yeast Extract Glucose Agar medium and was then incubated at 28°C. The inhibition of growth or overgrowth of isolates after a period of 72 h was the indicator of compatibility. The antagonism between bacteria and fungus was also tested by the same method and inhibition of fungus growth by bacteria as a zone of inhibition showed the antagonistic effects of bacteria.

Determination of lignin and lignin monomers

The total lignin and lignin monomer content was determined in plants from each treatment in three replicates by homogenizing the sample in 50 mM sodium phosphate buffer, pH 7.0 and centrifuged for 15 min, according to Kováčik and Klejdus (2008). The pellet obtained was considered as the protein freed from cell walls and lignin was quantified using thioglycolic acid and lignin monomers were quantified using alkaline nitrobenzene peroxidation.

Lignin histochemical staining

The lignin was stained with phloroglucinol stain according to standard protocols (Nakano and Meshitsuka 1992) and observed by taking transverse sections obtained by using a vibratome from internodes of plants and visualized under an image analyzer microscope (Carl Zeiss).

Analysis of isolates as biocontrol agents under greenhouse conditions

The bacterial isolates were assessed for their efficiency in suppressing *A. niger* infection in maize under greenhouse conditions, both alone and in combination. Inoculated plants were transferred to plastic pots containing sterilized sand-perlite (1 : 1) in a greenhouse. The *A. niger* spore suspension with a spore load of 10^4 conidia · ml^{-1} was sprayed on the plants, and caused more than 75% infection under greenhouse conditions. The disease index was calculated as grades 0 to 5 (Sriram *et al.* 1999) using the formula:

Disease index = Total grade × 100/No. of diseased leaves observed × maximum grade.

Phenylalanine ammonia lyase analysis

Phenylalanine ammonia lyase (PAL) activity was measured by making a mixture of 600 µl of 1 mM L-phenylalanine in 500 µl of 50 mM Tris HCl and 100 µl of plant extract and incubated at room temperature for 60 min. The reaction was stopped by adding 2N HCl. Then the assay mixture was extracted with 1.5 ml of toluene and toluene phase containing trans-cinnamic acid and measured at 290 nm against toluene as the blank. Enzyme activity was expressed as nmol trans-cinnamic acid released $min^{-1} \cdot g^{-1}$ fresh weight.

β-1,3-glucanase analysis

The laminarin-dinitroalisalicyclic acid method was used for the analysis of β-1,3-glucanase activity; by mixing 62.5 µl of plant extract and 62.5 µl of 4% laminarin incubated at 40°C for 10 min and 375 µl of dinitrosalicyclic reagent was used to stop the reaction. The dark yellow complex which formed was diluted with 4.5 ml of distilled water and the product was expressed as 1 nmol of reducing substances $min^{-1} \cdot mg^{-1}$ of fresh weight after taking absorption at 540 nm.

Field trials asssay

The effects of selected root associated bacterial treatment (consisting of a non-inoculated control, individual *B. megaterium, Pseudomonas aeruginosa* and mixtures of both isolates) in elicitation of ISR activity against target pathogen *A. niger* in maize were studied at a local diseased agricultural field over three rainy seasons, July to October 2014–2016. A randomized complete block was designed with three different treatments in three replicates. The plots were 2 m wide, 3 m long, and separated by 1 m. There were 4 rows separated by 40 cm. Plots were periodically watered and were enclosed with green sheet.

RAPD analysis of Narmada Moti

The RAPD analysis of Narmada Moti was done with the known susceptible (Pioneer 30 v92) and resistant (Kharif Shaktiman-1) varieties of maize with genomic DNA. The DNA obtained was qualified and quantified by agarose gel electrophoresis and UV spectrophotometer. RAPD profiles were generated using 5 decameric primer (AH1, AH2, AH3, AH4 and AH5) from MWG

Bangalore India. The reaction mixture contained 2 µl of primer (0.3 µM), 1 unit Taq DNA polymerase, 0.5 µl $MgCl_2$, 2 µl 4dNTPs, 5 µl 10X assay buffer, 2 µl DNA sample (100 ng), and adjusted to a final volume of 25 µl with nuclease free water. RAPD-PCR reaction was performed in an Eppendorf thermocycler. The standard conditions used 35 cycles and were as follows: initial denaturation at 95°C for 1 min, denaturation at 95°C for 30 sec, annealing at 36°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. Amplification products were analyzed on 2% agarose gel electrophoresis.

Statistical analysis

All the data were analyzed for significance using analysis of variance ANOVA followed by Fisher's least significant difference test ($p \leq 0.05$), using SAS software (SAS Institute, Cary, NC, USA).

Results

Molecular identification of bacterial isolates

Discrete bands of 1,500 bp were obtained for both isolates on agarose gel after separation of amplified PCR product by 16S rDNA specific primer. The root associated bacteria were identified as *P. aeruginosa* (Gen--Bank Accession Number: JQ790515) and *B. megaterium* (GenBank Accession Number: JQ790514) after nucleotide homology and phylogenetic analysis.

Antagonistic effects of isolates on fungus *in vitro*

Both the root associated bacteria *B. megaterium* and *P. aeruginosa* suppressed the growth of the *A. niger* fungal pathogen on the culture plate. A clear zone of inhibition was observed in the upper quadrant of the plate around the isolates (Table 1).

Localization of bacteria in the maize root *in vivo*

A dark red spot was observed in the transverse section of the root cortex region after TTC staining under the microscope, because it stains only living cells (respiring), while dead cells of the root cortex remain colorless (Fig. 1).

Table 1. Isolates showing antifungal activity against *Aspergillus niger*

Isolate	*A. niger*	ZOI* [mm^2]
Bacillus megaterium	+++	4.1
Pseudomonas aeruginosa	++	3.2

*zone of inhibition
(++) – small zone less effective; (+++) – larger zone more effective

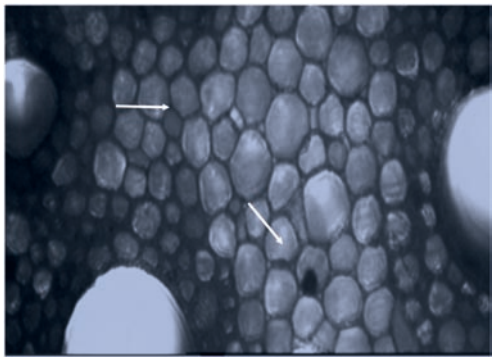

Fig. 1. Photomicrograph of a section of maize root showing the association of bacteria in the root cortex, seen as dark spot due to TTC staining

Lignin histochemical study

Transverse sections of maize root were stained with phloroglucinol. It stained almost all parts of the cells situated directly under the epidermis indicating that these parts of the cells were lignified after infection. Both isolates had similar effects on lignification (Fig. 2).

Both inoculated as well as infected maize have enhanced lignin and lignin monomers. Inoculation alone with bacterial isolates resulted in a significant increase in lignin content and infection of *A. niger* further increased lignin deposition in maize (Table 2).

Effect of biocontrol agents on maize growth parameters and disease index under greenhouse conditions

The maize plants inoculated with isolates showed considerably higher plant height, dry weight and root length. Plants showed 20% higher plant height, 15% longer root length and 11% enhanced dry weight after being inoculated with *B. megaterium.* Plants showed 30% enhanced plant height 46% longer root length and 25% higher dry weight, after being inoculated with *P. aeruginosa.* Plants had 20% higher plant height, 20% longer root length and 5% greater dry weight, when inoculated with both the isolates compared to the non-inoculated control (Table 3).

The bacterial isolates *B. megaterium* and *P. aeruginosa* as biocontrol agents considerably reduced the kernel rot of maize disease incidence under greenhouse conditions. The extent of reduction in disease incidence varied between the individual strain and mixture of bacteria *B. megaterium* and *P. aeruginosa.* For all treatments the most effective was *P. aeruginosa* which showed maximum reduction of disease

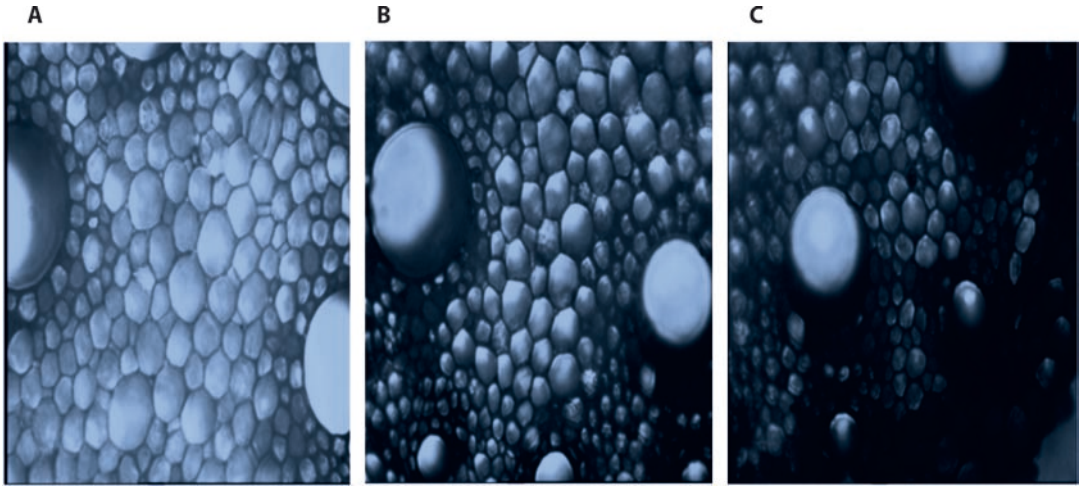

Fig. 2. Effect of root associated bacteria on lignin in maize cells after infection with *Aspergillus niger*. A – control, B – inoculated with *Pseudomonas aeruginosa*, C – inoculated with *Bacillus megaterium*

Table 2. Effect of root associated bacteria on disease index study (n = 3) in maize plants

Treatment	Disease index [%]	Lignin	Lignin monomers
Control	nill	1.124 e	0.81 e
Bacillus megaterium + pathogen	49.33	1.49 c	1.11 c
Pseudomonas aeruginosa + pathogen	58.33	1.53 b	1.29 ab
B. megaterium + *P. aeruginosa* + pathogen	66.66	1.59 a	1.31 a
Pathogen	89.74	1.32 cd	1.09 cd

Values are means of three replications. Means within columns sharing the same letters are not significantly different ($p \leq 0.05$; LSD test)

Table 3. Effect of root associated bacteria on various plant growth promoting activities in maize plants

Treatment	Germination [%]	Total plant height [cm]	Shoot weight [g]		Root weight [g]		Total biomass [g]	
			fresh weight [g]	dry weight [g]	fresh weight [g]	dry weight [g]	fresh weight [g]	dry weight [g]
Control	71.23 d	28.14 d	0.94 cd	0.23 d	0.56 d	0.11 cd	1.26 d	0.33 d
Bacillus megaterium	83.21 bc	48.26 c	1.27 c	0.38 bc	0.70 c	0.14 c	2.69 bc	0.48 c
Pseudomonas aeruginosa	86.33 b	51.21 d	2.95 ab	0.43 b	0.97 b	0.19 b	3.77 b	0.64 b
B. megaterium + *P. aeruginosa*	92.66 a	66.34 a	3.21 a	0.52 a	1.64 a	0.28 a	4.34 a	0.76 a

Values are means of three replications. Means within columns sharing the same letters are not significantly different ($p \leq 0.05$; LSD test)

incidence by reducing the disease index by 50–55%, where infection with fungus was 89% of the disease index in non-inoculated infected control maize plants (Table 2).

Induction of defense enzymes

There was a significantly enhanced ($p \leq 0.05$) enzyme level both in the presence and absence of the pathogen *A. niger*. The activity of both enzymes PAL and β-1,3-glucanase was higher in inoculated plants than in the control plants.

Induction of PAL in maize plants inoculated with *P. aeruginosa*, *B. megaterium* and infected with *A. niger* showed significant variation. Its activity was highest in plants inoculated with *B. megaterium* on the 45th day after infection, showing a threefold increase in its activity compared to the control. PAL activity was found to be more moderated in plants inoculated with *P. aeruginosa* (about one and a half fold) than in the

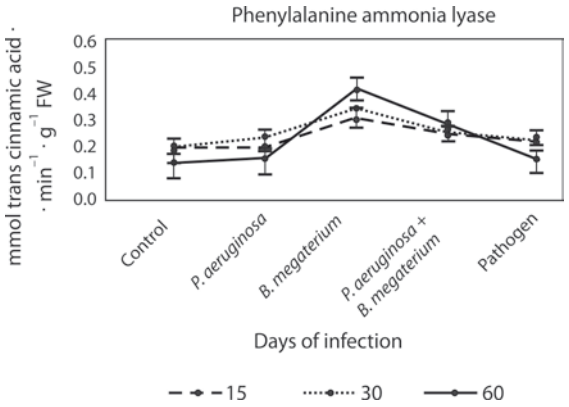

Fig. 3. Phenylalanine ammonia lyase (PAL) activity at the difference of 15 days up to 60 days of cultivation after infection with *Aspergillus niger* in maize plants. FW – fresh weight

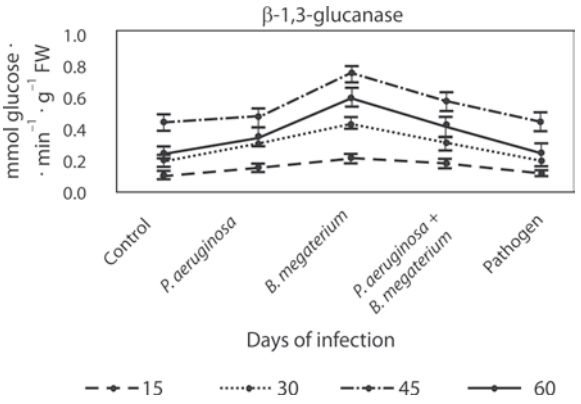

Fig. 4. β-1,3-glucanase activity at the difference of 15 days up to 60 days of cultivation after infection with *Aspergillus niger* in maize plants. FW – fresh weight

combination of both isolates (about twofold) compared to the control (Fig. 3).

In the case of β-1,3-glucanase the activity was again highest on the 45th day after infection and was about fivefold higher compared to the non-inoculated control. Its activity was highest in plants inoculated with *B. megaterium* (about fivefold), *P. aeruginosa* (about two and a half fold) and a combination of both *P. aeruginosa* and *B. megaterium* (about threefold) compared to the control (Fig. 4).

Field experiment

In the field assay, the non-inoculated control plants showed diseased symptoms 10 weeks after fungal infection, while plants inoculated with root associated bacteria showed disease symptoms only in a limited number of plants. The inoculated plants showed disease symptoms 12 weeks after infection. The disease incidence in all inoculated plants was significantly lower than the non-inoculated control. Individual isolates suppressed the disease by 64–77% while the mixture of both the isolates, by 52–54%. Plant dry and fresh weights were also recorded 45, 70 and 90 days after infection; they were 4.2–38% high in plants inoculated with *B. megaterium*, 0.6–15% in *P. aeruginosa,* and 2.7–24% in plants treated with both isolates. Similarly, enhanced yield (kg · ha^{-1}) of grain and straw was also recorded (Table 4).

RAPD analysis of Narmada Moti

The RAPD analysis of Narmada Moti (V) was done with the known susceptible Pioneer 30 v92 (S) and resistant (Kharif Shaktiman-1) (R) of maize. The PCR product was analyzed on agarose gel (Fig. 5). The tree, constructed by using Tree explorer 2.12 software, clearly indicated that the maize variety Narmada Moti (V) is closely related to susceptible variety Pioneer 30 v92 (S) (Fig. 6).

Discussion

Root associated microbes play an invisible role in plant establishment and health. When plants are invaded by microorganisms, major physiological changes occur which induce plant defense enzymes including PR

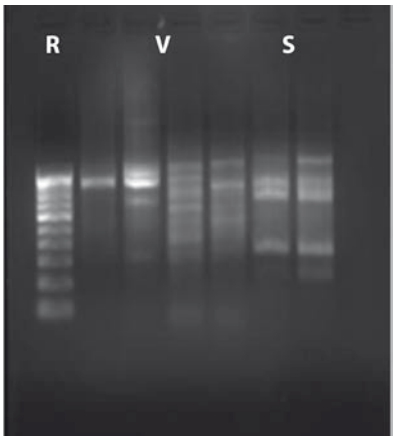

Fig. 5. Agarose gel of RAPD analysis with primer AH4 for Narmada Moti (V) with the known susceptible Pioneer 30 v92 (S) and resistant (Kharif Shaktiman-1) (R) of maize variety

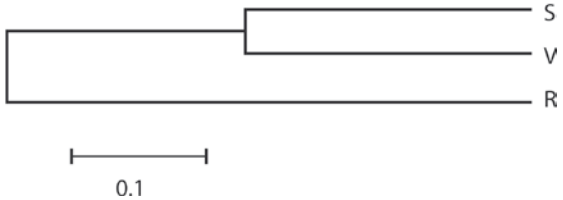

Fig. 6. The phylogenetic tree constructed using Tree explorer 2.12 software indicated that the maize variety Narmada Moti (V) is closely related to the known susceptible Pioneer 30 v92 (S); the resistant maize variety (R) – Kharif Shaktiman

Table 4. Effect of root associated bacteria as a biocontrol agent on maize against disease and yield in a field trail

Treatment	Disease incidence [%]	Plant dry weight/plant [g]			Yield [kg · ha^{-1}]	
		45 days	70 days	90 days	grain	straw
Control	100	0.215 a	0.628 a	0.889 a	734 a	1,313 a
Bacillus megaterium + pathogen	64	0.221 ab	0.697 b	1.247 b	941 b	1,550 b
Pseudomonas aeruginosa + pathogen	77	0.257 d	0.888 cd	1.674 d	993 d	1,673 cd
B. megaterium + *P. aeruginosa* + pathogen	58	0.242 c	0.880 c	1.433 c	922 c	1,576 bc

Values are means of three replications. Means within columns sharing the same letters are not significantly different ($p \leq 0.05$; LSD test)

proteins, which leads to the inception of induced systemic resistance in plants (Sudisha *et al.* 2012). Root associated microbial metabolites may activate the systemic resistance in plants under stress. In this study the bacterial association with the maize root was assured by TTC staining and the formation of a clear zone indicated that the *in vitro* growth inhibition of *A. niger* was associated with *in vivo* pathogenicity repression. The results showed that *B. megaterium* formed a larger zone of inhibition in its vicinity than *P. aeruginosa.* Hotterbeekx *et al.* (2017) established a direct relationship in radish between *in vitro* antagonism and *in vivo* disease reduction by *Pseudomonas,* similar to our observations in this study. This is also supported by the observation of the biocontrol assay, which showed that *in vivo* disease repression was directly proportional to *in vitro* growth suppression. The results of biocontrol assay indicated that root associated bacteria individually or in combination drastically reduced the disease frequency, but *B. megaterium* showed enhanced growth parameters such as plant height, root length, and biomass as well as maximum reduction in disease frequency. Root associated bacteria directly involved in siderophore production, which capture iron, made it unavailable for pathogens. Anti-fungal metabolites, like antibiotics, hydrogen cyanide or fungal cell wall – lysis enzyme, which restrict the growth of fungal pathogens are also produced. Their ability to effectively contend for nutrients or specific niches on the root or to induce systemic resistance against pathogens was also reported by Pieterse *et al.* (2014).

For millions of years, there has been a co-evolutionary battle between microbes and plants. Plants have developed a multifaceted defense system in which the development of cell wall is important as it serves manifold purposes in plant defense. It may serve as an active defense barrier as well as a structural barrier against plant pathogens. To establish the preferred pathogenic relationship, microbes have to cross the barrier of the cell wall of the host plants (Turra *et al.* 2015). An important cell wall component, lignin or lignin-like phenolic polymers, are frequently hastily deposited in response to pathogen attack and have numerous roles in plant protection. In the present study, histochemical analysis of maize root sections showed remarkable variation in lignin deposition in inoculated and non-inoculated plants after infection with *A. niger*. The phenylpropanoid pathway accountable for lignin biosynthesis is for defense purposes, and generates a physical barrier against pathogen incursion (Malinovsky *et al.* 2014). Accumulation of pathogenesis-related (PR) proteins is generally associated with systemic acquired resistance (SAR) in plants, but it is also induced in plants upon treatment with non-pathogenic bacteria. Root associated bacteria mediated ISR resembles that of pathogen induced SAR in that both types of induced resistance render uninfected plant parts more resistant towards a broad spectrum of plant pathogens (Choudhary *et al.* 2008). Induction of numerous defense enzymes like PAL, PPO and β-1-3-glucanase takes place by root associated bacteria to induce systemic resistance in plants. In the present study, augmented PAL activity was observed in the inoculated plants after being infected with the pathogen *A. niger. B. megaterium* has superior ability to *P. aeruginosa* to induce defense enzymes in treated maize plants under biotic stress. The biosynthesis of lignin is initiated from L-phenylalanine and induction of PAL at the early stages of development plays a critical role in activated defense. In the transformation of L- phenylalanine to trans-cinnamic acids, PAL is the first enzyme of this pathway which catalyzes the trans-elimination of ammonia from L-phenylalanine (Duan *et al.* 2014). It is the rate-limiting enzyme for the production of secondary metabolites and it is also responsible for phenolics and phytoalexin production. Therefore, it plays a critical role in plant stress management.

The β-1,3-glucanase is well known as a protective enzyme of plants. In the present study, plants inoculated with root associated bacteria showed elevated β-1,3-glucanase activity in the presence of *B. megaterium* followed by both isolates simultaneously with PAL enzyme. Fluorescent *Pseudomonas* stimulates systemic resistance by inducing production of protective enzymes and genes in numerous plants (Shi *et al*. 2017). The β-1,3-glucanase and chitinase like

enzyme induction by root associated bacteria are responsible for the development of systemic resistance in plants against a wide range of pathogens. Such protective enzymes have the potential for the hydrolysis of fungal cell walls. The RAPD analysis also clearly indicated that maize variety Narmada Moti (V) was susceptible to kernel rot of maize as it was closely related to the susceptible variety Pioneer 30 v92 (S). But it is able to survive after infection with pathogen *A. niger* due to association of root associated bacteria with the plant.

The field experiment also showed that *B. megaterium* has a better ability to protect plants from the pathogen than *P. aeruginosa*. At the same there was also higher dry mass and yield in the plants inoculated with *B. megaterium*. Delshadi *et al.* (2017) reported that a combination of root associated bacteria increased *Onobrychis sativa* L. yield under stress. Our field trial results were not very different than the greenhouse bioassay.

The enhanced activity of PAL and β-1,3-glucanase in plants in the presence of *B. megaterium* may be due to superior connection of such bacteria with plant roots. Our results indicated the potential use of root associated bacteria like *B. megaterium,* which helps to stimulate protective enzymes in the juvenile stage of the plant, will persist for long time to protect plants against various pathogens. Such activation of the defense system takes place in response to non- pathogenic bacteria. The present study also indicated that such isolates are better able to induce systemic resistance in plants for better protection against pathogens. Levy *et al.* (2007), suggested that such bacteria may induce systemic resistance through different mechanisms. They may also act as perfect vectors to manage crop protection, and can be used for seed coating or to be mixed with soil at the time of seedling and represent an attractive alternative to chemical pesticides.

One main benefit of such root associated bacteria is that once systemic resistance is induced, the inherent protective machinery of the plants functions for protracted periods even when the population of such beneficial bacteria declines over time. So definitely, root associated bacteria symbolize a potential protective tool for valuable crops of elevated importance in agriculture, where chemicals are used, and are responsible for pollution of the ecosystem.

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