World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Variability in bacterial endophytes in leaves of transgenic *Bt* and non-*Bt* cotton crop varieties and their role in suppression of bacterial leaf blight pathogen *X. a. pv. malvacearum* and the incited disease reaction

Suresh Govindarao Borkar [1,*], A. N. Bhosale [2] and Ajayasree T. S. [2]

[1] *Dr. Borkar's Laboratory and Research Centre, Endeavour Scientific Agriculture, 103, Prestige point Building, In front of Nashik road police station, Nashik (M.S), 422 101, India.*
[2] *Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist-Ahmednagar (M.S), 413 722, India.*

World Journal of Advanced Research and Reviews, 2023, 20(02), 626–636

Publication history: Received on 21 September 2023; revised on 31 October 2023; accepted on 03 November 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.2.2216

Abstract

Microbial epiphytes and endophytes are an integral part of the plant system and are known to play various roles in crop growth and crop health management. The transgenic crop plays an important role in crop pest management, however, environmentalists and ecologists have reservations about the cultivation of these crops. Whether the transgenic *Bt* cotton and non-*Bt* cotton vary in their microbial habitat ecology is not yet studied.

In the present investigations, the leaf endophytic bacteria were detected in the leaves of both transgenic *Bt* and non-*Bt* cotton hybrids. However, there were differences in the endophytic bacterial types and their population densities *i.e.* bacterial colony-forming units (cfu) in the leaves of *Bt* and non-*Bt* cotton varieties. At least ten different leaf endophytic bacteria were detected from two cotton varieties *i.e.* RCH-2 and Bunny (of transgenic *Bt* and non-*Bt* versions). A maximum of four types of leaf endophytic bacteria was present in RCH-2 *Bt* cotton leaves. The population density of leaf endophytic bacteria ranged from 50 cfu/leaf to 5 x 10^3 cfu/leaf and varied with individual leaf endophyte and cotton variety.

These bacterial leaf endophytes were observed to inhibit or suppress the growth of bacterial leaf blight pathogen *Xanthomonas axonopodis pv. malvacearum* (*Xam*) under *in-vitro* test. Leaf endophyte no.7 was more effective followed by leaf endophyte no.4 in suppressing the *Xam* population and population of other endophytes in the interaction studies.

Interaction of leaf endophytes and *Xam* in cotton leaves suggested that endophytes of transgenic Bunny-*Bt* were effective on *Xam* in transgenic Bunny–*Bt* hybrid only and so these changed the induction of susceptible water-soaking disease reaction into hypersensitive browning resistance reaction (HR). However, these endophytes of *Bt*-cotton were not effective in the non-*Bt* version in changing the susceptible reaction of *Xam* into an HR reaction. This indicated that the endophytes of the respective *Bt* and non-*Bt* crops were able to change the susceptible reaction of *Xam* into a hypersensitive one in their respective host, indicating that the use of leaf endophytes can be effective in their own habitat crop as a biocontrol agent against *Xam*. The specificity of leaf endophytes has to be considered in biological disease management programs.

Keywords: Leaf endophyte; transgenic *Bt* and non-*Bt* cotton; Bacterial blight; *X. a. pv. malvacearum*; Altered Disease reaction; Biological disease management

* Corresponding author: S. G. Borkar

1. Introduction

The cotton crop (*Gossypium sp.*) also known as white gold in the Indian sub-continent is grown in several countries of Asia region, parts of the USSR, some European countries, the American sub-continent, and some African countries (Khan et al., 2020). The fabrics and textile industry of the world is dependent on the production of this crop. The area under cotton crop in the world is around 32500 million hectares with production of 25 million tonnes (FAO, 2021). Several factors, among which pests and diseases are major, hampers the production of this crop. Cotton bollworm is a serious threat in its cultivation all over the world. To encounter its damage, a technological intervention was made in 1996 in the form of a transgenic cotton crop having the *Bt* gene (a gene derived from the bacteria *Bacillus thuringenensis (Bt)* and integrated into the cotton plant) which was resistant to bollworm pest, and was planted in Mexico and five other countries (James, 2016). However, environmentalists have raised several issues pertaining to transgenic *Bt* crop and their effect on the environment, ecosystem, and human health but the scientific assessment for these issues proved to be safe (Mendelsohn et al., 2003).

The presence of bacterial endophytes is known in the plant system (Chanway, 1998) and cotton is no exception (Misaghi and Donndelinger, 1990), with their role in plant growth and plant protection (Lodwyckx, 2002). However, is there any effect of transgenic *Bt* crops on bacterial endophytes is not yet studied. In the present investigation, we assess the leaf endophytic bacteria of transgenic *Bt* and non-*Bt* cotton varieties, their population densities, and their role in the suppression of bacterial leaf blight pathogen *Xanthomonas axonopodis pv. malvacearum (Xam)* and the incited disease reaction.

2. Material and Methods

2.1. Isolation of Leaf endophytes from transgenic *Bt* and non-*Bt* cotton variety

Leaves of two cotton hybrids *viz.* RCH-2 and Bunny with *Bt* and non-*Bt* versions were used for the isolation of endophytes. The lower leaves of these plants were removed, washed with tap water to remove dirt, dried in between tissue papers, and surface sterilized in $HgCl_2$ (0.1%) solution for two minutes to kill the leaf epiphytes. These were washed thrice with distilled sterilized water. The individual plant leaves were macerated in sterile mortar and pestle in 10 ml sterile water and allowed to stand for 10 minutes. With the help of a sterile pipette, about 0.2 ml of macerate suspension was taken, loaded on sterile nutrient agar (NA)-plates, and spread with an L-shaped glass rod. The replicated inoculated plates were incubated in BOD at 29±1˚C temperature and observed for the appearance of bacterial endophyte colonies for up to 3 days. Representative bacterial endophyte colonies that appeared in the plates were sub-cultured on NA slants, numbered properly, and used for further work. It is to be mentioned that the same endophytes appeared in *Bt*/non-*Bt* varieties were designated the same number (Figure. 1 and 2).

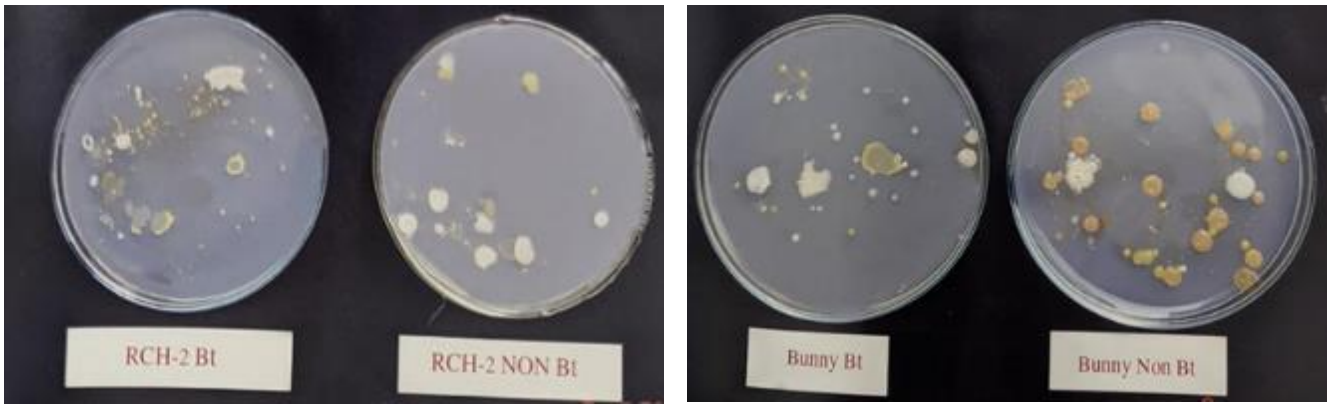

Figure 1 Leaf endophytes in RCH-2 *Bt*, RCH-2 non-*Bt* and Bunny *Bt*, Bunny non-*Bt* cotton hybrid at 74 days old crop

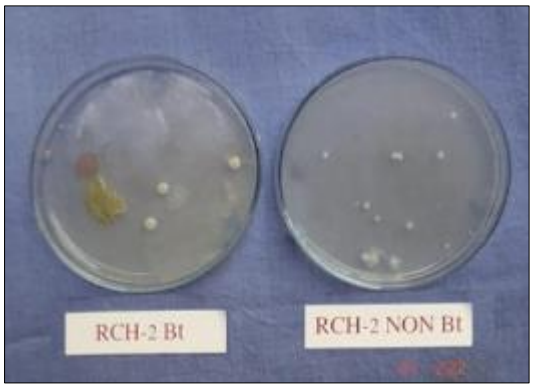

Figure 2 Leaf endophytes in RCH-2 *Bt* and non-*Bt* cotton hybrid at 154 days old crop

2.2. Reaction of leaf endophytes on cotton leaves

To study the reaction of leaf endophytes on cotton leaves, suspension of each leaf endophyte (0.1 OD at 620 nm) was inoculated into the leaf tissues of *Bt* and non-*Bt* cotton varieties by syringe infiltration method (Borkar, 2017). The observations for any visible reaction of endophytes on cotton leaves were recorded each day after inoculation for up to 4 days.

2.3. Isolation of cotton bacterial leaf blight pathogen *X. a. pv. malvacearum (Xam)* for interaction studies

Isolation of cotton bacterial leaf blight pathogen was carried out by the method described by Borkar et al., (1980). From the sample of *Bt* and non-*Bt* cotton hybrids showing angular leaf spot and vein blight (Figure. 3) were washed in running

Figure 3 Natural incidence of *X. a. pv. malvacearum* on cotton leaf showing angular leaf spot and vein blight

tap water to remove dust particles and epiphytes. These were pressed in sterilized blotter paper for drying and small pieces of diseased portion were cut with the help of a sterilized scalpel. The cut pieces were surface sterilized with 0.1 percent $HgCl_2$ solution for two minutes and then subsequently washed thrice in sterile water. These pieces were then removed and macerated in a sterile mortar and pestle with a sufficient quantity (5ml) of water. The above suspension was allowed to stand for 10 minutes. With the help of an inoculating needle one loop of the above suspension was taken and streaked on NA plates. The inoculated plates were incubated at 29±1˚C temp and observed for the development of typical bacterial colonies of *Xam* for up to 3 days.

Bacterial colonies having translucent, yellow, smooth, raised growth which developed after 72 h of incubation were purified by the streak plate method (Figure. 4).

Figure 4 Bacterial colonies of *X. a. pv. malvacearum* isolated from infected leaf

Single colonies with the above characters were transferred to the NA slant for their growth. The bacterial culture was tested for their pathogenicity on susceptible cotton plant Acala-44 and was confirmed as cotton bacterial pathogen *Xam*. The *Xam* culture tubes with fresh bacterial growth were preserved at 10 °C in a refrigerator and used for further experimentations of interaction studies.

2.4. *In-vitro* interaction of leaf endophytic bacteria with *Xam*

Leaf endophytic bacteria of bunny *Bt viz.* endophyte no. 1, 3, 4, and 5 were mixed individually with an equal proportion of *Xam* (1:1 at 10^7cfu/ml) in 5 ml nutrient broth. For this, one loopful of bacterial suspension of each endophyte was suspended in 4.5 ml of sterile water, and 1 ml of this was used to mix with *Xam* suspension. Similarly, a mixture of Bunny non-*Bt* endophytes 3, 4, 6, 7; RCH-2 *Bt* endophytes 1, 2, 3, and 4; and RCH-2 non-*Bt* endophytes 1, 2, and 4 was made in equal proportion (1:1 of each endophyte with *Xam*) with *Xam* in 5 ml nutrient broth medium. The interaction tubes (endophytes + *Xam*) were incubated in a BOD incubator at 29±1°C for 24 h. After 24 h, 0.1 ml reaction mixture was plated by spread plate method on NA medium (Borkar, 2017). The plates were incubated at 29±1 ˚C in a BOD incubator for 48 h and observed for bacterial growth of endophyte, *Xam,* or both.

2.5. In plants interaction of leaf endophytes with *Xam* (in *Bt* and non-*Bt* cotton leaves)

An equal proportion (1:1 at 10^7 cfu/ml) of *Xam* was mixed with leaf endophytic bacteria of Bunny *Bt*; with endophytic bacteria of Bunny non-*Bt*; with endophytic bacteria of RCH-2 *Bt* and with endophytic bacteria of RCH-2 non-*Bt* individually. These mixed (endophytic bacteria + *Xam*) suspensions were syringe infiltrated into the leaves of respective Bunny *Bt,* Bunny non-*Bt*, RCH-2 *Bt,* and RCH-2 non-*Bt* cotton plants (of 74 and 150 days old). Proper control with respective *Xam* was maintained in each host variety. After three days of inoculation, observations were taken for diseased water-soaking reaction/hypersensitive resistance reaction (HR).

2.6. Status of *Xam* and leaf endophytic bacteria in altered disease reaction (HR)

To study the status of *Xam* and leaf endophytic bacteria in a hypersensitive reaction (HR) area the method described by Borkar and Verma (1984) was followed. One centimeter HR area on the leaf was taken as a sample to assess the population of Xam and leaf endophytes. This sample was surface sterilized with 0.1 percent $HgCl_2$ followed by three washes with sterile water and was macerated in 5 ml sterile water in a sterile mortar and pestle. The leaf extract was then plated on NA medium by spread plate method. Plates were incubated for 48 h in a BOD incubator at 29±1˚C and were observed for types of bacterial endophytes, *Xam,* or both.

3. Result and Discussion

3.1. Isolates of leaf endophytic bacteria of *Bt* and non-*Bt* cotton hybrids

Two cotton hybrids *viz.* RCH-2 *Bt* and its non-*Bt* version as well as Bunny *Bt* and its non-*Bt* version were used for isolation and studies of the bacterial leaf endophytes in the leaves of these hybrids. The presence of different bacterial endophytes along with their population density in *Bt* and non-*Bt* leaves of these cotton hybrids were studied at 74 days (flowering and square formation stage) and 154 days (boll bursting stage) of crop stage.

At least 10 bacterial leaf endophytes having different bacterial colony morphology, different gram stain reactions, and bacterial cell shapes were isolated from two cotton varieties *viz.* RCH-2 (transgenic *Bt* and non-*Bt* version) and Bunny (transgenic *Bt* and non-*Bt* version) during the growth stages at 74 days and 154 days (table 1) old crops and the population density of these endophytes/leaf varies with the types of endophyte (table.2).

Table 1 Colony morphology of endophytic bacteria isolated from leaves of transgenic *Bt* and non-*Bt* cotton crop variety

Endophyte number	Bacterial colony morphology	Gram reaction and shape	Isolated from leaves of cotton variety	Crop growth stage (days)
1	Yellow suppressed irregular colony	Gram positive, cocci shape	RCH-2 *Bt*, RCH-2 non-*Bt,* Bunny *Bt*	74
2	White suppressed circular colony	Gram positive, cocci shape	RCH-2 *Bt,* RCH-2 non-*Bt*	74
3	White rough, irregular colony	Gram positive, rod shape	RCH-2 *Bt,* Bunny *Bt*, Bunny non-*Bt*	74
4	Yellow raised, glistering colony	Gram negative, rod shape	RCH-2 *Bt,* RCH-2 non-*Bt*, Bunny *Bt*, Bunny non-*Bt*	74
5	White suppressed, irregular, non-glistering colony	Gram positive, cocci shape	Bunny *Bt*	74
6	Orange, rough, glistering, circular colony	Gram positive, cocci shape	Bunny non-*Bt*	74
7	White, raised, glistering, circular colony	Gram negative, rod shape	Bunny non-*Bt*	74
8	Red, suppressed, irregular colony	Gram positive, cocci shaped	RCH-2 *Bt,*	154
9	Milky, raised, mucoid colony	Gram positive, cocci shaped	RCH-2 *Bt*, RCH-2 non-*Bt*	154
10	White, raised, circular colony	Gram positive, cocci shaped	Bunny *Bt*	154

Table 2 Variation in population density of different bacterial leaf endophyte in cotton leaves

Bacterial leaf endophyte number	Population density (cfu)/ leaf
1	2×10^2
2	1×10^2
3	1×10^2
4	5×10^3
5	5×10^1
6	7.5×10^1
7	1.5×10^1
8	5×10^1
9	2×10^2
10	1.5×10^2

The presence of bacterial leaf endophyte in cotton hybrids at 74 days indicated that in RCH-2 *Bt* leaves at least four types of endophytic bacteria were present. These endophytic bacteria were differentiated on the basis of their colony characters, gram staining reaction, and bacterial shape. The bacteria of endophyte no.1 produced yellow suppressed irregular colony. The population of this bacterium in leaves was 2×10^2 cfu/leaf. This endophytic bacterium was gram-

positive and cocci-shaped. The bacterium of endophyte no. 2 produced white-suppressed circular colonies. The population density of this endophyte was 1 x 10^2 cfu /leaf. This endophytic bacterium was gram-positive and cocci-shaped. The bacterial endophyte no. 3 produced white rough irregular colonies. The population of this endophyte was 1 x 10^2 cfu/leaf. The bacteria of this endophyte was gram-positive and rod-shaped. The bacterial endophyte no.4 produced a yellow-raised glistering colony. The population of this endophyte was 5 x 10^3 cfu/leaf. The bacteria of this endophyte was rod-shaped and gram-negative.

The bacterial endophyte no. 1, 2, and 4 were also present in the leaves of RCH-2 non-*Bt*, while endophyte no. 3 was not observed in the RCH-2 non-*Bt* hybrid. The variation in the number of colonies of endophyte 1 and 2 in the *Bt* and non-*Bt* cotton leaves was much less as compared to endophyte no.4 which had five times more population in RCH-2 *Bt* leaves than in non-*Bt* leaves.

In Bunny *Bt*, the bacterial leaf endophytes no.1, 3, and 4 were the same as observed in RCH-2 *Bt* leaves. However, their population was variable as compared to RCH-2 *Bt* leaves. The population of endophytes 1, 3, and 4 was 150 cfu/leaf, 2 x 10^2 cfu/leaf, and 25 x 10^1 cfu/leaf respectively. Endophyte no. 2 which was present in RCH-2 *Bt* leaves was not found in Bunny *Bt* leaves. Instead of this endophyte, another endophyte no 5 was present which produced white suppressed irregular non-glistering colonies. Their population was 50 cfu/leaf. The bacterium of this endophyte was gram-positive and cocci-shaped. In leaves of Bunny non-*Bt* version, endophyte no. 1, 2, and 5 were not present. However, it had endophyte no. 3 and 4 with endophytic population 5 x 10^2 cfu/leaf and 50 cfu/leaf respectively. Interestingly, Bunny non-*Bt* leaves had additional endophytic bacterium *i.e.* endophyte no.6 and 7. Endophyte no.6 produced orange, rough, glistering circular colonies. The population of this endophyte was 75 x 10^1 cfu/leaf. The bacterium was gram-positive and cocci-shaped. Endophyte no.7 produced white-raised circular glistering colonies. The population of this endophyte was 150 cfu/leaf and the endophytic bacterium was rod-shaped and gram-positive.

The leaf endophytes at 154 days were different than the leaf endophytes at 74 days. The results of bacterial leaf endophytes at 154 days indicated that endophyte no. 1 was found in RCH-2 *Bt* but not in RCH-2 non-*Bt* version. Other endophytes *i.e.* endophyte no. 2, 3, and 4 which were present in RCH-2 *Bt* at 74 days were also not present at 154 days in RCH-2 *Bt* as well as non-*Bt*. However, the other two endophytes *i.e.* endophyte no. 8 and 9 were present in RCH-2 *Bt* leaves. Endophyte no. 8 produced red-suppressed irregular colonies. The population of this endophyte was 5 x 10^1 cfu/leaf. It was gram-positive and cocci-shaped. Endophyte no.9 produced milky-raised mucoid colonies. The population of this endophyte was 2 x 10^2 cfu/leaf and the bacterium was gram-positive cocci-shaped. In RCH-2 non-*Bt* endophyte no. 8 was absent while endophyte no. 9 was present.

In Bunny *Bt* leaves, endophyte no. 3 and 5 which were present at 74 days were not present at 154 days. However, endophyte no. 4 was present with a lower population density of 2 x10^2 cfu/leaf. Interestingly, two more endophytes *i.e.* endophyte no.8 and 10 were present at 154 days in Bunny *Bt* leaves. Endophyte no. 10 produced white-raised circular colonies. The population of this endophyte was 15 x 10^1 cfu/leaf. The bacteria was gram-positive and cocci-shaped. In Bunny non-*Bt* leaves none of the above endophytes were present except endophyte no. 10 with a population of 50 cfu/leaf. Thus, there was a fluctuation in the population of endophytes in *Bt* and non-*Bt* versions and it varies with the type of endophyte.

3.2. Reaction of endophytes on cotton hybrids

Different endophytes *i.e.* endophyte no. 1 to 10 when infiltrated into leaves of cotton cv Akala-44 (universally susceptible to all known *Xam* race, producing water-soaking disease reaction) and on 101-102B (universally resistant to all known *Xam* races, producing hypersensitive browning reaction (HR), could not produce any visible reaction, further confirming that these were non-pathogenic bacterial endophytes habituating cotton leaves.

3.3. In-vitro interaction of cotton leaf endophytes with bacterial blight of cotton pathogen *X. a. pv. malvacearum*

For interaction studies of leaf endophytes with bacterial blight pathogen *X. a. pv. malvacearum*, the leaf endophyte no. 1, 3, 4, and 5 isolated from Bunny *Bt* cotton leaves at 74 days old plants were mixed with bacterium *Xam* in equal proportion (10^7 cfu/ml of each) in nutrient broth and incubated for 24 h for the bacterial interactions and interaction effect of endophytes on *Xam*. After 24 h the growth of mixed inoculum was plated on NA medium for detection/presence of endophyte/*Xam* population (Figure. 5).

The result (table 3) of interaction no. 1 indicated that colonies of the endophyte no. 4 and 5 were predominant. However, there were no colonies of endophyte no. 1 and 3, indicating that endophyte no. 4 and 5 suppressed/inhibited the growth of endophyte no. 1 and 3. Similarly, there were only seven colonies of *Xam* in the uncountable population of endophytes

no. 4 and 5 indicating that these two endophytes suppressed the multiplication and growth of *Xam* to a larger extent during their interaction.

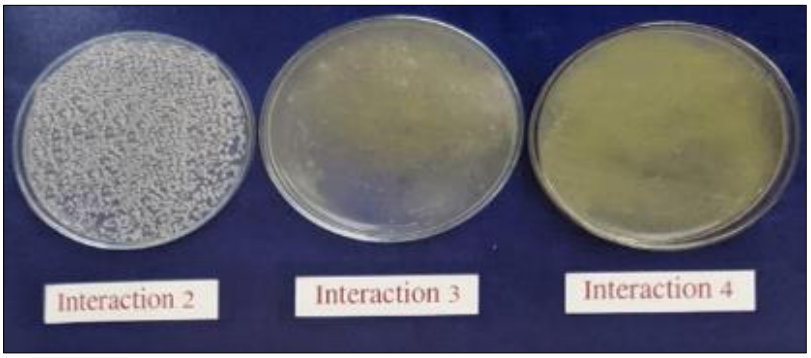

Figure 5 Interaction results of leaf endophytes and *Xam in-vitro*

In interaction no. 2, the endophytes no. 3, 4, 6, and 7 of non-*Bt* Bunny leaves when interacting with *Xam*, the colonies of *Xam* and endophytes no. 3, 4, and 6 did not appear at all. There were only colonies of endophyte no. 7 indicating that the endophyte no. 7 was more effective in suppressing/inhibiting the growth of *Xam* and the other three endophytes.

In interaction no. 3, endophytes no. 1, 2, 3, and 4 of RCH-2 *Bt* leaves when interacting with *Xam*, the colonies of *Xam* and endophytes no. 1, 2, and 3 did not appear. Only the colonies of endophyte no. 4 were predominant and suppressed/inhibited the growth of *Xam* and other endophytes.

In interaction no.4, endophytes no. 1, 2, 4 of RCH-2 non-*Bt* leaves when interacting with *Xam*, the colonies of *Xam* as well as endophyte no.1 and 2 did not appear. Only the population of endophyte no. 4 was predominantly present indicating that endophyte no. 4 inhibiting the *Xam* and endophyte no. 1 and 2.

Table 3 *In-vitro* interaction results of cotton leaf endophytes with *Xam*

Interaction number	Interacting endophytes with *Xam*	Interaction results
1	Bunny *Bt* endophyte no. 1 +3 +4 +5 with *Xam*	Colonies of endophyte no. 4 and 5 were predominant along with 7 colonies of *Xam*, suggesting that endophyte no. 4 and 5 reduced the population of *Xam*. It also suppressed/inhibited the growth of endophytes no.1 and 3.
2	Bunny non-*Bt* endophyte no. 3+4+6+7 with *Xam*	Colonies of endophyte no. 7 was predominant. Colonies of *Xam* and endophyte no. 3, 4, and 6 did not appear suggesting that endophyte no. 7 suppressed/inhibited the growth of *Xam* and the other three endophytes.
3	RCH-2 *Bt* endophyte no. 1+2+3+4 with *Xam*	Colonies of endophyte no. 4 were predominant. Colonies of *Xam* and endophyte no. 1, 2, and 3 did not appear, suggesting that endophyte no.4 suppressed the population of *Xam* and the other three endophytes.
4	RCH-2 non-*Bt* endophyte no. 1+2+4 with *Xam*	Colonies of endophyte no. 4 were predominant. Colonies of *Xam* and endophyte no. 1, and 2 did not appear, suggesting that endophyte no.4 suppressed the population of *Xam* and the other two endophytes.

It was evident from the above result that endophytes no. 7 and 4 were more effective in inhibiting/suppressing the growth of all other endophytes and *Xam*. However, whenever and wherever, the endophyte no. 7 was not available, the endophyte no. 4 was more effective in suppressing the growth of *Xam* and other endophytes.

3.4. Interaction of leaf endophytes and *Xam* in leaf tissues of cotton hybrids

Infiltration of 1:1 population (10^7 cfu/ml) of *Xam* and leaf endophytes of respective *Bt* and non-*Bt* cotton hybrids were made into the leaf tissues of *Bt* and non-*Bt* cotton hybrids to study the interaction effect *i.e.* either the production of susceptible water-soaking reaction (+) or browning hypersensitive reaction (HR).

Results (table 4 A) indicated that when leaf endophyte of Bunny *Bt* was mixed inoculated with *Xam* and infiltrated into Bunny *Bt* leaves (of 74 days old cotton crop) the endophytes did not allow the *Xam* to produce a water-soaking susceptible reaction and changed the reaction into a hypersensitive reaction. This could be due to the reaction of the interaction of a mixed bacterial population (Verma and Borkar, 1984) or secretion of water-soaking inhibitory metabolite against the *Xam* to convert the susceptible reaction into a hypersensitive reaction (Borkar and Verma, 1989, 1991). Similar results were also obtained when endophytes of Bunny non-*Bt* were mixed inoculated with *Xam* and inoculated on Bunny non-*Bt* leaves. When the same treatment was repeated on 150 days old cotton crop same results were obtained. Endophytes of Bunny *Bt* were effective on *Xam* in Bunny *Bt* leaves only and so they changed the induction of susceptible reaction into hypersensitive reaction. However, these endophytes were not effective on leaves of non *Bt* version and therefore the susceptible reaction was not changed into an HR reaction. These results indicated that the endophytes of the respective *Bt*/non-*Bt* crop were able to change the susceptible reaction of *Xam* into a hypersensitive one in their respective host indicating that the use of leaf endophytes can be explored as a biocontrol agent against *Xam* in their respective habitat.

Table 4 Interaction of leaf endophytes and Xam in cotton hybrid varieties

In Bunny *Bt* and non-*Bt cotton*					
SI. No.	Inoculation of	Reaction on cotton leaves			
		On 74 days crop		On 154 days crop	
		Of Bunny *Bt*	Of Bunny non-*Bt*	Of Bunny *Bt*	Of Bunny non-*Bt*
1	*Xam* (as control)	+	+	+	+
2	*Xam* with Bunny *Bt* endophyte no.1, 3, 4, and 5	HR	+	HR	+
3	*Xam* with Bunny non-*Bt* endophyte no 3, 4, 6, and 7	+	HR	+	HR
In RCH-2 *Bt* and non-*Bt*					
SI. No.	Inoculation of	Reaction on cotton leaves			
		On 74 days crop		On 154 days crop	
		Of RCH-2 *Bt*	Of RCH-2 non-*Bt*	Of RCH-2 *Bt*	Of RCH-2 non-*Bt*
1	*Xam* (as control)	+	+	+	+
2	*Xam* with RCH-2 *Bt* endophyte no.1,2,3, and 4	HR	+	HR	+
3	*Xam* with RCH-2 non-*Bt* endophyte no 1,2, and 3	+	HR	+	HR

Similar results (table 4 B) were also obtained for RCH-2 non-*Bt* crop where the leaf endophytes of RCH-2 non-*Bt* were effective in converting the susceptible reaction into hypersensitive reaction on RCH-2 non-*Bt* leaves, whereas endophytes of RCH-2 *Bt* was unable to convert the susceptible reaction into resistance HR reaction, indicating the role of varietal specificity in the conversion of susceptible reaction into hypersensitive reaction.

3.5. Status of *Xam* and endophytes in HR induced area in cotton leaves

It was evident from the earlier result that the endophytes were responsible for the inhibition/reduction of *Xam* population *in-vitro* as well as in cotton leaves and also for converting the susceptible water-soaking reaction into a hypersensitive reaction in the susceptible hybrids. Therefore, the status of *Xam* and endophytes was studied in HR-induced areas on susceptible cotton leaves. HR area of 1 cm diameter was used to determine the status of *Xam* and endophytes in this area.

The results (table 5) indicated that though there was a reduction in the population of infiltrated endophytes and *Xam* in the HR area, the presence of *Xam* and endophytes no. 4 and 5 were detected (Figure. 6). However, the endophyte no. 1 and 3 did not survive in HR area at all. These results were also confirmed with the interaction results of Bunny *Bt* endophytes with *Xam* under *in-vitro* studies.

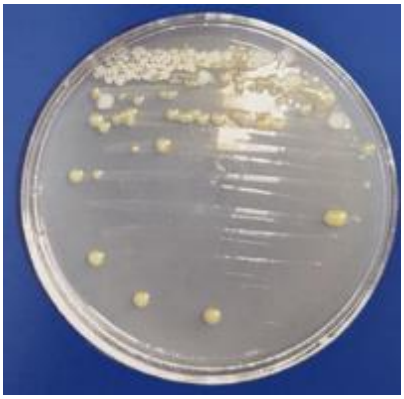

Figure 6 Status of *Xam* and endophytes in the HR-induced area

Table 5 Status of *Xam* and endophytes in the HR-induced area on cotton leaves

Interacting bacteria	Status of bacteria in HR-induced areas
Xam of Bunny *Bt* + Total endophytes of Bunny *Bt* (endophytes no. 1, 3, 4, and 5)	Few *Xam* and endophyte no. 4 and 5 present. Endophyte no. 1 and 3 did not appear at all.

Various scientists (Jacob et al., 1985; Fisher et al., 1992; McInroy and Kloepper.1995; Chanway, 1998; Helmann et al., 1997; Asis et al., 2000; Tapia-Hernandez et.al. 2000; Kobayashi and Palumbo, 2002; Arauja et al., 2001; Lodwyckx et al., 2002) reported the presence of endophytic bacteria in various agronomic crops. The presence of bacterial endophytes in various parts of plants including roots has been reported by various scientists (Barraquio et al., 1997; James et al., 1994; Reinhold Hurek and Hurek, 1998) for their usefulness in the growth of plants as diazotrophs and as a biocontrol agent for the management of root rot pathogens (Muthukumarswamy et al., 1999 and Chen et al., 1995).

Trevet and Hollis (1948) reported that bacterial endophytes reside within plant hosts without causing disease symptoms or substantial harm (Kobayashi and Palumbo, 2002). Our results also indicated that the 10 cotton leaf endophytes residing in the cotton leaves were harmless to the cotton and did not induce any visible reaction in the leaves when infiltrated. Misaghi and Donndelinger (1990) showed the presence of endophytic bacteria in symptom-free cotton plants. In addition to this, our results indicated that there were differences in the types of bacterial leaf endophytes, and their population densities associated with *Bt* and non-*Bt* cotton hybrids and further their role in suppression of bacterial blight pathogen *Xam* and conversion of susceptible water-soaking reaction into a resistant hypersensitive reaction. Furthermore, the cotton leaf endophyte no. 7 and 4 were predominant in cotton leaves which was effective in regulating the population of *Xam* in the leaf and in control of bacterial blight disease reaction.

4. Conclusion

Our experimental results indicated that the presence of bacterial leaf endophytes is specific to their host habitat, particularly in *Bt* and non-*Bt* cotton. Further, these act as biocontrol agents against the bacterial leaf blight pathogen *X. a. pv. malvacearum* in cotton host. However, these are effective biocontrol agents in their respective host habitat only indicating their host specificity in the management of this disease. Further, research on bacterial leaf endophytes host specificity as biocontrol agents will make advancements in this area.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Arauja, W. L., Maccheroni, W., Carops, J. I. 2001. Variability and interaction between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Canadian J. Microbiology.* 47(3):229-236.

[2] Asis, C. A., Kubota, J. M., Ohta, H., and Arima, Y. 2000. Endophytic nitrogen-fixing bacteria and cyanobacterial evaluation. *Plant Nutrition.* 46: 759-765.

[3] Barraquio, W. L., Revilla, L., and Ladha, J. K. 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant Soil.* 15-24pp.

[4] Borkar, S. G., Verma J. P., and Singh, R. P. 1980. Transmission of *Xanthomonas malvacearum* of cotton through spotted boll-worm. *Indian J. of Entomology.* 42(2): 390-397.

[5] Borkar, S. G and Verma, J. P. 1984. Population dynamics of *X. c. pv. malvacearum* in compatible/incompatible reaction of cotton cvs. *Acta Phytopathologica Academiae Scienatiarum Hungaricae.* 20(1-2): 31-34.

[6] Borkar, S. G and Verma, J. P. 1989. Water-soaking reaction inhibitory metabolite excreted by *X. c. pv. malvacearum. Folia Microbiologica.* 34: 515-520.

[7] Borkar, S. G and Verma, J. P. 1991. Inhibition of susceptible/hypersensitive reaction by exopolysaccharide of avirulent strain of *X. c. pv. malvacearum*. *Folia Microbiologica*. 36: 173-176.

[8] Borkar, S. G. 2017. Laboratory techniques in plant bacteriology. CRC Press, USA, 342 pages, ISBN: 978-135-179-838-9.

[9] Chanway, C. P. 1998. Bacterial endophytes: ecology and practical implications. *Sydowia.* 50: 149-170.

[10] Chen, C., Bauske, E. M., Musson, G., Rodriguez, K. R., and Kloepper, J. W. 1995. Biological control of *Fusarium* wilt of cotton by use of endophytic bacteria. *Biological Control*. 5(1): 83-91.

[11] FAO. 2021. Recent trends and prospects in the world cotton market and policy development. Rome. http://doi.org/10.4060/cb3269en.

[12] Fisher, P. J., Petrini, O., and Lappin, S. H. M. 1992. The distribution of some fungal and bacterial endophytes in maize. *New Phytologist.* 122: 299-305.

[13] Helmann, J., Hallman, A., Mahaffee, W. F., and Kloepper, J. W. 1997. Bacterial endophytes in agricultural crops. *Canadian J. Microbiology.* 43: 895- 914.

[14] Jacob, M. J., Bugbee, W. M., and Gabrielson, D. A. 1985. Enumeration, location and characterization of endophytic bacteria within sugar-beet root. *Canadian J. Botany.* 63: 1262-1265.

[15] James, E. K., Reis, V. M., Olivares, F.L., Baldani, J. I., and Dobereiner, J. 1994. Infection of sugarcane by the nitrogen fixing bacterium *Acetobacter diazotrophicus*. *J. Experimental Bot.* 45: 757-766.

[16] James, C. 2016. Global status of commercialised biotech/GM crop: 2016. Ithaca. NY: ISAAA Brief No. 52 ISAAA.

[17] Khan, M. A., Wahid, A., Ahmad, M., Tahir, M. T., Ahmad, M., Ahmad, S., and Hasanuzzaman, M. 2020. World cotton production and consumption: An overview. In book: Cotton production and uses. Publisher. Springer Nature, Singapore Pvt Ltd. DOI: 10.1007/978-981-15-1472-2-1.

[18] Kobayashi, D. Y. and Palumbo, J. D. 2002. Bacterial endophytes and their effects on plants and uses in agriculture. *Microbial Endophytes.* 199- 233pp.

[19] Lodwyckx, C., Vangronsveld, J., and Proteous, F. 2002. Endophytic bacteria and their potential application. *Critical Review in Plant Sciences.* 21(6): 583-606.

[20] McInroy, J. and J. W. Kloepper. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil.* 173: 337-342.

[21] Mendelsohn, M., Kough, J., Vaituzis, Z. and Matthews, K. 2003. Are *Bt* crops safe. *Nature Biotechnology*. 21(9):1003-1009.

[22] Misaghi, I. J and Donndelinger, C.R. 1990. Endophytic bacteria in symptom free cotton plants. *Phytopathology.* 43: 140-146.

[23] Muthukumarswamy, R., Revathi, G. and Lakshminarsimhan, C. 1999. Diazotrophic association in sugarcane cultivation in South India. *Tropical Agric.* 76: 171-178.

[24] Reinhold Hurek, B and Hurek, T. 1998. Life in grasses: diazotrophic endophytes. *Critical Review Plant Sci.* 17: 29-54.

[25] Tapia-Hernandez, A., Bustillos-Cristales, M. R., Jimenez Salgado, T., Cabellero Mellado, J., and Fuentes Ramierez. L.E. 2000. Natural endophytic occurrence of *Acetobacter diazotrophicus* in pineapple plants. *Microb. Ecology*. 39: 49-55.

[26] Trevet, I. W and Hollis, J. P. 1948. Bacteria in the storage organs of Healthy plants. *Phytopathology.* 38: 960-967.

[27] Verma, J. P and Borkar, S. G. 1984. Reaction of mixed races of *X. c. pv. malvacearum. Current Science*. 53:330-331.