

Rhizosphere Soil Bacterial Communities and Bacterial Root Endophytes in Cotton farming is Influence by Cultivation of Transgenic Bt and non-Bt Cotton Crop Varieties



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Abstract

Presence of different bacterial communities in soil and their association with plant roots plays an important role in crop growth and cultivation. These bacterial communities varies with the soil type, soil ecology, soil environment, and plant species grown. However, there are no studies on the variation of bacterial communities present in transgenic and non-transgenic crop soils. In the present investigation, we studied the variation in rhizosphere soil bacterial communities and bacterial root endophytes in transgenic Bt and non-Bt cotton crop cultivation. Rhizosphere bacterial inhabitant under cultivation of Bt and non-Bt cotton hybrids varied and was related to the cotton hybrids grown. In the rhizosphere of RCH-2 Bt cotton, 5 different kinds of bacterial communities were observed with various population density whereas in rhizosphere of RCH-2 non-Bt cotton, bacterial community of only one type was observed indicating that the exudates of RCH-2 Bt were more favourable for the growth of rhizosphere bacterial flora/communities. Similarly, there was variation in the types of rhizosphere bacterial communities of Bunny Bt and Bunny non-Bt hybrids. Two types of bacterial communities were associated with Bunny Bt hybrid rhizosphere while 3 types of bacterial communities were associated with Bunny non-Bt hybrid rhizosphere and had one common bacterial community in rhizosphere of both the hybrids. The presence of bacterial root endophytes was detected in Bt and non-Bt cotton hybrids. In RCH-2 Bt, 2 types of bacterial root endophytes were found whereas 4 types of bacterial root endophytes were found in RCH-2 non-Bt. In this hybrid, only isolate no.2 was common. In Bunny Bt only one type of bacterial root endophyte was found while in Bunny non-Bt, 2 types of bacterial root endophytes were found, and all were different. Thus, both the Bt and non-Bt cotton hybrids contain root endophytes, and the types of endophytes differ from Bt-hybrid to non-Bt hybrid with variable population. The role of these rhizosphere soil bacterial communities and cotton root endophytes needs to be explore for their potential as cotton crop growth stimulant/ in root disease management of cotton crop in western Maharashtra cotton tract.

Keywords: Soil Bacterial Communities; Bacterial Root Endophytes; Cotton Crop; Transgenic and non-Transgenic Hybrids

Introduction

Cotton crop (*Gossypium* sp) also known as white gold in Indian sub-continent is grown in several countries of Asia region, parts of USSR, some European countries, American sub-continent, and in some African countries Khan [1]. 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 32,500 million hectore with production of 25 million tons FAO [2] which comprises both transgenic Bt cotton and non-Bt cotton crop varieties. The acreage of cotton in India in 2019-20 was 12.58 million hectore

out of which 11.74 million hectore (i.e. around 93.6 percent area) was under Bt cotton only MoA&FW [3]. Farm land soils are the basic medium for the growth of crop plants and their production are influenced by soil ecology, soil fertility/composition Long [4] and soil microbial richness. There are different soil microbial communities which functions differently in the crop growth and crop protection. These microbes not only confine to soil, but colonies its host plants as epiphytes and endophytes. Presence of bacterial endophytes is known in the plant system Chanway [5] and cotton is no exception Misgahi & Donndelinger [6]. Similarly, the

presence of bacterial root endophytes are known in crops Haichun [7]. These bacterial endophytes are implicated with their role in plant growth and plant protection Lodwyckx [8]. The diversity of bacterial endophytes are affected by various factors including soil cultivation history Correa-Galeote [9] and the crop plant grown. However, is there any effect of transgenic Bt crop on rhizosphere soil bacterial communities and bacterial root endophytes has not yet studied. In the present investigation, therefore, we assess the bacterial communities in rhizosphere soil of Bt and non-Bt cotton hybrids grown in the same cotton cultivation plot and further the variation in bacterial root endophytes in transgenic Bt and non-Bt cotton varieties with their population densities. These results will help us to understand the influence of transgenic crop on the rhizosphere bacterial communities and communities of root endophytes.

Material and Methods

Studies on rhizosphere soil bacterial communities under cultivation of Bt and non-Bt cotton hybrids

To study rhizosphere soil bacterial communities, rhizosphere soil under two cotton hybrids, viz. RCH-2 and Bunny with their Bt and non-Bt counterparts were used. For the isolation of rhizosphere bacterial communities, 1 g soil from rhizosphere under each cotton variety was taken and serial dilution were made up to 10^{-5} dilution. 0.1 ml of each dilution was plated on Nutrient-Agar (NA) medium. The plates were incubated in BOD at $28 \pm 1^\circ\text{C}$ temperature for 48 hrs and the appearance of different bacterial colony type and population density was recorded as per standard procedure Borkar [10].

Studies on root endophytes of Bt and non-Bt cotton hybrids

Roots of 2 cotton hybrids viz. RCH-2 and Bunny with their Bt and non-Bt counterparts were used to isolate the root endophytes. Tap root of 1 cm length of each cotton variety was taken for isolation of root endophytes. The root portion was washed thoroughly in water to remove soil and dust particles from the root surface. The root portion was then surface sterilized with 0.1 percent HgCl_2 for 2 minutes, followed by 3 washing with distilled sterile water. This root portion was then macerated in 5 ml distilled sterile water in sterile mortar and pestle and 0.1 ml of macerated supernatant suspension was plated on NA medium by spread plate method. The plates were incubated in BOD at $28 \pm 1^\circ\text{C}$ temperature for 48 hrs and the observations were recorded for isolated root endophytes Jacob [11].

Results and Discussion

Rhizosphere soil bacterial communities under cultivation of Bt and non-Bt cotton hybrids

The rhizosphere soil bacterial type and their population (cfu/g of soil) from the root zone of RCH-2 Bt and non-Bt cotton

as well as Bunny Bt and non-Bt cotton was studied by dilution plate method. The results (Table 1) indicated that the rhizosphere soil bacterial community of RCH-2 Bt cotton was composed of different bacterial population which include white mucoid circular colonies with population density 4×10^5 cfu/g of soil, yellow non-glistening circular colonies with population density 2×10^5 cfu/g, white rough irregular colonies with population density 2×10^5 cfu/g, yellow glistening circular colonies with population density 3×10^5 cfu/g, and transparent irregular mucoid colonies with population density 2×10^5 cfu/g of soil. However, the rhizosphere soil bacterial communities of RCH-2 non-Bt cotton had only one bacterial population i.e. rough white irregular colonies forming bacteria with population density of 5×10^5 cfu/g of soil. Other bacteria population which were associated with RCH-2 Bt cotton rhizosphere were not found in RCH-2 non-Bt cotton rhizosphere soil of the same location. This indicated that the root exudates of the RCH-2 Bt cotton hybrid got ability to thrive various kind of bacterial population in its root rhizosphere as compared to RCH-2 non-Bt cotton hybrid. Similarly, the rhizosphere soil bacterial communities of Bunny Bt cotton composed of 2 bacterial population i.e. isolate no.3 with rough white irregular colonies with population density of 7×10^5 cfu/g; whereas another population of isolate no.6 was pink circular mucoid with population density of 1×10^5 cfu/g of soil. The bacterial population of isolate no.3 was universally present in rhizosphere of Bunny Bt and Bunny non-Bt, as well as RCH-2Bt and RCH-2 non-Bt cotton hybrids. However, isolate no.6 which was present in the rhizosphere of Bunny Bt was not present in the rhizosphere of Bunny non-Bt, RCH-2 Bt and RCH-2 non-Bt hybrids indicating that the type of Bt cotton hybrid had influence on the rhizosphere soil bacterial communities at the same cotton crop cultivation location.

Studies on root endophytes of Bt and non-Bt cotton hybrids

Presence of root endophytes in Bt and non-Bt cotton hybrids were estimated by spread plate technique. A root portion of 1 cm length was macerated in 5 ml of sterile water and suspension was plated by spreading on solidified NA medium to note the appearance of endophytes. The endophytic bacterium were differentiated on the basis of their colony characters. The results (Table 2) & (Figure 1) indicated that the RCH-2 Bt cotton root had 2 types of root endophytes. The one with pale yellow mucoid irregular colonies with a population density of 5×10^3 cfu/cm of root length and the other isolate no.2 forming white mucoid irregular colonies with population density of 50 cfu/cm of root length. In RCH-2 non-Bt cotton root four types of root endophytes were observed. Isolate no.2 had with population density 50 cfu/cm of root length. Endophyte isolate no.3 was transparent pale yellow suppressed irregular colonies with population density of 250 cfu/cm of root length. Endophyte isolate no.4 was white raised mucoid circular colonies with population density of 250 cfu/cm of root length. Endophyte isolate no.5 was yellowish

raised glistening circular colonies with population density 350 cfu/cm of root length. Bunny Bt root contained only 1 type of root endophyte i.e. isolate no.6 of rough white irregular colonies with a population density of $<5 \times 10^3$ cfu/cm of root length. This root endophyte was not present in Bunny non-Bt root. Instead of this, Bunny non-Bt root contained 2 other endophytes. The one was isolate no.2 with a population density of $<25 \times 10^2$ cfu/cm of root length and the other forming yellow raised irregular colony with population density of $<25 \times 10^2$ cfu/cm of root length. These

results clearly indicated that both Bt and non Bt hybrids contained root endophytes and the types of endophytes differed from hybrid to hybrid with variable population density. Studies on rhizosphere soil bacterial communities of Bt and non-Bt cotton hybrids indicated that the type of Bt cotton hybrids has influence on the rhizosphere bacterial communities at the same experimental site, which was noted for the first time in case of Bt cotton crop as no such reference is available.

Table 1: Bacterial inhabitant in rhizosphere of transgenic Bt and non-Bt cotton.

Rhizosphere Bacterial Communities	Bacterial colony morphology	Bacterial population densities (cfu/g of soil) In Rhizosphere of Cotton hybrids			
		RCH-2 Bt	RCH-2 non-Bt	Bunny Bt	Bunny non-Bt
Isolate no.1 type	White mucoid circular colonies	Present 4×10^5 cfu	Absent	Absent	Present 2×10^5 cfu
Isolate no.2 type	Yellow circular non-glistening colonies	Present 2×10^5 cfu	Absent	Absent	Absent
Isolate no.3 type	White rough irregular colonies	Present 2×10^5 cfu	Present 5×10^5 cfu	Present 7×10^5 cfu	Present 1×10^5 cfu
Isolate no.4 type	Yellow circular glistening colonies;	Present 3×10^5 cfu	Absent	Absent	Present 2×10^7 cfu
Isolate no.5 type	Transparent irregular mucoid colonies	Present 2×10^5 cfu	Absent	Absent	Absent
Isolate no.6 type	Pink mucoid circular colonies.	Absent	Absent	Present 1×10^5 cfu	Absent

Table 2: Types of Bacterial inhabitant as root endophytes in Bt and non-Bt cotton varieties.

Bacterial Root Endophytes	Bacterial colony morphology	Root endophyte's population density (cfu/cm of root length) In Roots of Cotton hybrids			
		RCH-2 Bt	RCH-2 non-Bt	Bunny Bt	Bunny non-Bt
Isolate no.1 type	Pale yellow mucoid circular colonies;	Present $<5 \times 10^3$ cfu	Absent	Absent	Absent
Isolate no.2 type	White mucoid irregular colonies;	Present 50 cfu	Present 50 cfu	Absent	Present $<25 \times 10^2$
Isolate no.3 type	Transparent pale yellow suppressed irregular colonies;	Absent	Present 250 cfu	Absent	Absent
Isolate no.4 type	White raised mucoid circular colonies	Absent	Present 250 cfu	Absent	Absent
Isolate no.5 type	Yellowish raised glistening circular colonies	Absent	Present 350 cfu	Absent	Absent
Isolate no.6 type	White rough irregular colonies	Absent	Absent	Present $<5 \times 10^3$ cfu	Absent
Isolate no.7 type	Yellow rough irregular colonies	Absent	Absent	Absent	Present $<25 \times 10^2$

Presence of endophytes in root was reported by many scientist. Tapia-Hernandez [12] reported the presence of endophytic diazotrophs in surface sterilized tissues of roots of pine-apple plant. Chanway [5] reported nitrogen fixing bacterial endophytes of sugarcane colonized the internal root, stem and leaf tissues.

Barraquio [13] isolated diazotrophic and non-diazotrophic bacterial endophytes from roots and culms of rice. Our results showed the presence of root endophytes in cotton roots which is also reported by McInray [14] McInray [15], and Chen [16]. Further studies showed that the types of root endophytes differed

with cotton hybrids with variable proportion. Similar results was also reported by Adams [17]. Papik [18] reported the diversity in endophytic bacteria. The role of root endophytes are reported in plant growth promoting and plant protection activities Kobayashi and Palumbo [19]; Gaiero [20]; Selim [21]; Dubey [22]. The

presence of diversified bacterial communities in rhizosphere soil can be used as a yardstick for soil richness for crop growth and the particular root endophytes as biocontrol agent to protect the crop for specific root pathogen.

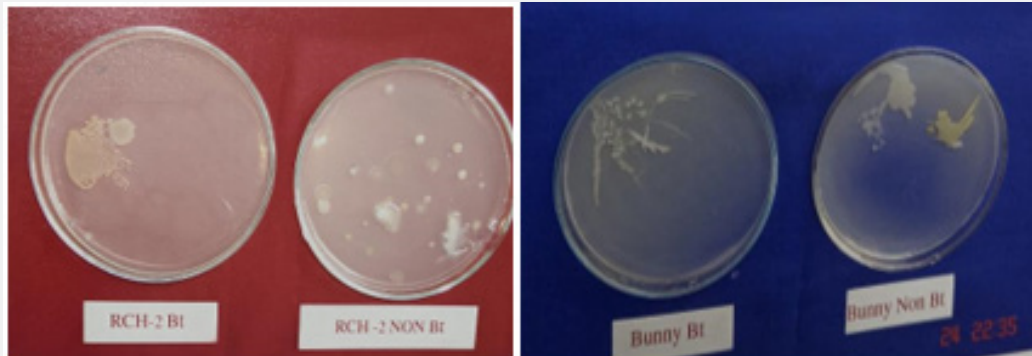


Figure 1: Variation in bacterial root endophytes of transgenic RCH 2 Bt, Bunny Bt and their non-Bt version.

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