

Status of microflora on Bt and non - Bt cotton

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ABSTRACT :

Transgenic Bt cotton expresses *CryIAc* protein from *Bacillus thuringiensis*. The diversity of ectophytic and endophytic fungi and bacteria in roots, stems and leaves from transgenic (Bt) and non transgenic (non Bt)cotton was evaluated during 30, 60 and 90 DAS to investigate possible non-target effects of genetically modified cotton on microbial communities. Total ten fungal and five bacterial organisms were isolated. This studies shows that the roots, stems and leaves of Bt and non Bt cotton plants harboured endophytes and ectophytes. Although the no.of endophytic and ectophytic species isolated from the two types of plant did not vary much. While Bt modifications had no effect on endophytes and ectophytes and it is seen from the observations that the Bt gene had not transferred from Bt plants to associated microflora. These results represent the first evaluation of the composition of endophytic and ectophytic fungi as well as bacteria associated with transgenic cotton plants. Also detection of Bt gene in associated microflora by using Bt Express strips.

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INTRODUCTION

Cotton (*Gossypium* spp.) is one of the world's important fibre crop from family *Malvaceae*. Cotton as a crop as well as a commodity plays an important role in the agrarian and industrial activities of the nation and has a unique place in the economy of our country. Cotton popularly known as "White gold" is grown mainly for fibre. India has been a traditional home of cottons and cotton textiles. India is the only country where all the four cultivated species of cotton are grown. Our economy is consistently influenced by cotton through its production

and processing sectors and by generating direct and indirect employment to more than sixty million people.

In India the total acreage under cotton in 2014-15 had been around 129.71 lakh hectares. Upto which 119.75 lakh ha area under Bt cotton. The production was 390 lakh bales, export was around 70.60 lakh bales of 70kg each and import was around 8 lakh bales. (Anonymous, 2015). Acreage under Bt cotton in 2013-14 had been around 92.5 per cent (*i.e.* 10.84 million hectares) of the total area of 11.73 million hectares while during 2012-13, it was around 88 per cent (*i.e.* 10.54

million hectares) of the total acreage of 11.98 million hectares. The export during the cotton season 2013-14 are estimated at 1.94 million MT (11.40 million bales of 170 kg). In Maharashtra area under Bt cotton in 2013-14 had been recorded 10.84 million hectares and production 1.43 million metric tones (8.40 million bales of 170 kg) (Anonymous, 2014).

For identification of Bt gene, protein based method named 'dipstick test' has been found quicker, simpler, less expensive and found suitable for on-site testing by untrained people. Present investigation is focused only on dipstick that is easier to perform than other techniques. (Kumar and Sinha, 2011). Keeping in view the economic importance of Bt cotton, present investigations were planned and conducted on microflora isolated from Bt cotton and non-Bt cotton and also effect of this gene on microflora. There is no previous report or work on this topic is available in India. The roots, stems and leaves sample of Bt and non-Bt cotton were collected from Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri.

MATERIAL AND METHODS

Ectophytic and endophytic fungi and bacteria were isolated from healthy leaves, stems and roots after surface disinfection by serial washing in 70 per cent ethanol for 1 min, sodium hypochloride solution for 2-5 min, followed by three rinses in sterilized distilled water. Plating aliquots of the sterile distilled water used in the final rinse on PDA and NA. After three to four days of incubation, the Petri plates containing PDA medium were observed. The well developed mycelial growth of fungi were observed, by following single hyphal-tip technique, the different fungi were transformed/subcultured aseptically on PDA medium plate and slant. Transferred different colonies on different plates. A single colony of these purified isolates was picked up and maintained on PDA and NA slants for further studies. Based on microscopic observations of fungal isolates and morphological characters of fungus, the fungus were identified upto genus level in Plant Pathology laboratory with the help of published literature (Barnett, 1960) and confirmed.

The observations on bacterial colony morphology and Gram reaction recorded as per the standard procedures given by Barthalomew and Mittewer (1950) and characterization has done according to Bergy's manual.

Procedure for detection of Bt gene from seed and microflora isolated from Bt cotton at 90 DAS.

- Pick up a cotton seed and break it open/take fungal colony/2-3 loopful of bacterial culture .
- Take the white coloured internal embryo matter (for seed) and transfer it in the vials provided.
- Add 0.5ml (Marked on the vial) of sample extraction buffer provided.
- Crush the embryo/fungal/bacterial culture using the pestle provided with the kit.
- Dip the 'CryIAC Bt instant-check' strip into vial ,to take care that only the end marked as sample is dipped into the sample. Wait until the sample solution travels till the top of the strip and filter pad at top-end is almost completely wet. This should take about 15-20 minutes.
- If one band develops halfway through the strip along with another at the top (two bands), it indicates presence of *CryIAC* in the sample. It is a positive Bt cotton sample.
- If only one band develops at the top end of the strip it indicates that the sample is negative for *CryIAC* and is a non Bt sample. Same procedure has been followed for detection of Bt gene from ectophytes and endophytes (fungal and bacterial) isolated from different parts of Bt cotton plant and detection of Bt gene carried out.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

The fungal microflora associated with the Bt and non-Bt cotton were *Aspergillus flavus*, *Aspergillus niger*, *Mucor* spp., *Rhizopus* spp., *Colletotrichum* spp., *Fusarium* spp., *Trichoderma viridae.*, *Trichoderma herzianum*, *Penicillium* spp.1 and *Penicillium* spp. 2 and Bt and the bacterial microflora associated with the Bt and non-Bt cotton were *Erwinia* spp., *Pseudomonas* spp., *Azotobacter* spp., *Xanthomonas* spp. and *Bacillus* spp. The results shows that, there was no much difference in microflora isolated from Bt and non-Bt cotton plants.

The results obtained by Suryanarayanan *et al.* (2011) were in accordance with present findings who reported that the number of endophyte species isolated from the respective Bt and non- Bt cotton tissues did not vary to great extent. Present investigations are similar

with Brusetti *et al.* (2005) and Icoz and Stotzky, (2008) who reported that the expression of *cry* genes did not alter bacterial endophyte communities. Donegan *et al.* (1995 and 1996) in agreement with *in situ* observations with transgenic Bt plants that generally showed no consistent and lasting effects on micro-organism. De Souza *et al.* (2011) proved that Bt modifications had no effect on endophytes, the cotton tissue. More or less similar results have been reported by Blackwood and Buyer (2004); Griffiths *et al.* (2006) that minor changes in soil microbial community structure of Bt maize compared to non Bt maize. Prischl *et al.* (2012) reported that diversity measures of endophytic isolates were not different in Bt versus non- Bt maize varieties. Koskella and Stotzky (2002) reported that no reaction on the bacteria, fungi and algae microbial development were observed. De Souza *et al.* (2011) reported that the integration of Bt gene in cotton has no influence on the

frequency of endophyte colonization.

Prischl *et al.* (2012) reported that the expression of Bt has no or minor influence on endophytic bacterial communities, in comparison to effects of plant cultivar or soil type. Donegan *et al.* (1995) and Tarafdar and Rathore (2012) reported that *in situ* observations with transgenic Bt plants that generally shows no consistent effects and lasting effects on micro-organisms. Other studies have found only minor changes in the soil microbial community structure (Blackwood and Buyer, 2004; Brusetti *et al.*, 2005; Castaldini *et al.*, 2005; Griffiths *et al.*, 2006 and Mulder *et al.*, 2006). More studies indicate that Bt cotton has no negative effects on soil flora and fauna and may even have beneficial effects (Saxena and Stotzky, 2001; Sarkar *et al.*, 2009 and Saxena and Stotzky, 2001) show that toxin released from roots and biomass of Bt corn appeared to have no deleterious effect on earthworms, nematodes and micro-

Table 1 : Detection of Bt gene in fungal isolates of Bt cotton at 90 DAS

Sr. No.	Name of fungal isolate	Detection of Bt gene in fungal isolates	
		No. of bands	Results
1.	<i>Aspergillus</i> sp. 1	1 Band	Negative
2.	<i>Aspergillus</i> sp. 2	1 Band	Negative
3.	<i>Mucor</i> sp.	1 Band	Negative
4.	<i>Rhizopus</i> sp.	1 Band	Negative
5.	<i>Colletotrichum</i> sp.	1 Band	Negative
6.	<i>Fusarium</i> sp.	1 Band	Negative
7.	<i>Trichoderma</i> sp. 1.	1 Band	Negative
8.	<i>Trichoderma</i> sp. 1.	1 Band	Negative
9.	<i>Penicillium</i> sp. 1.	1 Band	Negative
10.	<i>Penicillium</i> sp. 2.	1 Band	Negative

Table 2 : Detection of Bt gene in bacterial isolates of Bt cotton at 90 DAS

Sr. No.	Name of fungal isolate	Detection of Bt gene in bacterial isolates	
		No. of bands	Results
1.	<i>Erwinia</i> sp.	1 Band	Negative
2.	<i>Pseudomonas</i> sp.	1 Band	Negative
3.	<i>Azotobacter</i> sp.	1 Band	Negative
4.	<i>Xanthomonas</i> sp.	1 Band	Negative
5.	<i>Bacillus</i> sp.	1 Band	Negative

Table 3 : Detection of Bt gene in seeds of Bt and non- Bt cotton

Sr. No.	Detection of Bt gene		
	Cotton variety	No. of bands	Results
1.	Ajit-151 (Bt cotton)	2 Band	Positive
2.	Local (non- Bt cotton control)	1 Band	Negative

organisms. Reported effects of the *CryIAb* protein on microbial communities were transient and were exceeded by other environmental factors (Fang *et al.*, 2005; Filion, 2008; Griffiths *et al.*, 2006 and 2005 and Lilley *et al.*, 2006).

Detection of Bt gene :

Bt gene was not present in microflora means that Bt gene was not transferred from Bt cotton plants to associated fungal and bacterial microflora. Evidence of Bt gene was studied by several workers, out of which the results obtained from present investigations are consonance with Paget *et al.* (1998) who revealed that there is no evidence that the resistant gene from the plant had been transferred to soil bacteria in genetically modified tobacco also Widmere *et al.* (1997); Gebhard and Smalla (1999) reported that the transformation of plant DNA to native soil micro-organisms has not been found. No proof of a plant gene being transferred to a soil bacteria was found. More or less similar results have been reported by Blackwood and Buyer (2004) that the impacts of cry protein on the soil microbial communities and their interactions in soil and rhizosphere ecosystems were limited. Gebhard and Smalla (1999) reported that, it was not possible to obtain information on whether the transgenic DNA persisted as free DNA bound to soil particles, in decaying or rotting plant material, or in transformed bacteria in transgenic sugar beets.

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