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REGULAR ARTICLE

SEED PHYSIOLOGY OF DIFFERENT CROPS UNDER THE INFLUENCE OF BT AND NON-BT COTTON RHIZOSPHERIC FUNGI CULTURE FILTRATE

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SUMMARY

Present paper deals with the study of culture filtrate of fungi obtained from rhizosphere of Bt and non-Bt cotton varieties produced by growing these fungi on GN medium and these culture filtrates were tested for seed germination, shoot length and root length of three cereals and three oil seeds. Culture filtrates of fungi isolated from rhizosphere of Bt cotton varieties were found to be more inhibitory for germination of seeds as compared to culture filtrate of fungi isolated from rhizosphere of non Bt-cotton varieties.

Keywords: Bt cotton, non-Bt cotton, rhizosphere fungi, seed physiology.

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1. Introduction

Cotton is an important cash crop in India, which plays a significant role in national economy. It is clear from literature that similar to cotton nearly 50 such genetically modified crops have been accepted significantly by large number of growers. Bt cotton with Bt toxin gene oriented from a bacterium *Bacillus thurengenesis* was successfully incorporated in cotton, hence called as Bt cotton. With the incorporation of this gene the pest problem especially that of insects is nearly vanished. It was interesting to accept this new way of eco-friendly and economically feasible control of pests; however such plants are safe for microbes including fungi, bacteria and viruses growing in association with these plants i.e. rhizosphere and phyllosphere areas. Considering these facts the present topic was selected to find out the effect of biochemicals both intracellular (sap) as well as extra cellular (exudates) of Bt cotton. The effect on behavior of microbes in general and fungi in particular has been concentrated in detail.

2. Material and Methods

Bt cotton varieties NECH-2R BT, NECH-3R BT, and non-Bt cotton NECH-2R non-Bt, NECH-3R non-Bt were donated by Nath biotechnologies, Aurangabad and varieties like Rashi-2, Bollgaurd both Bt and non-Bt were purchased from local market were employed for the present studies. The seeds were sown in pots with natural microflora available in the soil.

(a) Collection of rhizosphere soil samples

Rhizosphere soil samples were collected from 90 days aged cotton plant. Five plants were randomly selected from each plot and the rhizosphere soils from the root zones of the selected plants were collected and mixed together to obtain a homogenous soil sample. Composite soil samples from the plot of each variety of Bt and its isoline non-Bt cotton were further assessed for their fungal population. The soil samples were collected from the surface of underground roots and their surroundings. Surface-sterilized scalpel was used to transfer rhizosphere soil to clean sterilized glass bottles and the bottles were sealed carefully. Same procedure was adopted for remaining cotton plants from each plot.

(b) Isolation of rhizosphere mycoflora by serial dilution agar plate method

Dilution plate method was used for isolation of rhizosphere mycoflora of cotton crop. For the isolation of fungi serial dilution factor 10-4 was used. Soil samples were collected randomly from rhizosphere of cotton plants. Composite samples from each plot were used for fungal analysis after each 15 days of growth stages.

At the time of serial dilution labeled the dilution blank, as 10-1, 10-2, 10-3 and 10-4 marked with marker pencil. 10 g of air-dry soil sample from composite sample was added into 90 ml sterilized distilled water and dilution blank labeled as 10-1. Thus diluting original sample 10 times (1: 10) and shaken thoroughly for 20 minutes to obtain a uniform distribution and release of microorganisms from adhering soil particles. From the first dilution 1 ml of suspension was added in 9 ml sterilized water to the dilution blank number 10-2 by sterile pipette. The dilution number second was shaken for 5 minutes.

The same procedure was repeated till the original sample was diluted to 10-4, every time a fresh sterile pipette was used. Finally from the dilution number 10-4 1 ml of suspension was transferred with the help of sterile pipette to sterilized Petri dishes containing potato dextrose agar medium. 1 ml of soil sample suspension was added in three sterile Petri plates. The inoculated Petri plates were incubated in inverted position at room temperature 27°C ± 2 for 2 -7 days. Pure cultures were obtained from inoculated plates by single spore isolation method.

(c) Assay of toxicity of culture filtrate

The toxicity of culture filtrate was determined by using seed germination method. Surface sterilized hundred seed pf each variety were soaked in crude toxin preparation for 24 hours. They were then placed on moist blotter in Petri plates. Seed soaked similarly in freshly prepared uninoculated liquid medium served as control. Percent germination or percent inhibition of germination, root and shoot length of seedlings was measured after 7 days of incubation at room temperature.

3. Results and Discussion

Table 1 shows that culture filtrates of fungi isolated from rhizosphere of Bt cotton varieties were found to be inhibitory for germination of cereal and oil seeds as compared to culture filtrate of fungi isolated from rhizosphere of non Bt-cotton varieties. Table 2 indicates that culture filtrate of rhizosphere fungi of Bt cotton varieties were

found to be inhibitory for shoot growth of cereals and oilseeds as compared to culture filtrate of fungi isolated from rhizosphere of non Bt-cotton varieties. Culture filtrate of rhizosphere fungi of Bt cotton varieties were found to be inhibitory for root growth of cereal and oilseeds as compared to culture filtrate of fungi isolated from rhizosphere of non Bt-cotton varieties.

Table 1. Effect of culture filtrates of cotton rhizosphere fungi on seed germination (%) of different crops.

Crops	Isolation from Bt			Isolation from non-Bt		
	A.alt	F.oxy	Tri	A.alt	F.oxy	Tri
Jowar	90	80	90	95	85	100
Wheat	75	70	80	70	80	75
Bajara	80	90	85	90	85	95
Groundnut	40	35	35	50	40	45
Sesame	70	85	70	80	90	85

A.alt- *Alternaria alternata*, F.oxy- *Fusarium oxysporum* and Tri- *Trichoderma viride*.

There are several reports that genetically modified plants changed behavior of microbe rhizosphere. Donegan et. al.(1996) studied the environmental release of GM plants may be accompanied by ecological effect including changes in plant associated microflora. Donegan et al (1999) in case of transgenic cotton containing Bt-toxin placed into soil in a laboratory study to observe decomposition of plant residue and found differences in carbon content between the decomposing parental and Bt cotton litter due to soil microbial populations. DiGiovanni et. al. (1999) found in case of *Medicago sativa* transgenic and normal varieties difference in soil microorganisms. Shen et al. (2006) studied microbial activities affected due to transgenic Bt cotton (Sukang-103) and its non-Bt cotton counterpart (Sumian-12) were investigated to evaluate potential risk of transgene on soil ecosystem. Savka & Farrand (1997) a very recently demonstrated that in a legume plant Lotus corniculatus that opine producing GM plant altered its microbial environment in rhizosphere.

Table 2. Effect of culture filtrates of rhizosphere fungi on shoot length (S mm) and root length (R mm) of different crops.

Crops	Isolation from Bt						Isolation from non-Bt					
	A.alt		F.oxy		Tri		A.alt		F.oxy		Tri	
	S	R	S	R	S	R	S	R	S	R	S	R
Jowar	3.40	3.8	4.20	3.2	3.80	4.8	4.10	3.9	4.70	5.5	4.50	4.2
Wheat	4.20	4.2	5.10	2.9	4.00	3.7	4.90	3.6	4.00	4.5	4.80	5.0
Bajara	5.90	3.2	4.80	4.1	4.20	5.3	6.50	4.3	5.30	4.9	4.60	5.2
Groundnut	0.90	2.9	0.70	2.7	0.50	3.3	1.30	3.5	1.00	3.8	1.10	3.9
Sesame	2.2	5.7	2.9	4.7	1.9	5.9	3.4	5.0	3.0	6.0	2.1	6.2

A.alt- *Alternaria alternata*, F.oxy- *Fusarium oxysporum* and Tri- *Trichoderma viride*.

It is clearly revealed from the results that the culture filtrates of fungi inhabiting rhizosphere of Bt cotton varieties secreted more toxic substance capable of inhibiting the germination, shoot length and root length of studied cereals and oils seeds as compared to that of culture filtrates of fungi inhabiting the rhizosphere of non-Bt cotton varieties.

These results inclined to conclude that the exudates of Bt cotton might be rich in other substance capable of modifying genetic makeup of rhizospheric fungi.

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