Recent Research in Science and Technology 2011, 3(5): 62-67
ISSN: 2076-5061
www.recent-science.com

PLANT PATHOLOGY

INCIDENCE OF SEED-BORNE MYCOFLORA ON FRENCH BEAN MUTANTS AND ITS ANTAGONISTIC ACTIVITY AGAINST *TRICHODERMA HARZIANUM*

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Abstract

Seed-borne mycoflora of two varieties of French bean viz. Waghya and Varun was studied. Seed mycoflora of nine mutants of Waghya and seven mutants of Varun was isolated on Glucose Nitrate Agar and Rose Bengal Agar Media. Thirteen fungi were isolated from Waghya variety and nine fungi were found to be associated with Varun variety. On both the varieties *Macrophomina phaseolina* showed its quantitative dominance which were followed by *Aspergillus niger* and *Fusarium oxysporum.* Broad pod mutant of variety Waghya showed maximum association of fungi while Long pod mutant showed minimum detection of fungi. Small leaf mutant of variety Varun was associated with maximum number of fungi while dwarf and branched mutant showed minimum detection of fungi. Waghya variety showed maximum incidence of fungi as compared to Varun variety. The antagonistic activity of *Trichoderma harzianum* against dominant fungi was also studied.

Keywords: Seed mycoflora, Waghya, Varun and *Trichoderma harzianum*

Introduction

French bean botanically described as *Phaseolus vulgaris* L. is a protein rich crop. It is also known as *Rajmash* or *Rajma* (Hindi) or haricot bean or kidney bean or common bean or snap bean, navy bean. The French bean, *Phaseolus vulgaris*, is an herbaceous annual plant domesticated independently in ancient Mesoamerica and the Andens, and now grown worldwide for its edible bean, popular both dry and as a green bean.18.3 million tonnes of dry French beans and 6.6 million tonnes of green beans were grown worldwide in 2007. Brazil and India are the largest producers of dry beans while China produces, by far, the largest amount of green beans, almost as much as the rest of the top ten growers altogether. Similar to other beans, the French bean is high in starch, protein and dietary fiber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B6, and folic acid. It is valued for its protein rich (23%) seeds. Seeds are also rich in calcium phosphorus and iron. After harvesting seed are stored at different storage conditions and if these storage conditions are not proper, various microorganisms like bacteria, viruses, nematodes and fungi interact with these seeds. Among these microorganisms fungi play dominant role in decreasing quality and longitivity of the seeds. Several seed-borne fungi, including species of *Alternaria, Aspergillus, Fusarium* and *Penicillium*, have been detected as seedling pathogens of cereals (Fahim et. al., 1983; Gulya, et. al., 1979; Martin and Johnston, 1982; Shurtleff, 1980). Many fungi are serious parasites of seed primordia, maturing and stored seeds and grains and their invasion can resulted various abnormalities including, reduce yields of seed in both quantitatively and qualititatively, discolorations, decrease germinability, mycotoxin production and total decay (Quenton *et al, 2003*; Castillo *et al.*, 2004). The fungal pathogens play a major role in the development of diseases on many important field and horticulture crops; resulting in severe plant yield losses. Extensive use of fungicides has resulted in accumulation of toxic compounds potentially hazardous to humans and environment and also in the increase of resistance in the pathogens (Anand and Jayarama, 2009). The increasing awareness of fungicide-related hazards has emphasized the need of adopting biological methods as an alternative disease control method. *Trichoderma* species are well documented fungal biocontrol agents (Papavizas, 1985; Elad and Kapat, 1999; Howell, 2002). The internal mycoflora depletes all the essential contents as well as reduces seed germination. However the different varieties may show varied response to the Mycoflora. Induced mutations plays an important role in changing the seed characteristics. The altered genotype may show the diverse response to inborn fungi as compared to the control seeds.

In present paper the internal mycoflora of two varieties of French bean and their mutants was studied on two different media viz.Glucose nitrate agar and Rose Bengal agar. The mutants of both the varieties along

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with control had shown the diverse response to development of internal mycoflora.

Materials and Methods

Isolation of mycoflora

In present research study, detection of seed mycoflora associated with the seed samples was done (ISTA, 1996). 10 seeds per pre-sterilized petri-plates were equispaced asceptically on autoclaved Glucose Nitrate Agar (GNA) and Rose Bengal Agar (RBA) media. The plates were then allowed to incubate at room temperature. Detail observations of fungal characters were done under the binocular microscope and their identification was confirmed with standard literature (Ellies, 1971; Mukadam et al., 2006). twenty two fungi were isolated from two varieties viz. Waghya and Varun of French bean by Agar plate methods on Potato Dextrose Agar and Rose Bengal Agar medium.

Antagonistic effects of *Trichoderma* against isolated fungi

Trichoderma harzianum was isolated from the sesame on PDA. A mycelial disc (1.2 cm diam), obtained from the peripheral region of 5-7-day-old cultures of *Aspergillus niger, Fusarium oxysporum, Fusarium equiseti, Alternaria dianthi, Macrophomina phaseolina, Rhizopus stolonifer, Curvularia lunata* and *Penicillium digitatum* on PDA, was placed on a fresh PDA plate (3 cm from the center) and incubated at 28~ for 48 h to initiate growth. Then a 1.2-cm-diam mycelial disc, obtained from the periphery of a 5-7-day-old culture of *Trichoderma harziaum* was placed 3 cm away from the inoculum of the pathogen, the plates were incubated at 28^{0}C and measurements were taken after 7 days. In the control experiment a sterile agar disc (l.2 cm diam) was placed in the dish. At the end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control as follows:

$$\frac{C-T}{C} \times 100 = \%\ \textit{Inhibition of radial mycelial growth}$$

Where, C = radial growth measurement of the pathogen in control

T = radial growth of the pathogen in the presence of *T. harzianum.*

Results and Discussion

Both the varieties and their mutants showed the incidence of seed-born fungi. Total twenty two fungi were recorded from both the varieties. Out of which the *Macrophomina phaseolina* showed its quantitative dominance which were followed by *Aspergillus niger* and *Fusarium oxysporum.* Ten mutants and one control sample of variety Waghya showed the occurrence of different fungi while eight mutants and one control sample of variety Varun was studied for fungal susceptibility. Broad pod mutant of variety Waghya showed maximum association of fungi while Long pod mutant showed minimum detection of fungi. Broad pod mutant of variety Waghya showed the occurrence of seven different fungi which includes *Macrophomina phaseolina, Aspergillus niger, Fusarium equsiti, Curvularia lunata, Alternaria dianthi, Rhizopus stolonifer* (Table 1). Small leaf mutant of variety Varun was associated with maximum number of fungi while Dwarf and Branched mutant showed minimum detection of fungi (Table 2). Mutants of Varun variety showed minimum occurrence of fungi as compared to mutants of Variety Waghya. From this result it can be concluded that Varun variety is resistant and Variety Waghya is susceptible to seed-borne fungi. Such type of work was earlier supported by several workers (Neergaard, 1973; Agrawal, 1976; Chavan and Kakde, 2009).

Table 1: Percentage incidence of seed-borne fungi on French bean mutants variety Waghya

Fungi	Mutant																			
	Control		Dwarf		Late maturing		Gigas		Long pod		Large leaf		Tall/Bold pod		Broad pod		Cream seed coat		Tall	
	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA
Alternaria alternata		--	--	--	--	--	--	--	--	10%	--	--	--	--	--	--	--	40%	--	--
Alternaria dianthi	20%	--	--	--	--	--	--	--	--	--	--	--	--	--	--	20%	--	--	--	--
Aspergillus niger	10%	--	50%	20%	10%	20%	20%	20%	--	--	--	--	20%	--	20%	--	30%	20%	--	--
Aspergillus flavus	20%	--	30%	20%	20%	20%	--	10%	--	--	--	--	--	20%	10%	--	--	10%	--	--
Aspergillus fumigatus	--	--	--	--	10%	--	10%	--	--	--	--	--	--	--	--	--	--	--	--	--
Aspergillus terreus	20%	--	20%	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Colletotrichum sp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	40%	--	--	--
Curvularia lunata	--	--	--	20%	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fusarium oxysporum	--	20%	20%	10%	--	20%	10%	10%	--	--	10%	10%	--	30%	--	20%	--	--	--	--
Fusarium equiseti	--		--	10%	--	--	--	--	--	--	--	--	--	--	--	10%	--	--	--	10%
Macrophomina phaseolina	--	20%	20%	30%	20%	30%	20%	50%	--	20%	70%	40%	40%	40%	30%	80%	20%	10%	100 %	100 %
Penicillium digitatum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	20%	--	--	--
Phytophthora sp.	10%	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Rhizopus stolonifer	10%	--	--	--	--	--	30%	--	--	--	30%	40%	30%	--	40%	--	--	--	20%	--

Table 2: Percentage incidence of seed-borne fungi on French bean mutants of variety Varun

Fungi	Mutant															
	Control		Leanear leaf		Small leaf		Dwarf		Large leaf		Branched		Early flowering		Long pod	
	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA	RBA	GNA
Aspergillus niger	20	10	30	--	20	30	--	--	80	70	20	--	20	--	--	10
Aspergillus flavus	10	--	20%	--	20%	20%	--	--	--	--	--	--	--	--	10%	10%
Aspergillus terreus	--	--	--	--	--	--	30%	--	20%	--	--	--	10%	--	--	--
Colletotrichum sp.	--	--	--	--	--	--	--	--	--	--	--	--	10%	--	--	--
Cercospora sp.	--	--	--	--	--	10%	--	--	--	--	--	--	--	--	--	--
Fusarium oxysporum	20%	30%	--	--	20%	20%	--	10%	10%	30%	20%	20%	--	--	--	--
Fusarium equiseti	--	--	--	--	--	--	--	--	--	--	--	--	--	30%	--	--
Macrophomina phaseolina	50%	40%	40%	70%	20%	30%	50%	50%	20%	--	40%	50%	30%	30%	50%	20%
Rhizopus stolonifer	--	--	30%	--	30%	--	--	--	--	--	--	--	--	--	20%	--

It is evident from the results that *Trichoderma harzianum* has antagonistic activity against seed-borne fungi. *Trichoderma harzianum* showed its greatest antifungal activity against *Fusarium oxysporum* which is followed by *Macrophomina phaseolina, Aspergillus niger* and *Alternaria alternata. Trichoderma harzianum* showed minimum radial growth inhibition against *Rhizopus stolonifer* and *Fusarium equiseti* (Table 3).

Fig. 1: Incidence of seed-borne mycoflora of French bean mutants on GNA

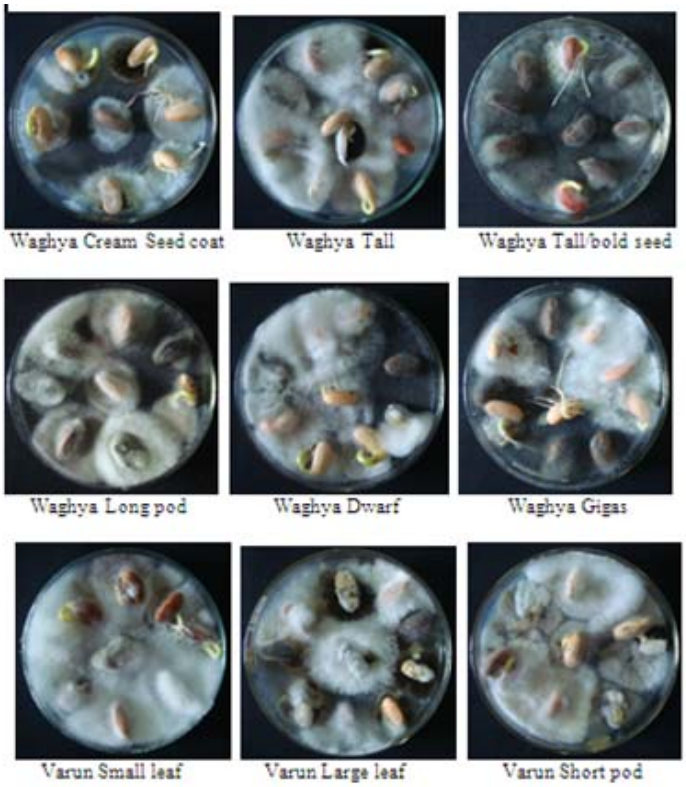

Fig. 2: Incidence of seed-borne mycoflora of French bean mutants on RBA

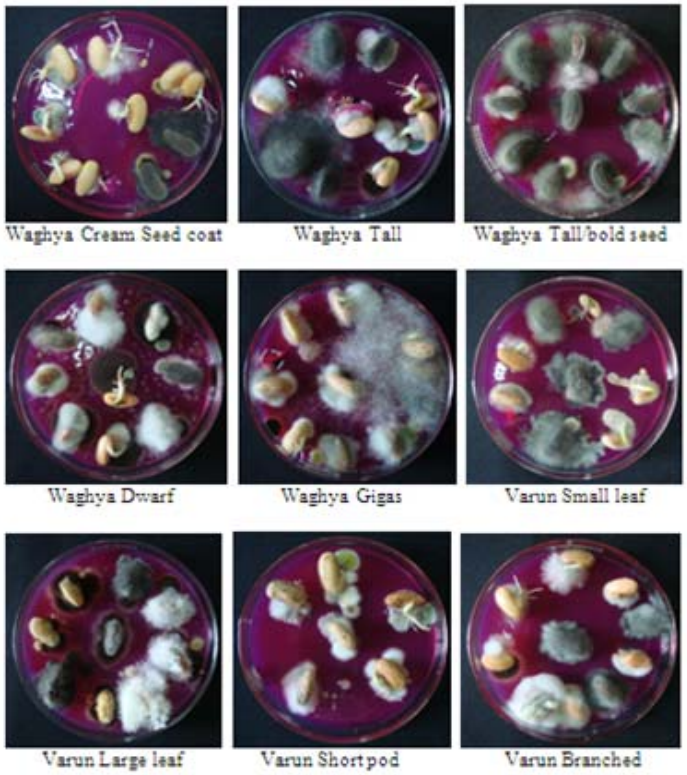

Fig. 3: Incidence of seed-borne mycoflora of French bean (Control) on RBA

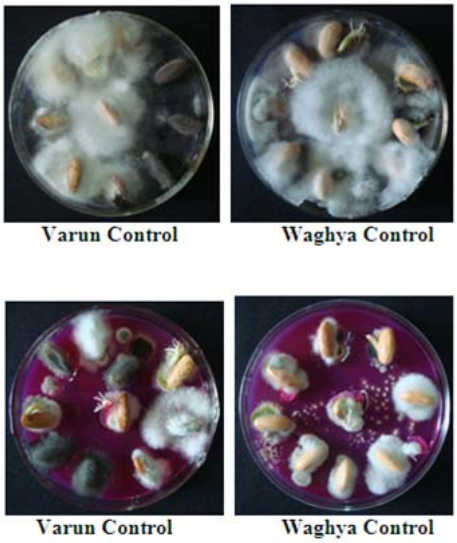

Fig. 4: Antagonistic activity of *Trichoderma harzianum* against Seed-borne fungi of French bean

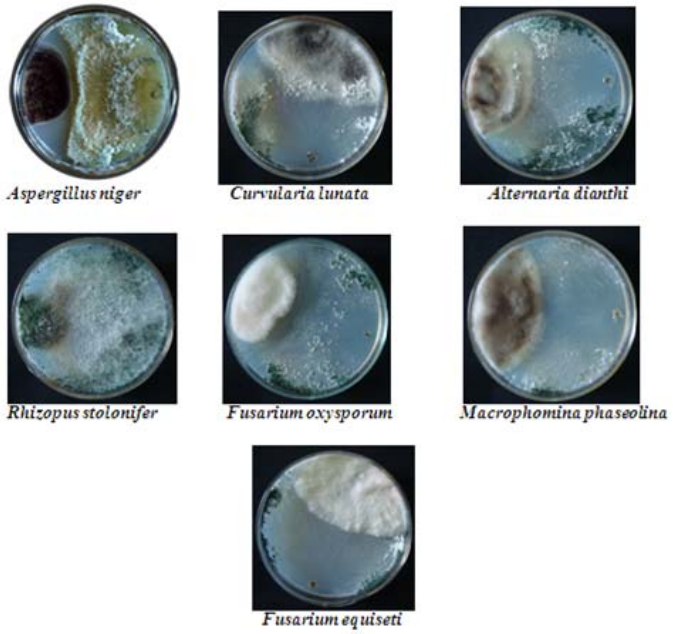

Table 3: Antagonistic activity of *Trichoderma harzianum* against Seed-borne fungi of French bean

Fungi	C (cm)	T (cm)	% Inhibition
Aspergillus niger	3.5	1.7	51.42
Alternaria alternata	5.2	2.6	50
Curvularia lunata	6.2	3.2	48.38
Fusarium oxysporum	5.1	2.1	58.82
Macrophomina phaseolina	5	2.4	52
Rhizopus stolonifer	7	5.4	22.85
Fusarium equiseti	5.2	3.5	32.69

C=Control; T=Treatment

Antagonism of *Trichoderma* species against several pathogens has been reported by several workers (Chet and Baker, 1980; Bell *et al*., 1982; Papavizas, 1985; Elad, 2000; El-Katatny *et al*., 2001; Howell, 2002). Dual culture method is widely used in antagonistic studies (Huang, 1978; Pachenari and Dix, 1980; Bell *et al*., 1982).

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