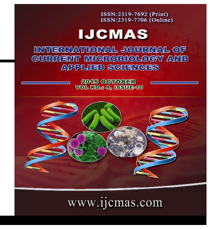
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 282-288



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 282-288**
http://www.ijcmas.com

Original Research Article

Virulence and Histopathological Study of *Pythium* Species which causes Soybean Damping-off

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Keywords

Pythium ultimum, Virulence, Host colonization, Root rot, *Glycine max* (L.) Merill.

A B S T R A C T

Two isolates of *Pythium ultimum* Trow. obtained from diseased soybean and chilli seedlings, were maintained and used against virulence study. When soybean seedlings were placed in culture filtrates of both isolates, soybean isolate given higher seedling mortality than the chilli isolate. The soybean isolate of *Pythium* on soybean also caused early and severe symptoms indicating the significant difference in the virulence. This study illustrates that the differential virulence of both isolates is related to host-pathogen specificity. In histopathological study, host-pathogen interaction between *Pythium* species and soybean germplasm lines (three susceptible and three resistant) were studied. Intensive epidermal and cortical infections by hyphae were observed in case of susceptible varieties. Infected roots ranged from a white to grey, water-soaked color. Extensively infected root tips typically were unhealthy, succulent and thin. Subsequently, resistant lines were shown limited root necrosis.

Introduction

Pythium species are ubiquitous soil-borne oomycetes that rank from opportunistic up to highly virulent pathogens on many plant species. They mainly infect young plant tissues and cause pre- and post-emergence damping-off or reduce the vigor and growth of surviving seedlings. Recently, *Pythium* was associated with progressive yield decline soybean fields (Avanzato *et al.*, 2008; Rosso *et al.*, 2008). Also closely related *Pythium* spp. exhibited a varying degree of virulence towards soybean seedlings, among which *P. ultimum* was the most virulent and inflicted a strong pre- and post-emergence damping-off. Few histopathological studies have monitored and revealed that this pathogen quickly invaded the rice rhizodermis via penetration hyphae, after which it generated a dense intracellular network in the inner root tissues and ultimately triggered necrosis (Mojdehi *et al.*, 1991).

When 2.5 per cent (v/v) culture filtrate was supplemented in rooting medium of geranium (*Pelaryonium* x *Hortorum*), plantlets appeared thinner, shorter and darker than the control. Several necrotic lesions developed on the root surface. Microscopy revealed absence of root hairs and a collapse of cortical and epidermal cells (Desilets and Belanger, 1991). *P. ultimum* caused more extensive degradation of pectins and cellulose, which occurred at a relatively greater distance from the hyphae (Campion *et al.*, 1998). Degradation of pectins was always more rapid in the cell walls than in the intercellular junctions. This causes loss of tissue integrity and tissue maceration by *P. ultimum* in carrot. *P. ultimum* has greater enzymic potential for degradation of cell wall polysaccharides in carrot than *P. violae.* Ray *et al.* (1998) studied ultrastructural and cytochemical changes due to infection by *Pythium* spp. Their cytological observations indicated a series of events.

However, scanty literature is available on virulence and histopathological interaction studies of *pythium* and soybean. The objective of the present research was to study the host specificity of *pythium ultimum* and reaction with soybean seedlings.

Materials and Methods

Virulence study

Two *Pythium* isolates obtained from soybean and chilli were maintained and used for virulence study. Twelve days old cultures in PDB of both isolates were homogenized by mixer grinder, separately. These culture filtrates were transferred to sterile test tubes containing 2/3 sterile sand. Ten days old soybean seedlings of var. JS-335 were transferred to test tubes. The experiment was planned in split plot design with two main and two sub treatments and five replications. Soybean and chilli as source of *Pythium* isolate served as main treatments. Inoculation with 100% culture filtrate and uninoculated control served as sub treatments. In each replication eight seedlings were maintained. Observations on seedling mortality were noted.

Histopathological study

From previous studies 3 resistance and 3 susceptible germplasm lines to damping-off disease of soybean *viz*. MAUS-47, MAUS-158, MAUS-162 (resistant) and MAUS-17, MAUS-136, SL-528 (susceptible) were selected for this study. Seven days old seedlings of these lines were transferred in sterile test tubes containing 100% culture filtrate of the pathogen. After seedlings were shown toppled symptoms, their transverse and longitudinal section cuttings were taken and observed under light microscope at various magnifications.

Results and Discussion

Virulence study on pathogen

Virulence study on pathogen was planned in split plot design with 5 replications. Treatments were as follows: Main treatments : 2 : Sources of isolate (S_{1S} = Soybean as source of *Pythium* isolate , S_{2C} = Chilli as source of *Pythium* isolate); Sub treatments : I_0 = Uninoculated control (Sterile water), I_1 = Inoculation with 100 % culture filtrate. For virulence studies, the *Pythium* isolated from soybean seed was labeled as S_{1S} and *Pythium* isolated from chilli seed was labeled as $S_{2C.}$ The observations on seedling mortality as induced by soybean and chilli culture filtrates were recorded which are given in table 1.

From table 1 it is revealed that the inoculation induced significant mortality over uninoculated control indicating virulent ability of the isolates. In general 42 %

mortality was induced. It can be concluded that soybean and chilli isolate differed significantly in respect of virulence. On the basis of interaction of S x I, soybean isolate has induced significantly higher mortality of soybean over chilli isolate indicating the expression of host specific virulence. Results are also shown with figure 1.

Table.1 Virulence as expressed with interaction Source × Inoculation (S×I)

Mortality (%) Arc sin value					
	Inoculation				
Source	I_0 Control	I_1 Inoculated	Mean	Main Treat.	
S_{1S} – Soybean	0.57	78.82	39.7		
S_{2C} – Chilli	0.57	47.93	24.3		
Mean	0.57	63.4	64	SE ±	CD@ 0.05
SE ±			1.7	2.96	9.25*
CD@ 0.05			5.6*		
Interaction (S×I)					
SE ±	4.36				
CD@ 0.05	13.08				

Table.2 Histopathological observations on soybean varieties

Sr. No	Name of Variety	Nature of damage					
		Cuticle	Epidermal cells	Root/Stem Cortex	Phloem	Pith	Xylem
1	MAUS-17	++++	++++	++++	++	+	-
2	MAUS-136	+++	+++	+++	+	+	-
3	SL-528	++++	+++	+++	+	+	-
4	MAUS-47	+++	++	+	-	-	-
5	MAUS-158	++	++	+	-	-	-
6	MAUS-162	+++	++	++	-	-	-

Control

Inoculation with soybean isolate

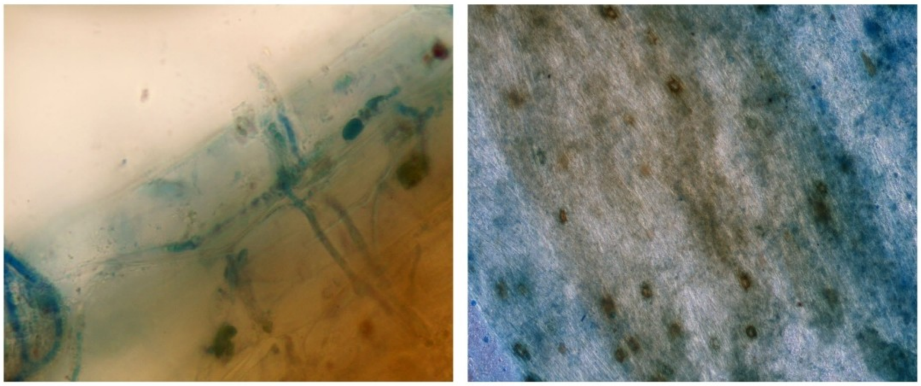
Soybean Isolate

Control

Inoculation with chilli isolate

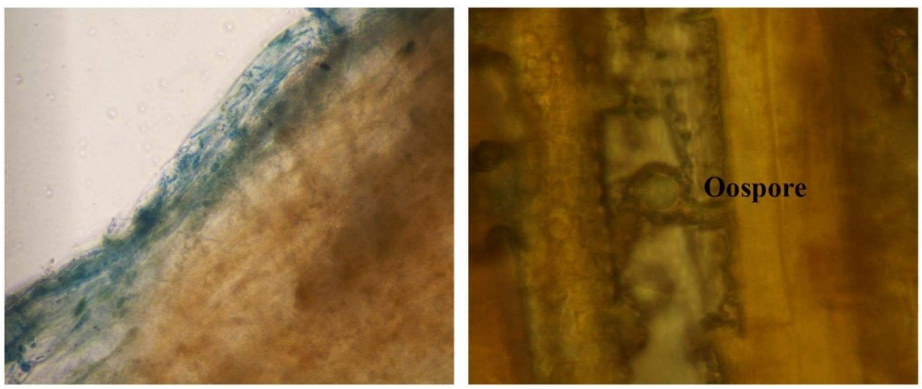
Chilli Isolate

Fig. 1. Testing of virulency of soybean and chilli isolates



Infection process at collar region **Development of necrotic regions**

A. Susceptible variety



Restriction of mycelium to upper epidermis **Development of Oospore on collar region**

B. Resistant variety

Fig 2 . Histopathological study

Histopathological study

To assess the nature of damage by pathogen in susceptible and resistant varieties histopathological investigation was undertaken. Three susceptible varieties *viz.* MAUS-17, MAUS-136, SL-528 and 3 resistant varieties *viz.* MAUS-47, MAUS-158, MAUS-162 were selected. They were subjected to root inoculation by submerging seedlings in culture homogenate for 3 days. After then the longitudinal as well as transverse sections were taken. The histopathological observations were recorded and depicted in table 2 and also exhibited with figure 2.

Due to thickness of root and stem epidermal cell walls as well as involvement of certain biochemicals in root and stem cortex, growth of *Pythium ultimum* is stopped completely in the cortex of tolerant plants. On the other hand in susceptible seedlings, the fungus rapidly colonizes entire root system and hyphal growth is unrestricted.

From table 2 it can be concluded that in susceptible varieties pathogen multiplied rigorously on surface and penetrated through epidermal layer and stem/ root cortex. The quick multiplication of the pathogen in susceptible stem and root caused disorganization of the epidermal cells and cells of root cortex. On the other hand in resistant varieties the mycelium did not ramify quickly, thereby restrict growth of pathogen to the surface. The oospores formation was more in case of resistant varieties as compared to susceptible one.

Virulence of the pathogen

Pythium ultimum Trow is known to be a polyphagous pathogen infecting a wide range of plant species. However, there were few reports wherein the pathogen was stated to differ in virulence and aggressiveness (Middleton, 1943; Brown and Kennedy, 1965). In the present investigation the virulence of soybean and chili isolate were tested.

Though soybean and chili isolate were reciprocally pathogenic, they differed in percentage mortality and severity of symptoms induced. The soybean isolate on soybean caused significantly higher mortality over chili isolate. The soybean isolate on soybean also caused early and severe symptoms on soybean indicating the significant difference in the virulence. These also indicated the host specificity for virulence. The variation among *Pythium* isolate in causing pre-emergence damping off has been reported by Brown and Kennedy (1965). McCarter and Littrell (1970) also showed variation in pathogenic ability within *Pythium* species. Zhang and Yang (2000) stated that 13% isolates were highly aggressive on soybean but weakly aggressive on corn. They also confirmed intraspecific variation in virulence and aggressiveness of *Pythium ultimum*.

Histopathological study

The histopathological investigations on susceptible and resistant varieties indicated that the mycelium multiplied by very fast on the surface of stem and roots of susceptible cultivars then it multiplied in root cortex and caused disorganization of the constituent cells. In resistant varieties the multiplication was slow and was confined to upper epidermis. The similar observations were made by Ray *et al*. (1998) who noted growth of the fungus in the epidermis and outer cortex tissue and which was associated with marked host cell disorganization and even break down. The Author also observed plugging of host cells with osmophillic electron granular, fibrilar materials. The degradation of pectin was more rapid in cell walls thus causing loss of tissues integrating and tissue maceration by *P. ultimum*. Hock and Klarman (1967) noted that *Pythium debaryanum* could not colonize suberized endodermal cells and lignified cells of 25 day old seedlings of virginia pine seedlings, thus suberized endodermis acted as barrier restricting the mycelium to the cells outside endodermis. Mellano *et al.* (1970) observed critically that the growth of *P. ultimum* was stopped completely in the cortex of mature portions of primary and secondary roots in case of tolerant plants in contrast hyphal growth was unrestricted in roots of susceptible plants. In susceptible seedlings, the fungus rapidly colonized the entire roots system, it formed oospores in the infected tissue and caused the plants to collapse and died.

Acknowledgments

Author is thankful to ASPEE, Mumbai for financial support. This work was supported by Dr. G.D. Deshpande, Head, Plant

Pathology and Dr. K.S. Baig, project coordinator AICRP Soybean VNMAU, Parbhani.

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