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ASSESSMENT OF VARIABILITY AMONG ISOLATES OF *PHOMOPSIS VEXANS* IN RESPECT TO PRODUCTION OF HYDROLYTIC ENZYMES AND TOXIN

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ABSTRACT

This study was conducted to investigate variability among five isolates of *Phomopsis vexans* in respect to the production of enzymes and toxins during pathogenesis. Results showed that five isolates *Phomopsis vexans* was varied among themselves to produce macerating enzyme (ME), pectin methylesterase [pectinesterase] (PME) and cellulase enzymes in the Richards' medium. The virulent isolates produce pectic and cellulose enzyme whereas some isolates are not capable of producing such enzyme. Such variability also observed in phytotoxin production by these isolates. The phytotoxin produces by those isolates inhibited the brinjal seedling. The symptoms produced on brinjal seedlings following dipping of seedlings in different concentration of toxin(s) preparation were shows variation from isolates to isolates.

KEYWORDS: *Phomopsis vexans*, Isolates, Hydrolytic Enzymes and Toxin

INTRODUCTION

The global area under brinjal cultivation has been estimated to be at 1.85m ha with total production of about 32 million metric tonnes. It is grown on nearly 550,000 hectares in India. Fruit rot and leaf blight disease caused by *Phomopsis vexans* is a major concern in brinjal production as it reduces yield and marketable value of the crop nearly 20-30%. Panwar *et al*. (1970) reported that the losses due to Phomopsis fruit-rot ranged to the extent of 10–20% in Punjab. The involvement of cell wall degrading enzymes in pathogenesis of eggplant fruit rot pathogen is an indicative from the extensive rotting it causes. The pathogen has a very restricted host range, its enzymatic activity does not appear to be very strong, possibility of toxin production-a toxin that plays a very important role in pathogenesis and disease development was a distinct possibility.

This possibility was explored and was well studied by many workers. In this study we tried to find out the variability among five different isolates of *P. vexans*, namely BhSPv, BSPv, GgSPv, KbFPv and TnSPv, with respect to their ability to produce pectic enzyme and cellulase *in vitro* were studied in Modified Richard's Synthetic medium amended with 0.5% citrate pectin and waters soluble Carboxy Methyl Cellulose (CMC)- Na salt. Also assess the variability among different isolates with respect to production of *in vitro* toxin using three different media namely Czapeck Dox solution, Richard's Synthetic solution and Modified Fries 3 solution.

MATERIALS AND METHODS

Enzyme

For *in vitro* studies of cellulolytic and pectic enzyme(s) activity, the test organism were grown in Czapeck-Dox medium (with 10gm/L sucrose) fortified with 1% Carboxymethyl cellulose sodium salt (CMC-Na) and pectin respectively. The flasks were inoculated with mycelia discs of *P. vexans* and incubated at 28±1ºC in a BOD incubator. After 15 days the culture medium was filtrated through folds of Watman No. 1 filter paper. The filtrate was centrifuged at 5ºC at 10000 rpm for 10 minutes in a model PR-2 International Refrigerated Centrifuge. The decant extract was collected and used as enzyme source. Viscosimetric assay (Bateman, 1966) was adopted to determine pectic enzyme and cellulase activity of *P. vexans* in an Ostwald's Viscosimeter. The enzyme and substrate added in Viscosimeter at a ratio of 1:1 and the flow time was recorded after specific time intervals (in min). The flow time of water and the flow time of a mixture of bufferised substrate and autoclaved enzyme were also determined.

Toxin

The pathogen was grown in three different media viz. - Richard's synthetic solution, Czapeck-Dox solution and Modified Frie's 3 solution. The flasks were inoculated with five isolates of *P. vexans* separately and were incubated at temperature of 28±1ºC for a period of 15 days.

The fungal mat was harvested by filtering through folds of Watmann No. 1 filter paper. The filtrate was centrifuged separately for each isolates and for each medium in centrifuge at 10000rpm for 20 minute in a model PR-2 International Refrigerated Centrifuge. The decant was collected and was used as source of toxin Toxin filtrate was brinjal seedling adopting the technique of Pringle and Braun (1957) with modification of Samaddar and Scheffer (1968) and on eggplant seedlings.

RESULTS AND DISCUSSIONS

Enzyme

The result presented in Table 1 reveals that highest pectic enzyme activity was recorded in isolate TnSPv ($PDFT_{50}$= 15 min) and that of specific enzyme activity 1.11. This was followed by isolate BSPv ($PDFT_{50}$= 90 min and specific enzyme activity (Px) = 0.18). The lowest pectic enzyme activity obtained in isolate GgSPv ($PDFT_{50}$= 210 min and specific enzyme activity (Px) = 0.079) while the Phomopsis isolate BhSPv failed to produce any pectic and cellulase enzyme.

Table 1: *In vitro* and *In vivo* Pectic and Cellulase Activity and $PDFT_{50}$ of Different Isolates of *Phomopsis vexans*

Isolates	*In vitro*			
	Pectic Enzyme		Cellulase Enzyme	
	$PDFT_{50}$ (in Min)	Specific Enzyme Activity*	$PDFT_{50}$ (in Min)	Specific Enzyme Activity*
BhSPv	……	……	……	……
BSPv	90	0.18	179.5	0.09
GgSPv	210	0.079	418	0.039
KbFPv	150	0.112	……	……
TnSPv	15	1.11	69.5	0.23

While considering the *in vitro* cellulase enzyme activity (Table 1) of this five isolates of *P. vexans*, again TnSPv was the highest producer of cellulase ($PDFT_{50}$= 69.5 min and Px= 0.23) followed by BSPv ($PDFT_{50}$= 179.5 min and Px= 0.09) and GgSPv ($PDFT_{50}$= 418 min, Px= 0.039). The other isolates KbFPv and BhSPv produce no significant amount of Cellulase enzyme *in vitro*.

Low level of cellulase activity by different isolate of *Phomopsis vexans* under the present study directly corroborated with the nature of symptom (Dry rot) produced by the pathogen. The pectic enzymes are apparently of prime importance in dry rot diseases in which parenchymatous tissue is rapidly followed by cell death and is likely to be colonized by secondary invaders.

Toxin

The pathogen *Phomopsis vexans* has a very restricted host range, its enzymatic activity do not appear to be very strong, possibility of toxin production-a toxin that plays a very important role in pathogenesis and disease development was a distinct possibility. In this study we tried to find out the variability among different isolate of *P. vexans* in respect to production of non-host specific phytotoxin and their differential effect on binjal seedling.

The effect of toxin produce by different isolates of *Phomopsis vexans* was described in Table 2 to Table 4 and showed in Figure 1 to 3.

Table 2: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Modified Fries 3 Media on Eggplant Seedlings (Pusa Purple Cluster)

Isolates	Concentration of Toxin*			
	0.25 N	0.5 N	0.75 N	N
BhSPv	Plants were mostly healthy	Tip of apical meristem turn brown	Curling in the apical leaf along with marginal chlorosis	Curling in the apical leaf along with marginal chlorosis
BSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Apical meristem wilted followed by intense chooses and burning
GgSPv	Plants were mostly healthy, no external visible symptom	Only apical meristem drops down	In addition to droop down of apical meristem, first two leaves from tip were chlorotic.	In addition to droop down of apical meristem, first two leaves from tip were chlorotic.
KbFPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Leaf tip burning in apical 1-2 leafs were noted.
TnSPv	Plants were mostly healthy, no external visible symptom.	Cotyledonary leafs became curl and crinkle.	Completely wilted and apical leaf become intense necrotic	Completely wilted and apical leaf become intense necrotic

Water (Control) = Plants were healthy
Uninoculated Media (Conrol) = Plant become wilted

Figure 1: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Modified Fries 3 Media on Eggplant Seedlings (Pusa Purple Cluster)

Figure 2: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Richard's Synthetic Media on Eggplant Seedlings (Pusa Purple Cluster)

Table 3: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Richard's Synthetic Media on Eggplant Seedlings (Pusa Purple Cluster)

Isolates	Concentration of Toxin*			
	0.25 N	0.5 N	0.75 N	N
BhSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.
BSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Completely wilted and apical leaf become intense necrotic
GgSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Completely wilted and apical leaf become intense necrotic
KbFPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Leaf tip burning in apical 1-2 leafs were noted.
TnSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Completely wilted and apical leaf become intense necrotic

Water (Control) = Plants were healthy
Uninoculated Media (Conrol) = Plant wilted completely

Table 4: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Czapeck Dox Media on Eggplant Seedlings (Pusa Purple Cluster)

Isolates	Concentration of Toxin*			
	0.25 N	0.5 N	0.75 N	N
BhSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.
BSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.
GgSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Completely wilted and apical leaf become intense necrotic
KbFPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.
TnSPv	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Plants were mostly healthy, no external visible symptom.	Completely wilted and apical leaf become intense necrotic

Water (Control) = Plants were healthy
Uninoculated Media (Conrol) = Plants were healthy

Figure 3: Effect of Different Concentrations of Toxins Secreted by Five Different Isolates of *P. vexans* in Czapeck Dox Media on Eggplant Seedlings (Pusa Purple Cluster)

The results indicated that the toxin (s) were translocable and accumulated in the plant tissue where it produces the observed symptoms. In the present investigations three media were used for obtaining toxin (s). The toxicity of the toxin produced by different isolates of *Phomopsis vexans* in three different media varied considerably with the composition of the medium and the reaction varied significantly within the isolates. This may be having a positive correlation with the level of virulence among different isolates of *P. vexans*.

CONCLUSIONS

The variability among isolates with respect to production of enzyme and toxin directly correlated with their virulence. Isolates which are more virulent and caused severe fruit rot produce more enzyme and toxin as compare to those isolates which are less virulent. So it can be concluded that enzyme and toxin are act as secondary determinant of disease caused by *P. vexans*.

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