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Studies on Morphological, Cultural and Pathogenic Variability in Isolates of *Fusarium oxysporum* f.sp. *pisi* causing Wilt of Pea from Different Districts of Manipur, India

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ABSTRACT

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Pea is one of the most important rabi pulse crops of India. Pea is being rich in protein are valuable for vegetable purpose. Fourteen isolates of *Fusarium oxysporum* f.sp.*pisi* from different districts of Manipur were studied for its cultural, morphological and pathogenic variability. The mycelia colour varied from white to light pink, purple and pale yellow colour. The radial growth of the isolates ranged from 5.4cm to 8.9cm at 8 days after inoculation at 26±10 °C in 90mm petriplates. Sporulation of all isolates showed moderate to profuse. The size of micro conidia ranged from 11.6x 3.1 to 25.2 x 6.2 µm and size of micro conidia ranged from 3.02x 2.1 µm to 9.2 x 5.6 µm. The number of septation of macroconidia was mostly 2-3 whereas in microconidia most of the isolates was found with no septum. The shape of the macroconidia is mostly sickle shaped. In microconidia most of the isolates are oval shaped. The conidial colours of all the isolates were found hyaline. The formation of chlamydospores ranged from ten to twentyone numbers of all the isolates on Potato Dextrose Agar medium. The dry weight mycelium showed variability in the weight of isolates which was ranged from 0.78 to 1.31 gm. In pathogenic variability, all the isolates exhibited wilt symptom and hence proved pathogenic on pea cultivars under artificial inoculation.

Introduction

Pea (*Pisum sativum* L.) is native of South Europe and grown a garden or field crop throughout the temperate regions of the world and was originally cultivated in the Mediterranean basin (Smartt, 1990; Sardana *et al.*, 2007). In India, pea is one of the most important rabi pulse crops, in which the crop is grown on a field scale for its dry seeds and

smaller scale for green peas. Peas being very rich in protein are valuable for vegetable purpose. It is rich source of protein, amino acid, sugar, carbohydrates, vitamins A and C, calcium and phosphorus besides having a small quantity of iron.

In Manipur field pea is one of the most important pulse crops grown in 26,000 ha. Area occupying about 85% of the total pulses

area (Anonymous, 2015). However, the productivity of pulses in Manipur is very low due to several factors that are known to affect pea cultivation such as lack of improved varieties, tolerance to soil acidity, the most important being the diseases. Due to high humidity of region several disease caused by fungi, bacteria and viruses. Among these diseases, wilt caused by *Fusarium oxysporum f.sp.pisi* is the most destructive disease of the crop and occurs as an epiphyte almost every year. *Fusarium* is a soil-inhabiting fungus, surviving from year to year in the soil as thick-walled, very hardy spores that can sit in the soil surviving all kinds of conditions for more than 10 years. When the plant is in its early growth stage, the fungus kills it out right, but attack at the later stage, results in shriveled grains and heavy yield losses (Linford, 1928). During recent years, wilt of pea has become very serious in many pea growing areas of Manipur, so far not much research has been done. Therefore, the present study was undertaken with the objective to study the cultural, morphological and pathogenic variability of fourteen isolates of wilt pathogen (*Fusarium oxysporum f.sp.pisi*) present in different districts of Manipur.

Materials and Methods

Collection of the samples and isolation of causal pathogen

Diseased plants were collected from the field of different districts of Manipur i.e. Imphal West, Imphal East, Bishnupur, Thoubal, Tamenglong and Ukhrul districts during the year 2014-2015. The diseased samples were cut into small pieces of 2-3mm size. These pieces were surface sterilized with 1% sodium hypochlorite solution for 2-3 minutes and then rinsed with distilled water. The sterilized pieces were then inoculated on PDA slants. The inoculated slants were incubated at $28 \pm 1^{\circ}\text{C}$ for 7 days. After 7 days the fungal isolates

appearing on the stem / root pieces were identified and transferred to PDA for purification. Identification of the causal fungus and pathogenicity test were carried out in the Department of Plant Pathology, College of Agriculture, CAU, Imphal. Culture was maintained on freshly prepared PDA slants inside the refrigerator and periodically sub cultured to fresh medium during the investigation.

Cultural and morphological variability

Fourteen isolates of *F.o.f. sp. pisi* were isolated from different districts of Manipur during the year 2014-2015. In order to study the cultural and morphological variability, 5mm diameter of mycelia of each isolates were taken from the actively growing culture and placed centrally on 90mm petridish containing solidified PDA medium and the inoculated plates were incubated at $26 \pm 1^{\circ}\text{C}$ for 7 days. Each plate is replicated three times. After seven days the growth pattern were observed daily and all the distinguishing characters such as pigmentation and sporulation were recorded. In morphological variability studies, average micro conidial, macroconidial size and chlamydospores number were recorded by observing under Binocular Microscope at 40x with the help of biowizard image analysis and measurement was done at 100x. For dry mycelium weight *F.oxysporum f.sp.pisi* isolates were grown on potato dextrose broth. Each 250 ml flask having 200 ml potato dextrose broth was inoculated with pure mycelia culture of *F.oxysporum* and incubated at $27 \pm 1^{\circ}\text{C}$. After 15 days by filtration through Watman no. 1 filter paper mycelia mats were collected and washed with sterile distilled water. The harvest mycelia filtrate was dried at room temperature for 20 days. After drying it was weighed with electric balance. The experiment was replicates three times.

Pathogenic variability

The pathogenic variability of fourteen isolates of *Fusarium oxysporum* f. sp. *pisi* were determined on the basis of pathogenicity test. In pathogenicity test, 14 isolates were mass multiplied on rice seed inoculum technique of (Weideman and Wehner, 1993) and inoculated 30 gm per pot and cover with transparent polythene sheet and incubated for seven days on natural condition. After incubation, pea cultivars are sown in pots and three replications were maintained. Pots without inoculums served as control. Disease was recorded after emergence of seedlings. Wilt symptom observed on plants were graded into different category. Ranked is as minus (-) for no symptom and plus (+) for wilt symptom on inoculated pot. The appearance of disease symptom was categorized again into four groups viz., up to 25 % wilt ranked as single plus (+), 25.1 to 50% ranked as double plus (++), 50.1 to 75% were ranked triple plus (+++) and more than 75% were ranked tetra plus sign (++++) and reacted as slow pathogenic, moderate pathogenic, pathogenic and highly pathogenic isolates respectively.

Results and Discussion

Identification of the causal pathogen

Diseased samples were collected from the different pea growing districts of Manipur during the survey and isolation of the causal pathogen was carried out in the laboratory on potato dextrose agar (PDA). With repeated isolations, *Fusarium oxysporum* was consistently found to be associated with infected plants of pea. *Fusarium oxysporum* isolated from wilted pea plant were identified microscopically by their morphologically characteristics such as abundance of micro and fewer macro conidia and white to creamy white colour culture on PDA medium. Microconidia were cylindrical, slightly

curved, hyaline and were produced on short unbranched monophialides

Cultural variability of *F. oxysporum* isolates

Among fourteen isolates of *F. oxysporum* little variation was observed. Colony of isolates THFOP-1, IEFOP-2 showed white compact mycelium with concentric ring, THFOP-2, IWFOP-2, IEFOP-1, SEFOP-1 showed white cottony mycelium growth, isolates BIFOP-1, BIFOP-2, IWFOP-1 and SEFOP-2 showed white cottony and fluffy mycelium growth, TAFOP-2, UKFOP-1 showed white cottony and fluffy mycelium growth with concentric ring while isolates TAFOP-1 showed sparsely distributed with white cottony mycelium growth and isolate UKFOP-1 showed white cottony mycelium with concentric ring. The radial growth of colony diameter on potato dextrose agar (PDA) medium ranged from 5.4 cm to 8.9 cm at 8 days after inoculation at 26 ± 10 °C in 90mm petriplates. Maximum radial growth was observed in IEFOP-1 (8.9 cm) followed by IWFOP-2, TAFOP-2, BIFOP-2 (8.6cm), SEFOP-1(8.5cm), BIFOP-1 (8.3cm), UKFOP-1 (8.1cm), TAFOP-1 (8.0cm), IWFOP-1 (7.7cm), UKFOP-2 (7.6 cm), THFOP-1, THFOP-2 (7.5cm) and isolate SEFOP-2 showed least radial growth in which diameter was 5.4cm. Variation in the colour of mycelium was observed in all the isolates in PDA medium. Initially the colour of all isolates was white which changed gradually with different pigmentation. Isolate THFOP-1 showed dark purple, THFOP-2 showed light pink, BIFOP-1 and TAFOP-2 showed purple colour, BIFOP-2, UKFOP-2 and SEFOP-2 showed pale yellow colour, TAFOP-2 showed light yellow colour while four isolates UKFOP-1, IWFOP-2, IEFOP-1 and IEFOP-2 showed light purple colour and isolates IWFOP-1 and SEFOP-1 showed pink colour. Sporulation of all isolates showed moderate to profuse. Moderate sporulation was observed in isolates THFOP-2, BIFOP-1, BIFOP-2

UKFOP-1, IWFOP-2. IEFOP-2, SEFOP-1 and SEFOP-2 whereas profuse sporulation was observed in isolates THFOP-1, TAFOP-1, TAFOP-2, UKFOP-2, IWFOP-1 and IEFOP-1 respectively. In a similar study Dubey et al., 2010; Mandhare et al., 2011 reported that

Fusarium wilt isolates were highly variable in their colony, growth pattern, size of colony, and pigmentation. Gupta *et al.*, (2011) were also studied on cultural variability of *Fusarium oxysporum* f.sp *pisi* isolates (Table 1 and 3).

Table.1 Cultural characteristics of different isolates of wilt pathogen (*Fusarium oxysporum f.sp.pisi*)

Sl. no	Isolates	Location	Colony character	Colony diameter (cm) 8 DAI	Pigmentation	Sporulation
1	THFOP1	Charangpat	White compact mycelium in concentric ring	7.5	Dark purple	Profuse
2	THFOP2	Wangjing	White cottony mycelium	7.5	Light pink	Moderate
3	BIFOP1	Toubul	White cottony and fluffy mycelium	8.3	Purple	Moderate
4	BIFOP2	Bishempur	White cottony and fluffy mycelium	8.6	Pale yellow	Moderate
5	TAFOP1	Awangkhol	Sparsely distributed white cottony mycelium	8.0	Purple	Profuse
6	TAFOP2	Kahulong	White cottony and fluffy mycelium in concentric ring	8.6	Light yellow	Profuse
7	UKFOP1	Kashung khullen	White cottony and fluffy mycelium in concentric ring	8.1	Light purple	Moderate
8	UKFOP2	Litan	White cottony mycelium in concentric ring	7.6	Pale yellow	Profuse
9	IWFOP1	Iroisemba	White cottony and fluffy mycelium	7.7	Pink	Profuse
10	IWFOP2	Nambol	White cottony mycelium	8.6	Light purple	Moderate
11	IEFOP1	Thongju	White cottony mycelium	8.9	Light purple	Profuse
12	IEFOP2	Kongba	White compact mycelium in concentric ring	8.6	Light purple	Moderate
13	SEFOP1	Kangpokpi	White cottony mycelium	8.5	Pink	Moderate
14	SEFOP2	Mao Maram	White cottony and fluffy mycelium	5.4	Pale yellow	Moderate

Table.3 Chlamyospores and Biomass production of *Fusarium oxysporum f.sp.pisi* isolates

Sl. No	Chlamyospore production (no.)	Biomass (dry weight/gm)
1	12	1.04
2	14	0.96
3	19	1.25
4	20	0.88
5	15	0.98
6	21	1.31
7	19	1.28
8	18	1.27
9	13	1.07
10	12	0.78
11	10	0.97
12	10	0.81
13	11	1.02
14	13	0.87

Table.2 Morphological characteristics of *Fusarium oxysporum f.sp pisi* isolates

Sl. no	Isolates	Location	Macroconidia			Micro conidia			Colour
			Size(µm)	Septation	Shape	Size(µm)	Septation	Shape	
1	THFOP1	Charangpat	11.6 x 3.1	2-3	Sickle shape with blunt end	3.0x 2.1	0	Oval	Hyaline
2	THFOP2	Wangjing	12.7x 3.7	3-4	Sickle shape	3.2x 2.5	0	Oval	Hyaline
3	BIFOP 1	Toubul	18.2x 5.3	3-4	Sickle shape	8.2x 5.9	0	Round	Hyaline
4	BIFOP 2	Bishempur	24.9x 5.7	3-4	Sickle shape	8.5x 5.5	0	Round	Hyaline
5	TAFOP1	Awangkhol	17.6x 5.1	2-3	Sickle shape	5.6x 2.7	0	Round	Hyaline
6	TAFOP2	Kahulong	24.5x 5.9	2-3	Elongated sickle shape	9.1x 5.6	0	Round	Hyaline
7	UKFOP1	Kashung khullen	17.4x 5.3	2.3	Sickle shape with blunt end	4.9x2.3	0	Oval	Hyaline
8	UKFOP2	Litan	13.6x3.9	2-3	Sickle shape	3.2x 2.6	0	Oval	Hyaline
9	IWFOP1	Iroisemba	15.8x4.3	2-3	Sickle shape	3.3x 2.5	0	Oval	Hyaline
10	IWFOP2	Nambol	21.9x 5.8	2-3	Elongated sickle shape	7.2x 5.2	0-1	Oval	Hyaline
11	IEFOP 1	Thongju	25.2x 6.2	2-3	Elongated sickle shape	9.2x 5 6	0	Round	Hyaline
12	IEFOP2	Kongba	23.4x 5.6	2-3	Sickle shape with blunt end	7.9x 5.1	0	Round	Hyaline
13	SEFOP1	Kangpokpi	20.4x5.2	2-3	Sickle shape with blunt end	8.4x 5.3	0-1	Oval	Hyaline
14	SEFOP2	Mao Maram	11.7x 3.1	2-3	Sickle shape with blunt end	3.1x 2.3	0-1	Oval	Hyaline

Table.4 Pathogenic variability of *Fusarium oxysporum f.sp. pisi* isolates

Sl. no	Isolates	Location	Pathogenecity	Reaction
1	THFOP1	Charangpat	+++	Pathogenic
2	THFOP2	Wangjing	+++	Pathogenic
3	BIFOP1	Toubul	++++	Highly pathogenic
4	BIFOP2	Bishempur	+++	Pathogenic
5	TAFOP1	Awangkhol	+++	Pathogenic
6	TAFOP2	Kahulong	+++	Pathogenic
7	UKFOP1	Kashung khullen	+++	Pathogenic
8	UKFOP2	Litan	+++	Pathogenic
9	IWFOP1	Iroisemba	+++	Pathogenic
10	IWFOP2	Nambol	++++	Highly pathogenic
11	IEFOP1	Thongju	++++	Highly pathogenic
12	IEFOP2	Kongba	+++	Pathogenic
13	SEFOP1	Kangpokpi	+++	Pathogenic
14	SEFOP2	Mao Maram	+++	Pathogenic

Up to 25 % wilt for + (single plus), 25.1 to 50% for ++ (double plus), 50.1 to 75% for +++ (triple plus) and more than 75% for ++++ (tetra plus).

Morphological variability of *F. oxysporum* isolates

Morphological variation like conidial shape, size, septation, colour and chlamyospores formation were studied using Potato Dextrose Agar medium. The size of macro conidia ranged from 11.6 x 3 µm to 25.2 x 6.2 µm and size of micro conidia ranged from 3.02x 2.1 µm to 9.2 x 5.6 µm. The largest size of macroconidia was found in IEFOP-1 (25.2 x 6.2 µm) whereas smallest size was found in THFOP-1 (11.6 x 3 µm) while the largest size of microconidia was also found in IEFOP-1 (9.2 x 5.6 µm) and smallest was also found in THFOP-1 (3.02x 2.1 µm). The number of septation was also varied in all the isolates. In macroconidia three to four septa are found in isolates of THFOP-2, BIFOP-1 and BIFOP-2 and two to three septa were found in all the remaining isolates whereas in microconidia most of the isolates was found with no septum but three isolates viz. IWFOP-2, SEFOP-1 and SEFOP-2 were found 0-1 septum. Isolates THFOP-2, BIFOP-1, BIFOP-2, TAFOP-1, UKFOP-2 and IWFOP-1 were found sickle shape macroconidia whereas isolates THFOP-1, UKFOP-1, IEFOP-2, SEFOP-1 and SEFOP-2 were found sickle shape with blunt end macroconidia while the three isolates TAFOP-2, IWFOP-2 and IEFOP-1 were found elongated sickle shape macroconidia. In microconidia most of the isolates are oval shape while isolates BIFOP-1, BIFOP-2, THFOP-1, THFOP-2, IEFOP-1 and IEFOP-2 are round shape. The conidial colour of all the isolates was found hyaline. The formation of chlamyospores ranged from ten to twentyone numbers of all the isolates on Potato Dextrose Agar medium. The dry weight mycelium showed variability in the weight of isolates. The highest dr mycelium weight was observed in TAFOP-2 (1.31g) followed by UKFOP-1(1.28g), UKFOP-2(1.27g), BIFOP-1(1.25g), IWFOP-1(1.07g), THFOP-1(1.04g) and SEFOP-1(1.02g) respectively whereas minimum dry mycelia weight of 0.78g, 0.81g, 0.87g, 0.88g, 0.96g, 0.97g and 0.98g was obtained from the isolates IWFOP-2, IEFOP-2, SEFOP-2, BIFOP-2, THFOP-2, IEFOP-1 and TAFOP-1

respectively. Similarly, the cultural variability and morphological characteristics of *Fusarium oxysporum* f.sp. *zingiberi* isolates was reported by Dohroo and Sharma (1992) and Siddique and Kaushal(2000). Shalini Verma and Dohroo (2003) was also reported that morphological and cultural variability existed among isolates of *Fusarium oxysporum* f.sp.*pisi*. from pea in which all the isolates showed slow to fast growth, variable pigmentation and morphology of the hyphae, microconidia and chlamyospores. S.K. Gupta et al.,(2011) studied on morphological variation of *Fusarium oxysporum* f.sp.*pisi* like mycelia colour, conidial size and formation of chlamyospores (Table 2).

Pathogenic variability of *F.oxysporum* isolates

In pathogenic variability, pot culture experiment was done. On the basis of percentage of wilt symptom observed on pea cultivars are graded into different category. Most of the isolates showed 50.1 to 75% wilt which was graded at pathogenic isolates whereas three isolates viz. BIFOP-1, IWFOP-2 and IEFOP-1 showed more than 75% wilt and graded at highly pathogenic isolates. Therefore all the isolates exhibited wilt symptom and hence proved pathogenic on susceptible pea cultivars under artificial inoculation. Similarly, Miedner et al. (1996) studied on 42 *Fusarium culmorum* isolates collected from diseased plants from the field of Australia and nine European countries on a synthetic winter rye population in which all the isolates were found pathogenic and differed in their ability to cause diseases. Shanthalaxmi Prasad et al. (2008) reported that the reaction of 29 isolates of *F. oxysporum* f.sp. *ricini* on different castor cultivars and indicated the existence of five pathotypes of the pathogen with different level of virulence (Table 4).

From the present study it can be concluded that the cultural, morphological and pathogenic variability of *Fusarium oxysporum* f.sp.*pisi* isolates causing wilt of pea showed different characters in mycelial growth pattern,

pigmentation, rate of growth on Potato dextrose agar. The radial growth was ranged from 5.4cm to 8.9 cm at 8 days after inoculation at $26 \pm 1^{\circ}$ C on 90mm petriplates. The colour of all the isolates are mostly white which changed gradually to light pink, purple colour, pale yellow colour etc. Sporulation of all isolates showed moderate to profuse. The size of macroconidia varied from $11.6 \times 3.1 \mu\text{m}$ to $24.9 \times 5.7 \mu\text{m}$ and microconidia was $3.02 \times 2.1 \mu\text{m}$ to $9.2 \times 5.6 \mu\text{m}$. In pathogenic variability all the isolates showed pathogenic causing wilt of pea. Thus the present study provides information about the epidemiology of *Fusarium* that helps in improving management strategies for wilt of pea.

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