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RESEARCH ARTICLE

Morphological and pathogenic variability in French bean isolates of *Sclerotinia sclerotiorum*

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SUMMARY

Studies conducted on morphological and pathogenic variability among 30 isolates of *Sclerotinia sclerotiorum* (Lib) de Bary, derived from infected French bean (*Phaseolus vulgaris*) plants showing typical symptoms of white mold, collected from different geographical locations of NE India. All the isolates showed variation in morphological characters based on their mycelial growth, colony character and sclerotial formations when grown on PDA. Out of 30 isolates, 14 isolates showed highly virulent reaction, each 8 isolate revealed moderately and less virulent reaction respectively on French bean under pot condition.

Key Words : *Sclerotinia sclerotiorum, Phaseolus vulgaris,*White mold, PDA, Virulent

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French bean is one of the most important edible legume crops of NE India. The crop is grown for the tender vegetable, shelled green beans and dry beans (rajma). However, the crop is susceptible to white mold caused by *S. sclerotiorum*,causing serious and unpredictable loss as high as 100 per cent (Purdy, 1979 and Tu, 1989). The disease have been found to be one of the most destructive in French bean as reported by several workers (Steadman, 1983 and Bag, 2000). The effects of weather on white mold incidence and development differs considerably (Abawi and Grogan, 1979). In the North Eastern part of the country, more particularly, in the surroundings of Jorhat and Golaghat districts of Assam, the disease is of common occurrence in epidemic form and has been posing a serious threat to the cultivation of French bean during the last several years (Dutta, 2006). Use of resistant varieties is one of theimportant alternatives to overcome this problemand for breeding resistant varieties, knowledge of variability in the pathogen is most essential.No information on morpho- and patho-genicvariability in *Sclerotinia sclerotiorum* of French bean is available from this particular areas. Keeping in view the variations in disease incidence, the studies have been conducted to ascertain themorphological and pathogenic variations among French bean isolates of *Sclerotinia sclerotiorum* from different areas of North East India.

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MATERIAL AND METHODS

Morphological variability :

Fresh cultures of 30 isolates of *S. sclerotiorum* collected from different geographical locations/states of NE India [Designated as Assam (AS_1 to AS_5), Nagaland (NL_1 to NL_5), Mizoram (MZ_1 to MZ_5),Arunachal Pradesh (AP_1 to AP_5), Manipur (MP_1 to MP_5) and Meghalaya (ML_1 to ML_5)] were separately subjected to detailed morphological and cultural characteristics *viz.*, radial colony growth (mm), number of sclerotia developed on potato dextrose agar (PDA) in 90 mm Petridishes, size of sclerotia (mm) and weight of single sclerotia (mg). These Petridishes were incubated at 25 ± 1°C. Data of radial colony growth were taken at 24, 48 and 72h after inoculation while number of Sclerotia and size of Sclerotia were recorded 10 to 15 days after inoculation. Fresh and dry mycelial weight was taken after 10 days growth of isolates grown at 25 ± 1°C on potato dextrose broth (PDB) medium.The experiment was repeated four times for each isolate.

Pathogenic variability :

Pathogenic variability of 30 isolates of *S. sclerotiorum* collected from different geographical locations/states of NE India were separately determined on French bean plant (var. Contender) grown on sterilized soil in pot condition. Earthen pots (25 cm dia.) containing 3 kg of sterilized soil were taken for the study. The pots containing sterilized soil were inoculated with 15 days old mass culture of *S. sclerotiorum* grown on MSM @ 2 per cent of soil (w/w). After 7 days of inoculation of *S. sclerotiorum*, French bean seeds were sown in each pot @ 5 seeds/ Pot. The experiment was conducted under shade house with CRD design in four replications. Observations were recorded on the basis of per cent disease incidence at 30, 60 and 90 days after sowing. The isolates were screened according to their virulence in relation to per cent disease incidence (PDI). The highly virulent isolate was used for further investigation in field condition.The pathogenic behaviours were calculated according to the modified scale of Iqbal *et al.* (2005).

Disease assessment :

$$\text{PDI (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

Less virulent : PDI (< 40%)

Moderately virulent: PDI (40-50 %)

Highly virulent : PDI (> 50 %).

RESULTS AND DISCUSSION

Table 1. clearly indicated that diversity among the isolates of *S. sclerotiorum* from different states of North East based on their mycelia growth, colony characters and sclerotial formations. Majority of the isolates had a fast growth rate with whitish mycelial growth but their texture were varied *viz.*, smooth, profused, fluffy and scattered. Variation in sclerotial formation *viz.*, days of formation, number, weight and size of Sclerotia among the isolates were also observed. Sclerotial formation of the isolates varied from 7-12 d after incubation, number of Sclerotia ranging from 10-42 and sizes varied from 3.42-6.54 mm diameter, respectively. Furthermore, bigger sizes of Sclerotia were observed in the same morphological group. High diversity among *S. sclerotiorum* isolates based on morphologic characteristics such as number, size and shape of sclerotia, mycelium growth rate and color of the colony have been reported (Price and Colhoun, 1975 and Kohn *et al.*, 1990). Tores (1990) also reported marked differences in the colony characters and sclerotial formation of the *S. sclerotiorum* isolates. This morphological variability might be due to different environmental conditions which influences vegetative and reproductive phase of the pathogen and presence of mycovirus resulting in hypovirulent strains of the host fungus showing reduced or delay morphological characters. Saharan and Mehta (2008) reported differences in morphologic aspects among isolates of *S. sclerotiorum*are attributed to variations in environment conditions and presence of mycoviruses. Xiao *et al*. (2010) also reported that a mycovirus (ssDNA) infected strain DT-8 of *S. Sclerotiorum* required for sclerotial initiation about 3–5 days longer than that of the normal strain and the Sclerotia were randomly distributed on the colony surface. Furthermore, the size of the Sclerotia produced by strain DT-8 was significantly smaller than that of the normal strain.

From the result of Table 2. It was clearly observed that large variability in virulence among the isolates. Out of 30 isolates, 14 isolates showed highly virulent reaction, each 8 isolate revealed moderately and less virulent reaction on French bean, respectively. However, a significantly higher per cent disease incidence was observed in AS_3 (Assam) isolates, while lower per cent

disease incidence was recorded in NL_1 (Nagaland) isolate as compared to others.Wide variability in virulence of different isolates of *S. sclerotiorum* have been reported by many workers (Price and Calhoun, 1975; Rai and Dhawan, 1976; Willets and Wrong, 1980 and Dhawan *et al.*, 1981). Variation in virulence among the isolates of *S. sclerotiorum* from different locations may be due to varying degree of ability to secrete oxalic acid and release of some enzymes to macerate the plant cell wall and tissue for infection. Cessna *et al*. (2000) reported that effective pathogenesis by *S. sclerotiorum* requires the secretion of pathogenicity factors like oxalic acid, extracellular lytic enzymes such as cellulases, hemi-cellulases and pectinases etc. which are highly active under the acidic conditions provided by oxalic acid and degrade the plant cell wall and tissues beneath it.Oxalic

Table 1 : Comparative study on mycelial growth, colony character and sclerotial formation of isolates of *S. sclerotiorum* from different states of NE *in vitro*

Name of isolates	Mycelial growth			Colony characters		Sclerotial formation			
	Growth on PDA (72 h , mm)	Growth on PDB (10 d)				No. of days for formation	Sclerotia/ plate	Wt. of sclerotia (mg)	Diameter (mm)
		Fresh wt.(g)	Dry wt.(g)	Colour	Texture				
AS_1	90.0^{a}	4.25^{abc}	0.46^{ab}	Whitish	Profused	7^{d}	42^{a}	0.54^{lm}	3.42^{kl}
AS_2	90.0^{a}	4.23^{cd}	0.44^{cd}	Whitish	Profused	7^{d}	41^{ab}	0.56^{lm}	3.44^{kl}
AS_3	90.0^{a}	4.12^{bc}	0.42^{cd}	Whitish	Smooth	7^{d}	39^{b}	0.58^{m}	3.56^{l}
AS_4	86.8^{d}	3.76^{f}	0.35^{f}	Whitish	Smooth	11^{ab}	18^{k}	1.17^{fg}	5.23^{gh}
AS_5	88.2^{c}	3.84^{ef}	0.36^{def}	Whitish	Smooth	10^{ab}	20^{jk}	1.11^{gh}	5.18^{hi}
NL_1	81.4^{f}	2.53^{l}	0.27^{l}	Whitish	Fluffy	10^{a}	21^{j}	1.05^{gh}	4.96^{hi}
NL_2	82.6^{e}	2.55^{l}	0.29^{l}	Whitish	Fluffy	10^{b}	22^{i}	1.04^{g}	4.94^{h}
NL_3	90.0^{a}	3.13^{j}	0.31^{j}	Whitish	Profused	9^{cd}	31^{def}	0.82^{hij}	4.64^{ijk}
NL_4	90.0^{a}	3.33^{i}	0.34^{ij}	Whitish	Profused	8^{d}	35^{c}	0.72^{m}	4.56^{l}
NL_5	90.0^{a}	3.30^{i}	0.32^{i}	Whitish	Smooth	8^{d}	34^{cd}	0.75^{lm}	4.59^{kl}
MZ_1	88.2^{c}	3.06^{j}	0.33^{j}	Whitish	Smooth	11^{ab}	18^{k}	1.14^{d}	5.21^{e}
MZ_2	90.0^{a}	3.71^{fg}	0.39^{g}	Whitish	Smooth	9^{cd}	28^{fg}	0.88^{gh}	4.75^{gh}
MZ_3	90.0^{a}	3.49^{h}	0.37^{hi}	Whitish	Smooth	9^{cd}	30^{ef}	0.85^{gh}	4.70^{hi}
MZ_4	90.0^{a}	4.36^{ab}	0.48^{a}	Whitish	Smooth	7^{d}	40^{ab}	0.55^{lm}	3.43^{l}
MZ_5	90.0^{a}	4.25^{abc}	0.46^{bc}	Whitish	Smooth	9^{cd}	29^{f}	0.84^{gh}	4.68^{hi}
AP_1	88.0^{c}	2.87^{k}	0.34^{kl}	Whitish	Fluffy	10^{ab}	21^{j}	1.04^{d}	4.98^{e}
AP_2	89.0^{b}	2.90^{k}	0.36^{k}	Whitish	Fluffy	10^{ab}	24^{hi}	0.96^{fg}	4.86^{gh}
AP_3	86.6^{d}	2.54^{l}	0.29^{l}	Whitish	Fluffy	10^{a}	21^{j}	1.05^{e}	5.10^{fg}
AP_4	89.6^{b}	3.00^{jk}	0.38^{jkl}	Whitish	Fluffy	10^{b}	25^{h}	0.99^{gh}	4.84^{hi}
AP_5	90.0^{a}	4.26^{abc}	0.47^{bc}	Whitish	Smooth	9^{cd}	28^{fg}	0.80^{kl}	4.75^{jk}
MP_1	80.0^{g}	2.57^{l}	0.27^{l}	Whitish	Scattered	12^{a}	10^{l}	1.62^{a}	6.54^{a}
MP_2	81.8^{f}	2.64^{l}	0.28^{l}	Whitish	Scattered	12^{a}	11^{l}	1.59^{ab}	6.50^{ab}
MP_3	90.0^{a}	3.45^{hi}	0.36^{hij}	Whitish	Scattered	10^{bc}	21^{j}	1.08^{b}	5.10^{bc}
MP_4	90.0^{a}	3.51^{h}	0.39^{h}	Whitish	Scattered	10^{bc}	22^{ij}	1.06^{c}	4.98^{d}
MP_5	90.0^{a}	3.50^{h}	0.38^{h}	Whitish	Scattered	10^{bc}	21^{j}	1.08^{bc}	5.11^{cd}
ML_1	90.0^{a}	4.40^{a}	0.50^{a}	Whitish	Smooth	8^{d}	34^{cd}	0.75^{lm}	4.59^{kl}
ML_2	90.0^{a}	3.98^{de}	0.40^{d}	Whitish	Smooth	9^{cd}	29^{f}	0.84^{gh}	4.65^{hi}
ML_3	90.0^{a}	4.13^{cd}	0.42^{cd}	Whitish	Smooth	8^{d}	32^{de}	0.79^{hij}	4.64^{jk}
ML_4	90.0^{a}	3.58^{gh}	0.37^{fh}	Whitish	Smooth	10^{bc}	22^{ij}	1.07^{e}	5.22^{f}
ML_5	90.0^{a}	4.31^{ab}	0.49^{ab}	Whitish	Smooth	9^{cd}	31^{d}	0.80^{hi}	4.63^{ij}
S.E.±	0.30	0.10	0.07	-	-	0.57	1.08	0.06	0.08
C.D.(P=0.05)	0.49	0.16	0.11	-	-	0.94	1.79	0.09	0.13

Values are mean of four replications

Means followed by same letter shown in superscript(s) are not significantly different

acid is a recognized virulence factor produced by several phytopathogenic fungi, including *S. sclerotiorum*, the causal agent of white mold and related diseases (Godoy *et al.*, 1990). Variation in virulence among the isolates of *S. sclerotiorum* might also be due to presence of mycovirus which attributed to hypovirulent strain to its host. Micovirus-mediated hypovirulence is a phenomenon in which the virulence of fungal pathogen is reduced or even completely lost as a consequence of virus infection (Nuss, 2005).

The results from the present investigation, it was observed that morphological characters exhibited on PDA do not necessarily relate to virulence of *S. sclerotiorum*. This may be due to the fact that when the pathogen come in contact with the host plant they released some toxic like oxalic acid and enzymes to degrade the cell wall and macerate the plant tissue which facilitate infection. So, the release of pathogenicity factors by *S.*

Table 2 : Per cent disease incidence (PDI) on French bean inoculated with different geographical isolates of *S. sclerotiorum* and their pathogenic behaviour under pot condition

State of NE	Name of isolates	Location	Per cent disease incidence (PDI)			**Pathogenic behaviour
			30 DAS	60 DAS	90 DAS	
Assam	AS_1	Digboi	20.00(26.57)^{g}	25.00(30.00)^{j}	49.25(44.57)^{l}	MV
	AS_2	Disoinagar	21.75(27.80)^{de}	29.25(32.74)^{g}	62.50(52.24)^{h}	HV
	AS_3	AAU	24.75(29.83)^{a}	34.00(35.67)^{a}	68.75(56.01)^{a}	HV
	AS_4	Rajaduar	22.50(28.32)^{bc}	31.50(34.14)^{cde}	63.00(52.54)^{gh}	HV
	AS_5	Silchar	16.00(23.58)^{l}	21.75(27.80)^{lm}	39.50(38.94)^{q}	LV
Nagaland	NL_1	Kohima	12.75(20.92)^{o}	19.75(26.39)^{o}	33.25(35.21)^{w}	LV
	NL_2	Jakhama	14.50(22.38)^{n}	21.00(27.27)^{mn}	34.00(35.67)^{w}	LV
	NL_3	Medziphema	21.25(27.45)^{ef}	26.25(30.82)^{i}	54.50(47.58)^{k}	HV
	NL_4	Chumukedima	19.25(26.02)^{hi}	25.00(30.00)^{j}	48.25(44.00)^{m}	MV
	NL_5	Dimapur	23.00(28.66)^{b}	31.25(33.99)^{de}	63.75(52.98)^{efg}	HV
Mizoram	MZ_1	Aizawl	14.75(22.59)^{n}	20.25(26.74)^{no}	37.50(37.76)^{r}	LV
	MZ_2	Sihphir	19.25(26.02)^{hi}	23.75(29.17)^{ij}	45.50(42.42)^{op}	MV
	MZ_3	Lunglei	22.25(28.14)^{cd}	32.00(34.45)^{cd}	65.00(53.73)^{cd}	HV
	MZ_4	Hnahthial	24.25(29.50)^{a}	33.25(35.21)^{b}	67.25(55.09)^{b}	HV
	MZ_5	Serkhan	20.00(26.57)^{g}	24.75(29.83)^{j}	46.75(43.14)^{o}	MV
Arunachal P	AP_1	Itanagar	14.75(22.59)^{n}	21.00(27.27)^{mn}	36.25(37.02)^{s}	LV
	AP_2	Parang	22.00(27.97)^{cd}	28.25(32.11)^{h}	61.75(51.80)^{h}	HV
	AP_3	Naharlagun	17.25(24.54)^{k}	22.25(28.14)^{l}	39.00(38.65)^{q}	LV
	AP_4	Sagalee	18.25(25.29)^{j}	24.75(29.83)^{j}	44.25(41.70)^{p}	MV
.	AP_5	Pasighat	24.00(29.33)^{a}	30.75(33.68)^{ef}	65.25(53.88)^{cd}	HV
Manipur	MP_1	Churachandpur	21.25(27.45)^{ef}	28.50(32.27)^{h}	59.75(50.62)^{i}	HV
	MP_2	Vengnom	20.75(27.10)^{f}	26.75(31.14)^{i}	57.25(49.17)^{j}	HV
	MP_3	Kawnpui	22.25(28.14)^{cd}	29.75(33.05)^{g}	62.75(52.39)^{h}	HV
	MP_4	Tuibong	15.50(23.18)^{m}	21.25(27.45)^{m}	38.00(38.06)^{r}	LV
	MP_5	Imphal	19.00(25.84)^{i}	23.75(29.17)^{k}	43.75(41.41)^{p}	MV
Meghalaya	ML_1	Jowai	18.75(25.66)^{hi}	23.00(28.66)^{h}	47.00(43.28)^{n}	MV
	ML_2	Laskein	23.00(28.66)^{b}	31.00(33.83)^{e}	64.00(53.13)^{ef}	HV
	ML_3	Shillong	24.25(29.50)^{a}	32.25(34.60)^{c}	65.75(54.18)^{c}	HV
	ML_4	ICAR, Barapani	19.75(26.39)^{gh}	23.50(29.00)^{k}	47.50(43.57)^{n}	MV
	ML_5	Mawjrong	15.00(22.79)^{mn}	19.50(26.21)^{o}	39.25(38.79)^{q}	LV
S.E.±		-	0.65	0.43	0.48	-
C.D.(P=0.05)			1.07	0.71	0.80	

Values are mean of four replications
Figures in the parenthesis are angular transformed values
Means followed by same letter shown in superscript(s) are not significantly different
** HV= Highly virulent, MV= Moderately virulent, LV= Less virulent

sclerotiorum is an important aspect for virulence of the pathogen. Harsh *et al*. (2009) reported that no correlation between colony diameter on PDA with the pathogenicity of different isolates of this pathogen as measured by diameter of cotyledon lesion on the host genotype. Therefore, the findings of the present investigation clearly revealed that morphological and pathogenic variability did exist in French bean isolates of *S. sclerotiorum* collected from different locations of North East India.

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