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Pathogenic and morphological variability of *Exserohilum turcicum* isolates causing leaf blight in sorghum (*Sorghum bicolor*)

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ABSTRACT

Pathogenic and morphological variability among 8 isolates of sorghum leaf blight pathogen *Exserohilum turcicum* (Pass.) Leonard and Suggs from Rajasthan, Gujarat, Maharashtra and Andhra Pradesh was studied during 2004–05. Pathogenic variability was studied in pot-grown plants by inoculating them of on a set of 14 differential lines comprising 12 sorghum (*Sorghum bicolor* L. Moench) germplasm accessions and 2 maize (*Zea mays* L.) cultivars. The isolates exhibited variations in cultural and morphological characteristics. The isolates also showed significant variations in symptoms in the natural conditions and in latent period and disease severity on the pot-grown plants of 14 test-lines. The differences in the latent period and mean disease score were statistically significant ($P = 0.05$) for the host lines, isolates and also for the host line×isolates interactions. Based on the disease severity and disease reaction the 8 isolates were distinguished into 5 pathotypes. Four isolates from Rajasthan were grouped into 3 different pathotyps and 2 from Maharashtra into 2 separate pathotypes, while the others from Gujarat, Maharashtra and Andhra Pradesh into a single pathotype. The isolate from Andhra Pradesh was the most virulent, followed by that in Rajasthan.

Key words: Differential lines, *Exserohilum turcicum*, Leaf blight, Pathotypes, *Sorghum bicolor,* Variability

Common leaf blight of sorghum, caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs have been reported from almost all the sorghum-growing counties (Frederiksen 2000). This pathogen also infects other hosts like maize, *sorghum halpencse*, teosinte, paspalum, *Echinoculoa, Triticum, Hordium, Avena, Oryza* and *Saccharum* (Echemendia *et al*. 2005). There are several reports about the possibility of existence of specialized races of *E. turcicum* in these crops. While extensive information is available on the variability in *E. turcicum* infecting maize (Fernandes and Balmer 2002, Abebe and Singburaudom 2006 and Harlapur *et al.* 2007), information about the isolates infecting sorghum is limited (Ayala *et al*. 1997). Epidemic incidence of common leaf blight have been reported in *rabi* cultivars (Harlapur *et al*. 2000) and *kharif* hybrid 'CSH 14' (Mathur and Bunker 2001), which were earlier resistant, indicating shift in virulence variables. Information about virulence in the populations prevalent in different regions is desirable. In this paper, morphological, cultural and pathogenic variability in *E. turcicum* prevalent in different states of India is reported and its significance discussed.

Based on a major part of Ph D thesis of the first author submitted to MPUAT, Udaipur during 2005 unpublished

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MATERIALS AND METHODS

Cultures of *E. turcicum* were isolated from the disease leaf samples of sorghum showing variable symptoms collected from different locations and designated as Et-01: Chittorgarh (Rajasthan), Et-02: Udaipur (Rajasthan), ET-03: Udaipur (Rajasthan), Et-04: Rajsamand (Rajasthan), Et-05: Surat (Gujarat), Et-06: Parbhani (Maharashtra), Et-07: Hyderabad (Andhra Pradesh) and Et-08: Akola (Maharashtra). The samples were scaled for lesion size (length and width) by measuring 15 randomly selected lesions and mean disease score for disease severity was recorded on a 1–5 disease rating scale recommended by the All India Coordinated Sorghum Improvement Project, where: 1 = free from disease, 2 = trace or 10% leaf area infected, 3=11–25% leaf area infected 4 = 26–50% of leaf area infected and 5= above 50% area of leaf infected (Table 1).

The cultural and morphological studies carried in laboratory by growing the isolates of *E. turcicumin* petridishes on potato dextrose agar medium keeping 3 replications of each isolates. The plates were incubated at 28 ± 1°C for 7 days and observations for colony diameter in mm, size of conidia and spore production by each isolate was recorded.

Pathogenic variability in *E. turcicum* isolates was studied

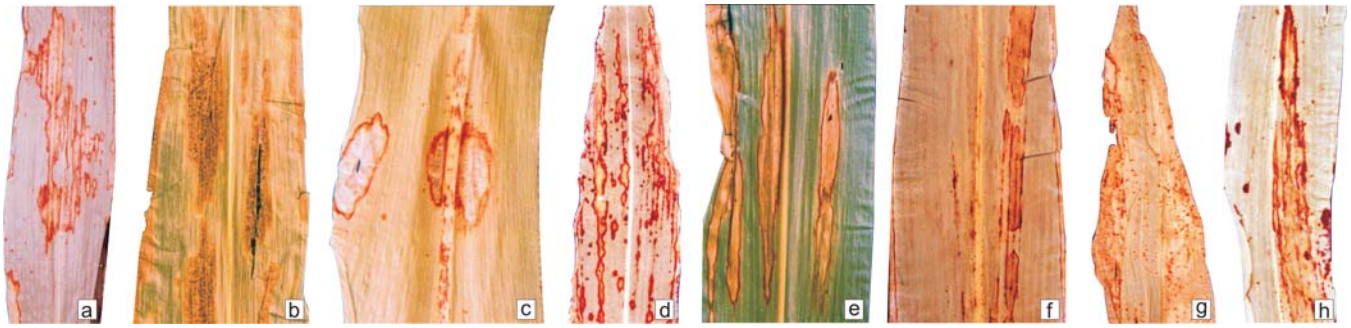

Fig 1 (a) Et-01 (Chittorgarh), (B)Et-03 (Udr. CSH-14), (c) Et-05 (Surat) SPV 1333, (d) Et-07 (Hyderabad SSV 84), (e) Et-02 (Udr. K. Local), (f) Et-04 (Rajsamand Local land race), (g) Et-06 (Parbhani) Local land race, (h) Et-08 (Akola) R–108-2, Local land race Variable symptoms of leaf blight on sorghum cultivar caused by different isolates of *Exserohilu turcicum*

Table 1 Variations in radial growth, sporulation and conidial morphology of 8 isolates of *Exserohilum turcicum*

Isolates	Colony charter		Size of conidia (μm)***		
	Diameter*(mm) days 7	Sporulation**	Length	Width	No. of septa
Et–01	63.2	6.7	70.0±8.8 (60–91)	19.7±2.2 (16–23)	6.6 ± 2.21 (3–8)
Et–02	55.7	9.3	65.4±8.3 (56–79)	19.4±1.5 (14–22)	6.8± 1.89 (2–8)
Et–03	73.0	8.1	78.5±5.9 (64–99)	21.0±1.8 (20–25)	7.2 ± 1.50 (3–1)
Et–04	56.2	7.3	63.3±5.5 (45–68)	18.5±2.5 (13–21)	6.2 ± 2.56 (3–6)
Et–05	50.0	7.8	65.0±4.1 (58–73)	19.6±2.2 (15–23)	5.8 ± 2.32 (2–6)
Et–06	55.0	8.2	67.1±6.6 (52–78)	20.0±2.2 (17–22)	6.8 ± 2.17 (2–8)
Et–07	58.0	11.3	76.7± 6.0 (60–92)	20.5±1.9 (18–23)	8.5 ± 1.99 (3–10)
Et–08	54.2	9.9	73.7 ±6.0 (59–94)	19.2±1.7 (18–23)	7.0 ± 1.8 (3–8)
LSD (*P*=0.05)	2.98	0.36	2.58	0.80	0.82

*Mean of 5 replications, ** Mean no. of 50 conidia, ***Mean no. of 50 conidia and ± SD of mean values, data in parentheses are range of conidia

by inoculating these on different host plants in cage house. Twelve sorghum germplasm accessions, (IS 13904, IS 13996, IS 9303, IS 26866, IS 10284, IS 10755, IS 13057, IS 12466, IS 15745, IS 30403, IS 25069 and IS 30351) and 2 maize cultivars—Navjot and Kiran were used for the experiment. These lines represented wide geographical diversity and sorghum race types (Table 2). Surface sterilized seeds were sown in pots containing garden soil-farmyard manure mixture (3 : 1). There were 5 plants/pot and 2 pots served as 2 replications. Twentyeight-days-old plants were spray inoculated with spore suspension (1×10^4 conidia/ml) containing tween–20 (1 ml/litre). Polyethylene sheets were used to separate plants when inoculating with different isolates to avoid inoculums drift. Following inoculation, the plants were kept in humid chamber for 24 hr and then arranged in cage house following completely randomized design, keeping isolates as blocks. Since the weather was mostly dry, water spraying was given thrice a day to provide proper humidity.

Table 2 Sorghum lines, their race type and country of origin used in sorghum leaf blight virulence study

Host differential lines	Race-type	Country of origin
IS 13904	Kafir	South Africa
IS 13996*		
IS 9303	Kafir	South Africa
IS 26866	Guinea-caudatum	Nigeria
IS 10284	Guinea-caudatum	Sudan
IS 10755	Guinea	Chad
IS 13057	Kafir	South Africa
IS 12466	Gaudatum	Sudan
IS 15745	Gaudatum	Cameroon
IS 30403	Bicolor	China
IS 25069	Guinea-caudatum	Ghana
IS 30351*		
Maize: 'Kiran'	Local land race from	India
'Navjot'	Uttranchal Composite	India

*Not known

Observations for latent period (time in hours for development of first chlorotic or necrotic lesion after inoculations) (Table 3) were started on 24 hr after inoculation and recorded at 12 hr interval up to 5 days of inoculation. Disease severity on the standard 1–5 scale (Mathur *et al.* 2007) based on per cent leaf area covered by the disease. In this: 1 = free from disease, 2 = trace or 10% leaf area infected, 3 = 11–25% leaf area infected 4 = 26–50% of leaf area infected

Table 3 Latent period of eight isolates of *Exserohilum turcicum* on sorghum and maize cultivars.

Host line	Latent period in hours*								
	Et-01	Et-02	Et-03	Et-04	Et-05	Et-06	Et-07	Et-08	Mean
IS 13904	36	36	42	24	30	30	36	24	32.2
IS 13996	36	24	30	36	24	30	24	36	30.0
IS 9303	30	36	36	42	48	36	30	36	36.7
IS 26866	_a	_a	_a	_a	_a	_a	_a	_a	_a
IS 10284	30	30	36	24	36	36	24	30	30.7
IS 10755	36	30	36	48	42	36	24	42	36.7
IS 13057	36	36	30	24	48	36	24	36	33.7
IS 12466	24	36	24	30	48	36	36	48	35.2
IS 15745	36	30	24	36	24	30	24	24	28.5
IS 30403	36	42	42	30	42	36	36	36	37.5
IS 25069	42	30	42	30	30	42	24	42	35.2
IS 30351	36	48	48	36	42	42	30	36	39.7
Kiran (maize)	42	36	36	42	48	36	36	30	38.2
Navjot (maize)	48	24	36	42	36	24	24	30	33.0
Mean	36.0	33.7	35.5	34.1	38.3	36.6	28.6	34.6	

LSD (P=0.05); Host line, 6.19; isolate, 4.85; Host line × isolate, 17.51
*Mean of 2 replications; 5 plants/pot; (_a) no infection, resistant reaction

and 5 = above 50% area of leaf infected, was recorded after 20 days of inoculations on individual line. The disease scores of 1.0 to 2.0 were considered as resistant (R) reaction, 2.1 to 3.0 as moderately resistant (MR) and 3.1 to 5.0 as susceptible (S) reaction. The disease-causing potential of an isolate was described in terms of virulence and aggressiveness. Virulence (qualitative disease causing potential) and aggressiveness (quantitative disease causing potential) was determined from the means severity score on a host differential. The isolates causing mean disease severity score of ≤ 2.0 were considered avirulent and then causing score ≥ 2–1 as virulent. Correspondingly, the host differential lines were considered as resistant (R/MR) or susceptible (S). The infected plants were then carefully uprooted, placed in polypropylene bags and autoclaved to destroy the pathogen. The experiment was repeated once in the same season to confirm the results.

Data analysis: The date of latent period and disease severity score were subjected to analysis and least significant difference (critical deviation) determined on 5% (P = 0.05) possibility for host lines, isolates and host lines × isolate interaction.

RESULTS AND DISCUSSION

The isolates showed considerable variations in leaf blight symptoms, the lesion size ranged from 5 to 155 mm in length and 2 to 20 mm in width across the isolates. The maximum mean lesion size (90.5×10.8 mm) with the range of 25–158 mm × 5–20 mm was recorded for the isolate Et-03 (CSH 14). The typical lesion size described for *E. turcicum* on sorghum is 25 to 150 mm in length and 12 mm in width Bergquist (2000). These variations may be due to host - sorghum genotypes interaction (Fig 1).

All the isolates of *E. turcicum* showed significant variations in colony diameter and conidial morphology. The maximum colony diameter (73.0 mm) was recorded for the isolate Et-03 (Udaipur, 'CSH 14'), while the least (50.0 mm) was in Et-05 (Surat) after 7 days. The isolate Et-07 (Hyderabad) produced maximum number of spore (11.33×10^4 conidia/mm^2) with maximum mean number of septa (8.5)/conidia, while the lowest (6.76×10^4 conidia/mm^2) was by Et-01 (Chittorgarh). In all isolates, the size of conidia ranged from 63.3 (45.2– 68.4) to 78.5 (63.8–99.2) µm in length and 18.5 (13.5–21.3) to 21.0 (19.7–24.8) ≤ m in width, and mean number of septa/conidium ranged from 2 to 11 (Table 1). Despite these variations, the size of conidia agreed well with the standard description of conidial size (28–153×10–20 µm) of *E. turcicum* (Bergquist 2000). It was interesting to hope that isolate Et.-07 produce maximum number of conidia/mm^2 was also found to be the most virulent on the tested host lines. Morphological variations among different isolates/strains of several pathogenic fungi are known, and these may or may not be related to virulence diversity (Caten 1996).

The isolates exhibited considerable variations in latent period (hr), disease severity, and accordingly in disease reaction on the 14 tested differential lines. The mean latent period ranged from 24 to 48 hr, depending on the isolate×host genotype interaction. The shortest mean latent period across the test lines was of isolate Et-07 (28.6 hr), followed by Et-02 (33.7 hr), Et-04 (34.1 hr), Et-08 (34.6 hr), Et-03 (35.5 hr), Et-01 (36.0 h) and Et-06 (36.6 h). The longest mean latent period (38.3 hr) was of isolate Et-05. Among the test lines, longest mean latent period was on IS 30351 (39.7 hr), followed by 38.2 hr on 'Kiran' maize and 37.5 hr on IS 30403. The shortest mean latent period (28.5 hr) was recorded on IS 15745, while on IS 26866 no symptoms were observed

Table 4 Reaction of sorghum and maize differential lines to 8 isolates of leaf blight pathogen *Exserohilum turcicum*

Differentials host lines	Isolates (disease score 1–5 scale)/disease reaction								Mean score
	Et-01	Et-02	Et-03	Et-04	Et-05	Et-06	Et-07	Et-08	
IS 13904	2.2 (MR)	2.5 (MR)	2.7 (MR)	2.4 (MR)	2.7 (MR)	2.7 (MR)	2.9 (MR)	2.0 (R)	2.5[b]
IS 13996	1.6 (R)	2.2 (MR)	2.0 (R)	1.7 (R)	2.1 (MR)	1.9 (R)	2.4 (MR)	2.1 (MR)	2.0[a]
IS 9303	2.6 (MR)	2.6 (MR)	2.6 (MR)	2.7 (MR)	3.0 (MR)	2.4 (MR)	2.7 (MR)	2.5 (MR)	2.6[c]
IS 26866	1.0 (R)	1.3 (R)	2.0 (R)	1.7 (R)	2.0 (R)	2.0 (R)	2.0 (R)	1.6 (R)	1.7[a]
IS 10284	3.1 (S)	3.1 (S)	3.0 (MR)	3.2 (S)	4.0 (S)	3.7 (S)	4.0 (S)	3.0 (MR)	3.4[d]
IS 10755	2.4 (MR)	2.5 (MR)	3.2 (S)	2.6 (MR)	2.7 (MR)	2.8 (MR)	2.6 (MR)	2.2 (MR)	2.6[b]
IS 13057	2.5 (MR)	2.0 (R)	2.2 (MR)	2.6 (MR)	2.1 (MR)	2.6 (MR)	2.9 (MR)	2.5 (MR)	2.4[b]
IS 12466	2.5 (MR)	2.6 (MR)	1.7 (R)	2.5 (MR)	2.2 (MR)	1.9 (R)	2.0 (R)	3.2 (S)	2.3[b]
IS 15745	1.1 (R)	2.2 (MR)	3.0 (MR)	3.4 (S)	2.5 (MR)	2.7 (MR)	2.7 (MR)	2.7 (MR)	2.5[b]
IS 30403	2.6 (MR)	3.2 (S)	4.0 (S)	2.2 (MR)	3.2 (S)	2.2 (MR)	2.2 (MR)	3.4 (S)	2.9[c]
IS 25069	3.5 (S)	3.7 (S)	3.0 (MR)	2.2 (MR)	2.5 (MR)	2.2 (MR)	3.7 (S)	3.0 (MR)	3.0[c]
IS 30351	3.1 (S)	2.7 (MR)	2.5 (MR)	2.3 (MR)	2.0 (R)	2.7 (MR)	3.5 (S)	2.7 (MR)	2.7[c]
Kiran (maize)	3.0 (MR)	3.2 (S)	2.6 (MR)	2.2 (MR)	2.5 (MR)	2.7 (MR)	3.1 (S)	3.0 (MR)	2.8[c]
Navjot (maize)	2.7 (MR)	3.0 (MR)	2.5 (MR)	2.2 (MR)	2.5 (MR)	2.0 (R)	2.7 (MR)	2.5 (MR)	2.5[b]
Mean score	2.4	2.6	2.7	2.4	2.6	2.5	2.8	2.6	
Pathotypes designate	I	III	IV	I	III	II	V	III	

LSD (P=0.05) Host line =0.23, Isolate =0.18, Host line × Isolate =0.66

*Mean of two replication, 5 plant/replication; Disease rating scale (1.0–2.0 =R. 2.1–3.0 = MR, 3.1–5 =S)

Data in parentheses are disease reaction corresponding to disease score based on disease rating scale (1–5)

[a,b,c] = figures with some letter are not different from each other

(Table 4). The Et-07 showing shorter latent period was most virulent, and shorter latent periods is considered to be of benefit to the pathogen (Agrios 2000) on host part, longer latent periods indicate implification of dilatory resistance and this has been reported in variability studies in sorghum anthracnose caused by *Colletotrichum graminicola* also (Thakur *et al.* 2007). The Isolate Et-07 (Hyderabad) was the most virulent. It could infect all the test lines, and caused susceptible (S) reaction (score 3.1–4.0) on 4 lines—IS 10284, IS 25069, IS 30351 and maize 'Kiran' (Table 5). The highest mean disease score across the 14 differentials was of isolate Et-07, closely followed by Et-03 (2.7) and 2.6 by Et-08, Et-05 and Et-02. Among the host lines highest disease score (3.4) across the isolates was on line IS 10284, and the lowest (1.7, chlorotic flecks only) on IS 26866. The differences in mean disease score were statistically significant (P=0.05) for the host lines, isolates and also for the host line×isolates interactions.

Masias and Bergquist (1974) distinguished races of *E. turcicum* using isolates which infect both, maize and sorghum, and those capable of differentiating between maize lines with and without the resistance genes. They proposed that the common occurrence of isolates with virulence to both, maize and sorghum should be treated as a third specialized form and suggested the Trinomial, *Setosphaeria turcica* f. sp. *complexa*. In further studies on host range of sorghum isolates *E. trucicum,* (Bunker and Mathur 2006) some isolates could infect both maize and sorghum. The ability of the isolates to infect hosts other than sorghum can be used to distinguish the pathotypes, and in the present study, the isolates Et-07 and Et-08 showed wider host range than the others. More number of isolates needs to be further tested to elucidate this.

Two sorghum Germplasm accessions, 'IS 26866' and 'IS 13996' developed lowest disease score (ranging from 1.0 to 2.0 and 1.6–2.4, respectively) across all the 8 isolates, and thus showed stable resistance with respect to tested isolates, while 'IS 10284' was susceptible to 7 isolates, and moderately resistant to one. The 2 isolates from Udaipur (Et-02 from 'Kekri local' and Et-03 from 'CSH 14') showed clear variations in pathogenicity across the 14 test lines, and were designated as 2 distinct pathotypes. Thus, based on the disease reaction, the 8 isolates of *E. turcicum* were assigned in to 5 pathotypes—Pathotype I Et-01 (Chittorgarh), and Et-04 Rajsamand (Rajasthan); Pathotype II Et-06 Parbhani (Maharashtra); Pathotype III Et-02 Udaipur, Kekri local (Rajasthan), Et-05 Surat (Gujarat) and Et-08 Akola (Maharashtra); Pathotype IV Et-03 Udaipur CSH 14 (Rajasthan); and, Pathotype V Et-07 Hyderabad (Andhra Pradesh). These observations are crucial as isolates from same states (Rajasthan) and Maharashtra fell into different pathotypes, while these from 3 different states—Gujarat, Maharashtra and Rajasthan were grouped into a single pathotype. These results suggest that considerable variability exist in populations of *E. turcicum* in India. More studies on virulence analysis using more number of differentials for determining higher levels of variability would further add to knowledge of population biology of *E. turcicum*. From the

results, it appeared that 'IS 26866' and 'IS 13996' have different genes for leaf blight resistance, and IS 9303 has partial resistance. In sorghum, leaf blight resistance has been reported to be governed by dominant genes (Frederiksen 2000), but there is no information on the number of resistant loci, as well as for the genes in lines showing partial/moderate resistant (MR) reaction or delayed disease development (longer latent period).

Variability in fungal pathogens is studied through several characters, such as pathogenicity, fungicide resistance, culture characteristics, vegetative compatibility, isozymes ds RNAs and mycoviruses, Nuclear DNA, polymorphism, mitochondrial DNA polymorphism and karyotype polymorphism (Caten 1996). These characters may be independent or correlated in different fungi. While all those parameters have their own importance and applications, the use of virulence (pathogenicity) to access genetic variability provides direct information on the effect of host selection. This variation is detected on the basis of differential disease reaction among a set of host cultivars. Since there has been no work on identification of biotypes in *E. turcicum* infecting sorghum, no information was available on differential lines. The lines selected for this study showed differential reaction across the isolates. Because of the lack of information on the resistant genes, the isolates in this study have been designated as 'pathotypes' and not as 'race'. It would be useful to develop and evaluate a multi-locational virulence nursery at national and regional (Asian) levels, like the one for sorghum anthracnose (Thakur *et al.* 2007), to further evaluate variability in *E. turcicum* populations, supported by molecular studies.

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