

Race Typing and Evaluation of Aggressiveness of *Exserohilum turcicum* Isolates of Kenyan, German and Austrian Origin*

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Abstract: *Exserohilum turcicum* the causal agent of northern corn leaf blight is a threat to corn production in many areas of the world. The pathogen has a high genetic variability in terms of virulence, genetic structure and several races have been reported. Diseased corn plants from Kenya, Germany and Austria were used to isolate the pathogen following the standard isolation procedures. Detached leaf technique using leaves of maize plants with (Ht0) genes for resistance were used to evaluate aggressiveness and the parameters assessed included incubation period, size of chlorotic and necrotic lesions, lesion density, area under disease progress curve (AUDPC) and rate of lesion expansion. Differential cultivars bearing *Ht1*, *Ht2*, *Ht3*, *HtN* and *Ht0* resistance genes were used to perform race typing on 87 isolates under greenhouse conditions. Isolates from the three countries showed a great variation in aggressiveness with incubation periods ranging from 2 to 6 days, lesion size ranging from 1.81 mm² to 57.04 mm², rate of lesion expansion ranging from 0.29 mm²/day to 21.67 mm²/day and AUDPC ranging from 31.3 mm² to 133.9 mm². Twelve races namely 0,1,2,3,N,12,13,13N,3N,123,23,23N were identified from the three countries. Race 2 was the most common and had 27% frequency of occurrence followed by race 0 and 1 which had frequency occurrence of 22% and 12% respectively. There was no distinct correlation between the type of the race and its origin and different races scattered within the various countries. The high level of genetic variability may explain the occurrence of the disease in the different geographical localities and the ability of the pathogen to infect most of the germplasm including the resistant varieties.

Key words: Cultivars • Differentials • Corn leaf blight • *Exserohilum turcicum* resistance genes • Variability

INTRODUCTION

Maize (*Zea mays* L.) is a popular crop [1, 2] and ranks third in production among the major cereal crops worldwide closely following wheat and rice [3, 4]. Northern corn leaf blight of maize caused by *Exserohilum turcicum* (Pass.) causes yield loss of up to 50% especially when disease setsearly in the season [5]. *Exserohilum turcicum* (Pass.) has a wide host range and a high pathogenic variability with several races already reported in different parts of the world [5, 6].

The disease also causes qualitative alterations of the seeds, resulting in reduced total sugar content, lowered germinative capacity [7] and nutritive value and the heavily infected plants are predisposed to stalk rots [1, 8]. The disease is controlled mainly through the use of

resistant varieties derived from qualitative and quantitative genes acting together or separately [9]. Qualitative resistance is typically race-specific and controlled by single genes whereas quantitative resistance is race-non specific and controlled by many genes [10]. Most of the qualitative genes such as *Ht1*, *Ht2*, *Ht3*, *Htn* and *Htm* are dominant or partially dominant and have a great phenotypic effect but might be overcome by virulent genes present in specific races of the pathogen [6, 9].

Race typing work using differentials have revealed existence of races 0, 1, 2, 3, 4, 12, 23 and 23N in different parts of the world. Some of the differentials that have been used in race typing includes H4460, H4460*Ht1*, H4460*Ht2*, H4460*Ht3*, A697 (W22*HtN*), A619*Ht1*, A619*Ht2*, A619*Ht3*, A619*Htn*, PA91, B37, A632v430302,

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B37Ht1, PA91Ht1, B37Ht2, PA91Ht2, B37Ht3, PA91Ht3, B37HtN, A632Ht1HtN and B102 [11, 12, 13]. Race typing in *E. turcicum* is done under controlled environmental conditions since reactions associated with *Ht1*, *Ht2* and *Ht3* genes are sensitive to variation in temperature and light intensity [14].

Emergence of new races of the pathogen has posed a big challenge to the use of resistant varieties in managing northern corn leaf blight [15]. Hence identification of the *E. turcicum* races present in an area and the understanding of their geographical distribution is critical in screening for resistance and deployment of resistant genes. Information on aggressiveness of the various strains of *Exserohilum turcicum* (Pass.) is scanty since little has been done to this regard. The wide pathogenic variability can render host resistance ineffective as a tool in disease management [1]. The objective of this study was to identify races of *E. turcicum* isolates and determine their aggressiveness.

MATERIALS AND METHODS

The race typing work and determination of aggressiveness was done in the Department of Crop Science, Division of Plant Pathology University of Goettingen in Germany.

Isolation of *Exserohilum Turcicum* Isolates: Infected leaf samples were collected from different parts of Kenya, Germany and Austria and isolation done by cutting leaf discs (1mm²) along advancing margins of the disease and surface sterilizing with 2.5% sodium hypochlorite for three minutes. The tissues were rinsed in 3 changes of sterile distilled water and then plated on Potato Dextrose Agar medium (glucose 20 g, agar 15 g and water 1 litre) in 9-mm diameter petri dishes. Incubation was done at room temperature (22°C ± 2°C) for 7 days and pure cultures obtained by sub-culturing onto fresh PDA medium. Monospore cultures were obtained by preparing a dilute conidial suspension (1000 conidia per ml) and seeding this on water agar (agar 20 g, water 1 litre). After 12 hours of incubation at room temperature, individual conidia were located using a compound microscope at ×40 and single conidium transferred onto clean PDA medium using sterile mounted needles.

Determination of Aggressiveness of *Exserohilum Turcicum* Isolates: A modification of the detached leaf technique by [18] was adopted. Leaves from 21-day

old maize plants cultivars PA91, B37, H102 and A632v430302 (no *Ht* genes) were excised and cut into 6 –cm long pieces and placed inside moisture chambers lined with wet blotting paper. The leaves were placed with the adaxial surfaces facing upward and three leaves were prepared per isolate and replicated three times. Conidial suspension was prepared by flooding the cultures with sterile distilled water, scrapping the surface with microscopic slides to dislodge the conidia and then filtering using cheese cloth. The concentration of the conidial suspension was then adjusted to 2×10⁴ conidia per ml using a haemocytometer. Tween 20 (Polyoxyethylene sorbitan monolaurate) was added at the rate of two drops per 10 ml of water to assist in dispersion of the spore suspension. Conidia from the various *E. turcicum* isolates were prepared separately and two uniform drops (500µl) from each of the conidial suspension were placed on the leaf surface at equidistant points and the lids of the moisture chambers replaced. Moist conditions were maintained and incubation was done at 25°C in growth chambers illuminated 12 hours per day with cool fluorescent light (150µ EM⁻² S⁻¹). Completely randomized design was used.

The inoculated leaves were incubated for seven days and symptom development monitored on a daily basis. Seventy nine isolates including three standard isolates were used in the evaluation of aggressiveness. The parameters assessed were the incubation period, size of the chlorotic and necrotic lesions, number of chlorotic and necrotic spots and severity score based on a scale of 0 to 5, where 0 is no symptoms observed, 1- small chlorotic spots, 2- large chlorotic spots, 3- chlorotic lesions which had coalesced with some necrosis, 4- necrotic lesions and 5- necrosis of the whole leaf. A modification of a key by Bigirwa *et al.* [17] was used.

Race Typing of *Exserohilum Turcicum* Isolates:

Two sets each of 5 differential cultivars namely B37Ht1 and PA91Ht1 bearing *Ht1* gene, B37Ht2 and PA91Ht2 bearing *Ht2* gene, B37Ht3 and PA91Ht3 bearing *Ht3* gene B37HtN and A632Ht1HtN bearing *HtN* and *Ht1* genes and PA91, B102, B37 and A632v430302 without *Ht* genes were used to race type the *E. turcicum* isolates in the greenhouse. This was to ensure that reactions of isolates could be observed on at least two differential sources of *Ht* genes. The differentials were grown in 500g of media composed of a mixture of peat and tuff (1:1 v/v). Two seeds were sown per pot and three pots were used as replicates for each particular treatment. All the necessary agronomic practices carried out.

Table 1: Assessment of reactions of *E. turcicum* on *Ht0*, *Ht1*, *Ht2*, *Ht3* and *HtN* maize differentials

Score	Grouping	Phenotype
0	R	++No symptoms
1	R	+Chlorotic lesions without necrosis
2	R	Chlorotic lesions with some necrosis
3	R	-Chlorotic lesions with considerable necrosis
4	S	Wilted and necrotic lesions without chlorosis
5	S	Elliptical tan colored lesions

Inocula from different *E. turcicum* isolates were prepared separately as above and adjusted to 1×10^4 conidia per ml. Plants at the five to seven leaf stage were inoculated with the conidial suspension. Inoculation was accomplished by spraying the conidial suspension onto the two sets of differentials until run-off using a hand sprayer. The inoculated plants were covered with moistened polythene papers for 48 hours to raise the relative humidity necessary for infection. Three plots comprised the experimental unit and these were replicated three times in a completely randomized design. The experiment was conducted in the inoculation chambers and proper isolation distances maintained to avoid cross infections. After inoculation, plants were transferred into the greenhouse and maintained at 25°C and 12 hours of light and 12 hours of darkness.

A set of maize differentials similarly inoculated with spores of known isolates of *E. turcicum* race 0, 1, 23 were included as standard checks. In watering the plants, leaves were wetted to promote conditions necessary for infection and symptom development. Monitoring was done closely and evaluation for resistant or susceptible reactions based on the production of necrotic lesions on lines without an *Ht* gene, chlorotic or necrotic reactions on lines with *Ht1*, *Ht2* and *Ht3* and resistant or susceptible lesions on lines with *HtN*. Scoring for lesion types was done on a weekly basis and a modification of a key described by [11] was used (Table 1).

RESULTS

Evaluation of Aggressiveness of the Various *Exserohilum Turcicum* Isolates: The isolates significantly ($P \leq 0.05$) differed in the incubation period, size of the chlorotic and necrotic lesions, number of chlorotic and necrotic spots and severity but not in the rate of lesion expansion. Incubation period ranged from 2 to 6 days for the most aggressive and the least aggressive isolates respectively. The average incubation period was 3.8 days for most of the isolates. The lesions

began to appear on the third day of incubation and lesion expansion continued up to the 5th day. Based on the incubation period, isolates G7, B5, B3 and G3 were the most aggressive with an average incubation periods of 2, 2.3, 2.4 and 2.6 days, respectively. Isolates G5, S62, Sorte 2 and MMCZ were the least aggressive with average incubation periods of 5.3, 5, 5.3 and 5.4 days respectively (Table 2). The different races and isolates from different localities showed varying incubation periods.

Different isolates induced varied lesion sizes with the smallest lesion sizes being 1.81 mm² (isolate G33) and the highest lesion size being 57.04 mm² (isolate ETDZ) and the mean lesion sizes was 19.81mm². Lesion density also differed significantly ($P \leq 0.05$) among the various *E. turcicum* isolates with the highest and lowest lesion density being 2.67 lesions/4cm² (isolate B5) and 0.39 lesions/4cm² (isolate S62) respectively. The disease intensity varied significantly ($P \leq 0.05$) among the various *E. turcicum* isolates as exhibited by the different area under disease progress curve values (AUDPC) (Table 2). The highest AUDPC was 284.6 mm² and the lowest was 5.6mm² observed from isolates ETDZ and G33 respectively whereas the overall mean was 89.6mm². The various isolates showed variation in aggressiveness based on the rate of lesion expansion with the highest and the lowest being 21.67 mm²/day and 0.29 mm²/day observed from isolates G38 and G33 respectively. The mean rate of lesion expansion was 9.1 mm²/day.

The isolates showed a great variation in aggressiveness and in isolates G7, B5, B3, the lesions coalesced to produce large chlorotic and necrotic lesions whereas in isolates G5, S62, Sorte 2, the lesions remained as small chlorotic flecks localized in the areas covered by the droplet containing the spore suspension. Ranking of races for aggressiveness based on AUDPC showed that, the races N, 13, 2 and 1 were the most aggressive with mean AUDPC of 133.9mm², 132.4mm², 102.9mm² and 100.1 mm² respectively. Races 13N, 3N, 23N and 23 were found to be the least aggressive with mean AUDPC of 31.3 mm², 57.7 mm², 59.2 mm² and 71 mm² respectively. Races 3, 123, 0 and 12 were moderate in aggressiveness with mean AUDPC of 74.8mm², 80.6 mm², 89.6 mm² and 87.1 mm² respectively (Table 2).

There was a significant ($P \leq 0.05$) and positive correlation ($r = 0.87$) between lesion sizes and disease severity. Lesion sizes as exemplified by the AUDPC were also positively correlated to the lesion density ($r = 0.556$). Similarly, there was a strong positive correlation ($r = 0.75$) between lesion density and disease severity.

Table 2: Aggressiveness based on incubation period, lesion sizes, lesion density, area under disease progress curve and rate of lesion expansion induced by the various *E. turcicum* isolates

Isolate	Race	Incubation period (days)	Lesion sizes (mm ²)	Lesion density	AUDPC (mm ²)	Rate of lesion expansion (mm ² /day)
KTCZ	0	4.67	8.19	0.78	34.00	5.92
G4	0	3.28	10.14	0.89	40.60	4.21
G13	0	3.33	19.62	2.11	88.40	11.17
G43	0	2.67	11.17	1.39	43.80	5.08
G21	0	4.33	19.11	1.00	88.80	11.25
G27	0	4.00	9.28	2.11	40.80	4.35
G37	0	2.67	37.00	2.50	143.20	6.92
G11	0	5.33	3.31	0.61	6.30	1.50
G1	0	3.06	18.48	1.11	80.30	13.96
G38	0	3.00	44.86	2.06	204.00	21.67
G48	0	3.50	15.03	1.44	58.30	5.42
G20	0	5.11	6.93	0.55	26.00	6.92
ETDZ	0	3.94	57.04	1.67	284.60	19.33
SORTE 2	0	5.33	3.24	0.83	11.80	2.00
ENG9	0	4.28	28.31	1.22	128.20	16.67
B2	0	3.67	36.84	1.94	169.60	16.67
S53	0	2.67	23.69	1.78	113.50	8.42
G19	1	3.67	4.14	1.17	13.20	0.92
G8	1	3.67	25.14	1.00	116.30	6.50
ENG6	1	3.67	28.41	1.50	133.40	14.50
SORTE 3	1	2.61	21.81	1.89	100.80	12.58
P5	1	2.67	31.57	2.11	151.40	10.71
RZ 260 DELITOP	1	3.00	28.61	1.61	133.50	13.20
S62	1	5.33	8.44	0.39	35.30	7.00
4	1	4.39	24.46	1.50	119.40	12.67
S13	1	3.78	8.57	1.44	37.40	3.42
S55	1	4.67	37.01	1.56	160.40	17.50
KGMGCZ	2	4.17	23.81	1.22	108.10	13.29
G12	2	4.72	14.58	1.00	63.00	7.92
G23	2	3.94	30.78	2.44	140.80	7.46
G26	2	3.33	28.19	1.39	129.70	13.17
G32	2	4.06	14.50	1.00	66.80	6.75
MMCZ	2	5.39	6.50	0.61	31.30	2.58
G47	2	4.06	37.22	0.89	177.70	17.25
G15	2	4.33	31.58	1.22	149.20	15.75
G7	2	2.00	27.01	2.11	130.00	13.77
G29	2	3.06	29.46	2.06	136.80	14.57
RZ 300 BENICIA	2	2.72	20.55	1.94	99.40	9.83
B5	2	2.28	23.12	2.67	109.10	9.54
SORTE 1B	2	4.00	20.78	1.17	94.80	10.58
RZ 280 MORISAT	2	3.06	14.68	1.61	64.70	3.58
SORTE 6	2	3.61	36.40	1.33	181.00	15.00
RZ 230 NUESTRO	2	3.11	13.42	1.06	62.40	5.12
ENG 1	2	3.78	15.59	1.89	60.60	11.83
RZ250 DC2949	2	4.00	23.02	1.78	100.50	12.95
S2	2	3.67	28.66	1.89	130.80	18.33
S1	2	5.28	5.53	0.61	20.70	2.33
G36	3	4.33	3.75	0.78	16.20	1.75
G46	3	3.33	37.33	1.22	186.00	17.50
G17	3	2.67	21.95	2.28	96.20	9.25
G42	3	4.72	9.19	0.78	38.70	5.33
G10	3	3.67	8.75	1.94	37.20	5.15
G14	N	3.67	30.51	1.94	133.90	10.08
G49	12	3.06	11.63	2.33	55.70	4.78
ENG4	12	5.33	13.39	0.78	52.30	5.74

Table 2: continued

Isolate	Race	Incubation period (days)	Lesion sizes (mm ²)	Lesion density	AUDPC (mm ²)	Rate of lesion expansion (mm ² /day)
B1	12	3.00	32.84	2.39	148.40	18.72
ORT	12	4.00	18.64	1.39	92.00	7.58
G50	13	3.61	27.84	1.67	132.40	13.58
MMSZ	123	3.94	28.07	1.28	139.80	12.67
G41	123	4.33	13.75	0.94	62.00	6.92
G39	123	4.67	21.17	0.83	99.20	10.92
G9	23	3.61	28.31	1.17	135.30	14.92
G35	23	2.72	9.01	1.89	39.10	3.81
G40	23	4.33	4.42	0.67	19.70	1.83
G18	23	4.06	15.38	1.17	103.10	12.75
ENG7	23	3.17	13.84	1.39	58.10	8.67
ENG 4	23	5.33	13.39	0.78	52.30	5.75
G5	23N	5.33	4.64	0.61	18.70	1.83
G3	23N	2.56	41.85	2.61	193.40	18.86
G33	23N	4.17	1.81	1.00	5.60	0.29
G51	23N	3.33	9.51	1.56	33.60	3.96
ENG8	23N	4.67	13.67	1.11	63.20	7.58
P6	23N	3.67	11.17	1.44	40.50	4.75
G6	13N	2.89	8.36	1.11	31.30	2.17
G30	3N	4.67	12.65	1.11	45.20	4.83
G34	3N	4.00	19.48	1.72	70.10	6.25
Standard	Race 1	3.61	22.19	1.29	100.00	9.08
Standard	Race 23	3.67	24.80	1.28	114.90	12.17
Standard	Race 0	2.33	27.39	2.67	120.80	9.06
Mean	3.76	19.81	1.44	89.60	9.10	
LSD _(P=0.05)	0.77	15.22	0.59	131.47	13.61	
CV(%)	31.2	77.40	62.80	91.00	92.80	

Table 3: Race determination of *Exserohilum turcicum* isolates using differential cultivars

Race	Country of origin	Isolates as coded
0	Kenya	KTCZ, G4, G13, G43, G21, G45, G27, G7, G11, G1, G38, G48, G44, G20, ETDZ
0	Germany	SOTE 2, ENG 9, B2
0	Austria	S53
1	Kenya	G19, G8
1	Germany	SORTE 3, SORTE 4, P5, RZ 260 DELITOP
1	Austria	S62, S64, S13, S55
2	Kenya	KGMGCZ, G12, G23, G26, G32, MMCZ, G47, G15, G7, G29
2	Germany	RZ 250 PHANTOM, RZ 300 BENICIA, B5, RZ 280 LUGAN, B4, SORTE 1B, RZ 280 MORISAT, SORTE 6, RZ 230 NUESTRO, ENG 1, RZ 250 DC2949
2	Austria	S31, S2, S1
3	Kenya	G36, G46, G2, G17, G42, G10
N	Kenya	G14
12	Kenya	G49
12	Germany	ENG 4, B1, ORT
13	Kenya	G50
13	Germany	SORTE 5
123	Kenya	MMSZ, G41, G39
23	Kenya	G9, G35, G40, G18, G16
23	Germany	ENG 7, ENG 11
23N	Kenya	G5, G3, G33, G51
23N	Germany	ENG 8, P6, ENG 5
13N	Kenya	G6
3N	Kenya	G30, G34

Table 4: Race typing of *E. turcicum* isolates from different origins. Listed are the numbers of isolates assigned to certain races

Race	Isolate origin			Total	% Total
	Kenya	Germany	Austria		
0	15	3	1	19	22
1	2	4	4	10	12
2	10	11	3	24	28
3	6	Nd*	Nd*	6	7
N	1	Nd*	Nd*	1	1
12	1	3	Nd*	4	5
13	1	1	Nd*	2	2
123	3	Nd*	Nd*	3	3
23	5	2	Nd*	8	8
23N	4	3	Nd*	8	8
13N	1	Nd*	Nd*	1	1
3N	2	Nd*	Nd*	2	2
Total	51	27	8	86	
%	59	31	9	100	

Nd* = not detected

The correlation ($r = -0.297$) between incubation period and disease severity was negative since isolates with a shorter incubation period generally had the highest disease severity. Likewise, incubation period was negatively correlated ($r = -0.514$) with the lesion density. There was a strong positive correlation ($r = 0.863$) between the AUDPC and the rate of lesion expansion meaning that the isolates with higher aggressiveness as shown by higher rate of lesion expansion induced more disease on the plants thus resulting in high AUDPC.

Physiological Race Typing of *E. Turcicum* Isolates:

Race typing revealed a great diversity among the *E. turcicum* isolates from the different localities. Twelve races namely 0,1,2,3,N,12,13,13N,3N,123,23,23N were identified in samples from Kenya, Germany and Austria. The diversity of races differed from the different regions, however some races were found to be more dominant than others. Race 2 was the most dominant and it comprised 26.6% of all the races identified. This was followed by race 0 and race 1 with occurrence frequencies of 21.07% and 12.26% respectively. Races N and 13N were identified in very few localities and both had occurrence frequencies of 1.11% each (Table 3).

Race 0 was the most common in Kenya and it constituted 28.8% of all the races identified, race 2 and 1 were the most common in Germany and Austria respectively and each constituted 42.3% and 50% of occurrence respectively (Table 4). Kenya had the most diverse number of isolates with a total of 12 races being reported followed by Germany and Austria which had 7 and 3 races respectively.

DISCUSSION

The *E. turcicum* isolates from Kenya, Germany and Austria showed a varied response in the differentials indicating a high virulence complexity and variability of the pathogen. In total, 12 physiological races were identified based on the reaction on the differentials. Races 0, 1, 2 and 3 were found to be the most dominant in the three countries and this confirms findings by [6] who reported that races 0,1,2,3 and 12 are the most common. Kenya had the most diverse number of *E. turcicum* races with all the 12 races being represented and this probably can be explained by the fact that the widespread and continuous cultivation of maize, presents a high selection pressure for the pathogen with the resultant involvement of many races since the conditions in the tropics provides conducive disease conditions almost all the year round as also observed by [12]. Other researchers have used differential cultivars of maize in race typing *E. turcicum* isolates in other parts of the world. The presence of race 1,2,3 in Ohio and other states in Central and Eastern USA was reported while [18] indicated the presence of race 0 and 1 in China, races 23N, 23, 2N in Mexico, race 23, 23N and 0 in Zambia and race 0,2,N, 23N in Uganda.

Since the reaction of differentials bearing the different Ht genes can be modified by light and temperature conditions, race typing work has to be done under identical conditions for the results to be comparable [14]. The present study revealed that, there was no distinct correlation between the race and the geographical origin of the isolates and different races of *E. turcicum* occurs naturally in many places as reported previously [17].

Aggressiveness of the various *E. turcicum* isolates differed in terms of lesion density, AUDPC, lesion sizes, length of incubation period and rate of lesion expansion. A high number of these isolates had a moderate to high levels of aggressiveness and this has serious implications since the more aggressive isolates induces disease faster leading to higher disease levels with subsequent yield losses. The correlation between rate of lesion expansion and AUDPC was highly positive ($r = 0.86$) meaning that, those isolates which were more aggressive in terms of lesion expansion resulted in more leaf damage thus contributing to higher yield losses.

The length of incubation period was a good indicator of aggressiveness and most of the isolates with short incubation periods having higher AUDPC, rate of lesion expansion and area of lesions. In evaluating for aggressiveness, it is necessary to consider several parameters since the study revealed a few isolates with a higher rate of lesion expansion but with comparatively long incubation periods. AUDPC and rate of lesion expansion are important parameters in assessing aggressiveness, as they give a reflection of the area of the plant covered by the disease which closely correlates with the damage caused as also reported [19].

Races N, 13, 2 and 1 were found to be more aggressive as shown by lower incubation periods, higher lesion sizes, lesion density, AUDPC and rate of lesion expansion. Intensive and comprehensive breeding programmes are required to introgress genes for resistance to northern leaf blight to forestall breakdown of resistance likely to occur due to evolution of new races that would overcome the Ht genes. Maize breeding programmes must also incorporate vigorous testing to determine levels of resistance to the various races of the *E. turcicum* pathogen.

Aggressiveness studies requires very controlled and uniform environments rendering detached leaf technique very useful since it is easy to achieve these conditions as compared to glasshouse conditions an observation similarly reported [20]. The technique yields results that are comparable to studies done on intact foliage and allows handling of a large number of isolates especially where facilities are limiting [16].

CONCLUSION

The parameters used in assessing aggressiveness can be used for characterizing isolates into virulence groups in cases where a large number of isolates are to be handled and where facilities for using molecular markers and differential cultivars are unavailable [20].

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