Collections, Isolations, Morphological Study of *Exserohilum turcicum* and Screening Resistant Varieties of Corn to Northern Corn Leaf Blight Disease

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Abstract Collections of Northern Corn Leaf Blight (NCLB) samples were accomplished from 11 and 6 provinces in the north and northeast of Thailand, respectively, from which 478 isolates were collected. Pure cultures of the pathogen were obtained using the single spore isolation technique. Morphological study was performed by observing mycelia and conidia under a compound microscope. Characteristics of the fungus found in this study were similar to *Exserohilum turcicum* (Pass.) Leonard and Suggs, the causal agent of Northern Corn Leaf Blight disease. The highly virulent H1 and KL1 isolates identified through a pathogenicity test were selected for the study of growth and sporulation on eight media; CMA, CLIA, MEA, OMA, PDA, PDYeA, RBA and V-8 agar, under laboratory condition. Screening of corn varieties, resistant to NCLB disease, was carried out on 160 varieties in the field. The results revealed that nine varieties were highly resistant at level 1 to *E. turcicum* isolate H1 and KL1 in both vegetative and reproductive stages, namely IAS CO 6, SW05D-D10 199-36, SW05D-C8 1055-11, Syngenta 1, Syngenta 2, Syngenta 3, Syngenta 4, Syngenta 5 and Syngenta 6.

Keywords: Northern Corn Leaf Blight, *Exserohilum turcicum*, Resistance Screening

Introduction

Corn could rank second after rice for the export grains of Thailand. Due to high local demand, Thailand has increased the acreage of field corn production from 190,297 acres in 2009 to 2,886,047 acres in 2014 with the expected yield of 4.98 m tons in 2014 (Office of Agricultural Economics, 2009 and 2014). However, planting corn continuously in a monocrop system has created yield losses resulting from disease and insect damage (Nakhon Sawan

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Field Crops Research Center Newsletter, 2008.)

Northern Corn Leaf Blight (NCLB) is an important disease of corn and causes significant economic loss worldwide. It is caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass). This pathogen can infect the corn plant within 6-18h after inoculation; under favorable conditions severe symptoms are shown in 3 d after inoculation (Lipps and Mills, 2002). Observation of the symptoms of the diseased leaves indicate that there are many lesions on the heavily damaged leaves. The spindle-shaped lesions are light brown to dark brown with a width of about 1.5-15.0 cm, and are parallel to the midrib. The symptoms begin from the lower leaves and spread to the upper leaves, resulting in total foliar blight and plant death. Spores of the pathogen produced in diseased tissue are disseminated by wind and/or contaminated seed. The conductive conditions for pathogen development include high temperature (22-30° C) and a high relative humidity (90-100%). Disease outbreaks in the production areas have caused reduction of yield ranging from 40-80% (Sitthikul, 1996).

Therefore, the main purpose of this study is to investigate the pathogenic variation of the pathogen by collecting the fungus from numerous areas with different conditions and testing their pathogenicity on different corn varieties. Resistant cultivars are used to limit losses from the disease. Genetic resistance to NCLB disease encompasses at least seven physiological races of *E. turcicum* that are pathogenic to corn. The race distinction is based on their pathogenicity to different maize genotypes possessing different resistant genes. To date, four genes have been identified that are responsible for major resistance to Turcicum leaf blight: *Ht*1, *Ht*2, *Ht*3 and *Ht*N. Race 0 of *E. turcicum* is virulent to all *Ht* genotypes while race 2, 3 is virulent only to *Ht*2 and *Ht*3 genotypes (Welz and Geiger, 2000).

This research will benefit breeding programs developing NCLB diseaseresistant corn varieties. Such resistant varieties will be introduced to the farmers by the extension program; this will also lead to reduction of chemical fungicide usage, maintenance of beneficial microorganisms in the soil and increased yields without increasing planting areas.

Material and methods

Surveying and collecting northern corn leaf blight (NCLB) samples and isolating the causal fungal pathogen

Surveying and collecting the NCLB samples

Diseased corn leaves showing the typical symptoms of NCLB were collected from planting areas in northern and north-eastern parts of Thailand. All necessary information including typical symptoms, locations, plant cultivars and disease severities were recorded. The diseased leaves were cut by scissors and kept in plastic bags then brought back to the laboratory for further study.

Isolation and morphological study of the pathogenic fungus

Infected corn leaves were collected from the fields, different lesions were separately cut, sterilized in a 0.5% sodium hypochlorite solution (1:3,v/v) for 1 min, washed three times with sterile water and placed on moist paper towels in petri dishes for 48 h to allow sporulation. Single spores were picked from the lesions and placed on potato dextrose agar (PDA) plates, incubated at room temperature (Carson, 1995) and examined by using the slide culture technique under a compound microscope.

Growth, sporulation and characteristics of E. turcicum on different media

A fungal disc of *E. turcicum* was transferred to each Petri dish containing different culture media, with four replications for each medium. Growth of fungal colonies was measured after10 d of incubation. Eight different media were employed, e.g. corn meal agar (CMA), corn leaf infusion agar (CLIA), malt extract agar (MEA), oat meal agar (OMA), potato dextrose agar (PDA), potato dextrose yeast extract agar (PDYeA), rice bran agar (RBA), and Vegetable-8 agar (V-8 agar). The fungal growth was observed at 2 d intervals for 10 d. The fungal colonies were measured and photographs taken. For sporulation, 10 ml of sterile water was added to each culture plate and the spores were scraped off by using and L-shaped glass rod. The spore suspension was filtered through 2-layers cheese cloth then counted using a haemacytometer, under acompound microscope.

A screening test of E. turcicum isolates H1 and KL1 to identify resistant corn varieties

Pre-screening of the corn varieties

One hundred and sixty varieties of corn seeds obtained from various public institutes and private sectors were used in this study. The corns were planted in $3x5 \text{ m}^2$ planting plots with a 80x25 cm space. The experiment was performed as split plot design with two replications of 10 plants each.

The seedlings were watered and nourished with 46-0-0 and 15-15-15 fertilizer regularly and were inoculated at the age of 2 wks with two isolates of *E. turcicum*, H1 and KL1, previously selected based on their high virulence.

Preparation of spore suspension (inoculum)

Spore suspensions of *E. turcicum* cultured on V-8 agar medium were prepared according to the method developed by Ogliari *et al.* (2005). The inoculum was adjusted to the concentration of 10^5 spore/ml.

Field evaluation of the corn varieties to the E. turcicum

Disease assessment and evaluation and data collection and analysis

The experimental plots were located at the Rajamangala University of Technology Lanna, Lampang campus, and the seedlings were inoculated at the ages of 14 and 28 d old. In order to evaluate disease severity, inoculated plants were examined and scored at 30 and 60 d after inoculation. Disease severity was assessed using a scale of 0 to 9 where (R) resistant = level 1-2, (MR) moderately resistant (moderate severity) = level 3, (MS) moderately susceptible = level 4-5, (S) susceptible (high severity) = level 6-9 (modified from Fetch and Steffenson, 1999) (Fig. 1).

Disease rating scales were divided into three categories as follows:

(R)	Resistant	= level 1-2
(MR)	Moderately resistant (moderate <i>severity</i>)	= level 3
(MS)	Moderately susceptible	= level 4-5
(S)	Susceptible (high <i>severity</i>)	= level 6-9



Figure 1. Assessment of virulent level of symptom severity (Fetch and Steffenson, 1999).

- Level 1 Small, brown, spherical spot
- Level 2 Brown square spot, 0.3-0.5x0.3-0.7 cm.
- Level 3 Round spot, 0.5-0.7x0.8-1.3 cm, chlorosis around the spot

- Level 4-5 Brown, obovoid spot, 0.3-0.7x0.7-1.3 cm. level 5 has bigger spot size than level 4 and having chlorosis
- Level 6-9 Brown, oblong spot, 1.4-3.2x0.4-0.8 mm, chlorosis, with 0.5-1.0 cm bleached leaf tissue surrounding the spot. Spots always expand and connect with each other becoming the bigger spot on the leaf

Results and discussion

Surveying and collecting northern corn leaf blight (NCLB) samples and isolating the causal fungal pathogen

Surveying and collecting the NCLB samples

NCLB samples were randomly collected from 17 major production areas in 11 provinces in the north: Chiang Mai, Chiang Rai, Lampang, Lamphun, Payao, Nan, Uttaradit, Tak, Sukhothai, Phitsanulok and Nakhon Sawan, and 6 provinces in the northeast of Thailand: Nakhon Ratchasima, Nong Khai, Nakhon Panom, Loei, Khon Kaen and Phetchabun (Fig. 2).



Figure 2. Locations where the samples of northern corn leaf blight disease were collected.

Isolation and morphological study of the pathogenic fungus

Results from isolating the causal pathogen from the diseased leaves showing NCLB symptoms, using single spore isolation technique, revealed that the fungus produced small grayish white colonies in 4 d (Fig. 3A). The pure isolates grown on PDA plates for 10 d initially produced grayish white colonies with a thick mycelium which subsequently changed to greenish white colonies with a very thick mycelium, and the color of PDA changed to dark green (Fig. 3B).



Figure 3. Fungal pathogen from single spore isolation on PDA medium (A); characteristics of the fungal colony (B) upper surface (left) lower surface (right).

Total Northern Corn Leaf Blight (NCLB) pathogen isolates from the samples collected from 17 provinces of Thailand; 478 isolates were obtained, 381 of which were from Northern provinces and 97 isolates were from north-eastern provinces (Fig. 4).



Figure 4. Proportion of the isolates of *E. turcicum* obtained from the diseased corn leaves collected from the northern (A) and the northeastern (B) parts of Thailand.

Morphological studies indicated that the mycelia produced spores/conidia that were spindle-shaped, greenish-grey in color; the number of septa ranged from 3-8, with a size of 20-25x110-129 μ m. There were both single and groups of spores produced on the greenish-grey conidiophores which have 2-4 septa, with a size of 7.9-8.9 x 119-129 μ m. In the segment area between the spore and conidiophore is the hilum which can be clearly seen (Fig. 5). The fungus was classified following Abebe *et al.* (2008) as identical to *Exserohilum turcicum* (Pass.) Leonard and Suggs (*Helminthosporium turcicum*), causing Northern Corn Leaf Blight (NCLB) disease.



Fig. 5 Conidia and conidiophores of *E. turcicum*, causal pathogen of NCLB disease: Conidium on conidiophores (A); characteristics of conidia showing septa and hilum (B).

Growth, sporulation and characteristics of E. turcicum on different media

Growth and sporulation of the H1 and KL1, the two isolates of *E. turcicum*, were studied on eight different media e.g. CMA, CLIA, MEA, OMA, PDA, PDYeA, RBA and V-8 agar under laboratory conditions. Growth and sporulation among different media were found to be significantly different, LSD (p = 0.05), in the two isolates tested. Isolate H1 showed the highest growth rate on PDA after 10 d of cultivation followed closely by PDYeA. On OMA, CMA, CLIA, MEA and RBA; the fungus showed similarly moderate growth, and rather slow growth was found on V-8. The highest sporulation was found on the V-8 followed by PDA and PDYeA. No sporulation was found on RBA (Table 1 and 2; Fig. 6).

Madia	Colony diameter (cm) ¹						
Media	2 d	4 d	6 d	8 d	$10 d^*$		
CLIA	1.15±0.01	2.75±0.12	4.30±0.12	5.28±0.11	5.69 ± 0.03^{d}		
CMA	1.17±0.02	2.65±0.16	3.98±0.11	5.13±0.15	$5.80\pm0.00^{\circ}$		
MEA	0.85±0.10	2.15±0.08	3.85±0.21	4.75±0.10	$5.15 \pm 0.06^{\circ}$		
OMA	1.10±0.05	2.10±0.11	3.65±0.19	5.45±0.06	6.14±0.05 ^b		
PDA	1.70±0.02	3.20±0.12	5.23±0.21	7.40±0.15	9.00 ± 0.00^{a}		
PDYeA	1.70±0.15	3.10±0.21	5.20±0.13	7.21±0.09	8.70 ± 0.00^{a}		
RBA	1.55±0.00	2.55±0.11	3.58±0.13	4.60±0.13	5.14 ± 0.10^{e}		
V-8	0.90±0.03	1.90±0.10	3.25±0.17	4.18±0.22	4.94 ± 0.05^{f}		
LSD $(p = 0.05)$ 0.41					.41		
%CV 24.55				4.55			

Table 1. Comparison of the growth of *E. turcicum* isolate H1 on different media under laboratory conditions.

¹ Means of 4 replications

* Means followed by the same letter in the last column are not significantly different by LSD (p = 0.05)

Media	Characteristics of colony	spore production (spores/ml) ¹
CLIA	Irregular shaped, rough and slightly fluffy black mycelium	$4.95 \times 10^{5} d^*$
СМА	Irregular shaped, rough and slightly fluffy grayish- brown mycelium	3.45×10^{56}
MEA	Irregular shaped, rough and slightly fluffy black mycelium	2.05×10^{5f}
OMA	Irregular shaped, rough and slightly fluffy greyish- brown mycelium	$0.50 \mathrm{x} 10^{5 \mathrm{g}}$
PDA	Irregular shaped, rough and slightly fluffy greyish- black mycelium	10.00×10^{5b}
PDYeA	Irregular shaped, rough and slightly fluffy greyish- black mycelium	7.40×10^{5c}
RBA	Irregular shaped, rough and slightly fluffy whitish- grey mycelium	0 ^g
V-8	Irregular shaped, rough and slightly fluffy black mycelium	16.3×10^{5a}
LSD ($p = 0.0$	05)	2.93
%CV		93.94

Table 2. Characteristics of *E. turcicum* colony, isolate H1, on different media under laboratory conditions, and comparison of spore production.

¹ Means of 4 replications of each treatment.

* Means followed by the same letter in the last column are not significantly different by LSD (p = 0.05)



Figure 6. Characteristics of *E. turcicum* colonies isolate H1grown on 8 media (10 d): CMA (A), CLIA (B), MEA (C), OMA (D), PDA (E), PDYeA (F), RBA (G) and V-8 (H) under laboratory condition.

Similar results were found in the isolate KL1; the maximum growth rate was found on the PDA and PDYeA which were not statistically different. The highest sporulation was also found on V-8 agar which was statistically different from the second ranked PDA. The other media didnot support a high level of sporulation especially RBA where no sporulation was found (Table 3 and 4; Fig. 7).

	5			1			
Madia	Colony diameter(cm) ¹						
Ivieuta	2 d	4 d	6 d	8 d	$10 d^*$		
CLIA	1.20±0.02	2.80 ± 0.11	4.45 ± 0.26	5.20 ± 0.37	5.69 ± 0.03^{d}		
CMA	1.37±0.00	2.70 ± 0.10	4.13 ± 0.12	5.00 ± 0.18	$5.80 \pm 0.00^{\circ}$		
MEA	1.00±0.01	2.45 ± 0.02	4.37 ± 0.21	5.55 ± 0.17	5.15 ± 0.06^{e}		
OMA	1.12±0.00	2.20 ± 0.11	3.70 ± 0.12	5.06 ± 0.16	6.14±0.05 ^b		
PDA	1.65±0.01	3.18 ± 0.19	5.24 ± 0.22	7.40 ± 0.16	9.00 ± 0.00^{a}		
PDYeA	1.65 ± 0.02	3.18 ± 0.14	5.23 ± 0.16	7.25 ± 0.23	8.70 ± 0.00^{a}		
RBA	1.50 ± 0.00	2.92 ± 0.14	4.90 ± 0.23	5.90 ± 0.35	5.14 ± 0.10^{e}		
V-8	0.85 ± 0.00	1.75 ± 0.02	2.89 ± 0.21	4.19 ± 0.17	$5.00 \pm 0.08^{\rm f}$		
LSD $(p = 0.05)$ 0.53					.53		
%CV 22.22				2.22			

Table 3. Comparison of the growth of *E. turcicum* isolate KL1 on different media under laboratory conditions.

¹ Means of 4 replications

* Means followed by the same letter in the last column are not significantly different by LSD (p = 0.05)

Media	Characteristics of colony	spore production (spores/ml) ¹
CLIA	Irregular shaped, rough and slightly fluffy whitish grey mycelium	$4.55 \text{ x} 10^{5} \text{ d}^{*}$
СМА	Irregular shaped, rough and slightly fluffy black mycelium	3.24×10^{56}
MEA	Irregular shaped, rough and slightly fluffy greyish- black mycelium	1.95x10 ^{5f}
OMA	Irregular shaped, rough and slightly fluffy whitish grey mycelium	0.5x10 ^{5g}
PDA	Irregular shaped, rough and slightly fluffy black mycelium	9.90x10 ^{5b}
PDYeA	Irregular shaped, rough and slightly fluffy greyish- black mycelium	7.30x10 ^{5c}
RBA	Irregular shaped, rough and slightly fluffy greyish- black mycelium	0 ^g
V-8	Irregular shaped, rough and slightly fluffy black mycelium	18.3x10 ^{5a}
LSD(p=0.0))5)	3.86
%CV		101.10

Table 4. Characteristics of *E. turcicum* colony, isolate KL1, on different media under laboratory conditions, and comparison of spore production.

¹ Means of 4 replications of each treatment.

* Means followed by the same letter in the last column are not significantly different by LSD (p = 0.05)



Figure 7. Characteristics of *E. turcicum* colonies isolate KL1grown on 8 media (10 d): CMA (A), CLIA (B), MEA (C), OMA (D), PDA (E), PDYeA (F), RBA (G) and V-8 (H) under laboratory condition.

A screening test of E. turcicum isolates H1 and KL1 for resistant variety in corn

E. turcicum isolates H1 and KL1 expressed highly virulent in previous test were used in inoculating 160 varieties of corn in the resistant variety screening. The results of inoculation showed that the severity index difference came out at 3 levels e.g. R, MR, MS and S which some corn varieties died soon after inoculation, therefore no plant survived at monitoring time. The early symptoms occurred as quickly as few d after inoculation the symptoms appeared on the leaves in the inoculated plants but did not show in the control ones (Fig. 8). However, it was found that the control plants started showing symptoms by the end of the observation period due to infection of dispersing spores from the nearby inoculated plants.



Figure 8. Comparison of corn plants after inoculation with *E. turcicum* and non-inoculated plant; the inoculated ones show stunting and having blight symptoms at 30 d after inoculation. The experiment was conducted at Rajamangala University of Technology Lanna (RMUTL), Lampang campus.

Of all 160 varieties, results showed that at 30 d after inoculation there were numbers of the corn varieties showing highly resistant to both H1 and KL1 and only some showing moderate resistant while few of susceptible ones. At 60 d after inoculation there were less varieties of highly resistant to the two isolates,

but higher numbers of varieties showing moderately resistant together with susceptible and dead numbers of the corn varieties to both isolates (Table 5).

Table 5. Number of corn varieties at three different resistant levels to northern corn leaf blight disease caused by *E. turcicum* isolates H1 and KL1 at 30 and 60 d after inoculation.

Desistent	H1 isolate		KL1 isolate	
Resistant	30 d	60 d	30 d	60 d
R (level 1-2)	68	12	70	11
MR (level 3)	26	1	21	2
MS (level 4-5)	60	71	65	84
S (level 6-8)	6	65	4	53
Death	-	11	-	10

The corn varieties that showed different level of resistance to NCLB disease were divided into 3 groups as follows: (1) highly resistant at level 1 to both isolates – SW05D-D10 199-36, SW05D-C8 1055-11, Syngenta 1, Syngenta 2, Syngenta 3, Syngenta 4, Syngenta 5, Syngenta 6 and IAS Co. 1. (2) highly resistant at level 2 to isolate H1 – LARTC 10, SW05D-D10 206-5 and SW05D-D10 206-11; highly resistant at level 2 to isolate KL1 – Pacific Co. 4 and Pacific Co. 7 and (3) highly resistant at level 3 to isolate H1 – SK07C2ER 21; highly resistant at level 3 to isolate KL1 – Pacific Co. 6 as shown in Table 6.

Table 6. Varieties showing highly resistant (HR) to northern corn leaf blight disease caused by *E. turcicum* isolates H1 and KL1 at different level.

UD loval	H1 isolate	KL1 isolate
nk level	varieties	varieties
1	SW05D-D10 199-36	SW05D-D10 199-36
	SW05D-C8 1055-11	SW05D-C8 1055-11
	Syngenta 1	Syngenta 1
	Syngenta 2	Syngenta 2
	Syngenta 3	Syngenta 3
	Syngenta 4	Syngenta 4
	Syngenta 5	Syngenta 5
	Syngenta 6	Syngenta 6
	IAS Co. 1	IAS Co. 1
2	LARTC 10	Pacific Co. 4
	SW05D-D10 206-5	Pacific Co. 7
	SW05D-D10 206-11	
3	SK07C2ER 21	Pacific Co. 5
		Pacific Co. 6

¹ Grading of resistant variety (See Materials and methods)

Conclusion

Surveying and collecting Northern Corn Leaf Blight samples from 17 provinces of northern and north-eastern parts of Thailand cover the major areas of corn plantations during the time of survey. The purpose of collecting many diseased samples is to study pathotypic variation of the fungus *E. turcicum*. This study can be useful for the growers and the others involved with corn production on making plan for control of this disease.

Isolations of the causal pathogen on PDA and purifications, using single spore isolation technique given 97 isolates from the sample collected from north-eastern provinces and 381 isolates from Northern provinces. Of all the isolates from the North's samples, most are from Chiang Mai and Chiang Rai while the isolates mostly received from the northeast's samples are from Nakhon Panom and Nong Khai

Results from studying growth and sporulation of *E. turcicum* isolate H1 and KL1 on 8 different media – CMA, CLIA, MEA, OMA, PDA, PDYeA, RBA and V-8 under laboratory condition showed that the fungus grew most quickly on PDA followed by PDYeA. For spore production, the fungus produced highest spores number on V-8 followed by PDA but much less on PDA compared to V-8. Anyhow, comparing to V-8, preparing PDA is easier, less time consuming and lower cost, so PDA was chosen to be used in most experiments.

One hundred and sixty varieties of corn were tested for disease resistance in the field with the use of most virulent *E. turcicum* isolates H1 (from the Northeast) and KL1 (from the North). The results revealed significant difference in response of corn varieties to the individual pathogen. The pathotypic variations were classified into 3 levels of virulence categories: R, MR, MS and S. Nine varieties of corn include IAS CO. 6, SW05D-D10 199-36, SW05D-C8 1055-11, Syngenta 1, Syngenta 2, Syngenta 3, Syngenta 4, Syngenta 5 and Syngenta 6 showed highly resistant to *E. turcicum* isolate H1 and KL1, both in vegetative and reproductive stages which should be useful for the future breeding program.

Disease development was determined essentially by 4 factors; infection, latent period, sporulation of pathogen, and loss of infected tissue (Van der Plank, 1963). In addition, plant-pathogen interaction was concerned with resistant gene (Ht); the resistant levels were dependent on Ht1, Ht2, Ht3 and HtN, respectively. The result implied that the pathogenic divergences of each *E. turcicum* isolate affected variation of virulence level of the disease. The results of this study came out in agreement with Pedersen and Brandenburg (1986). Moreover, Lević *et al.* (2008) used the differential maize lines to

determine the *E. turcicum* race classification. They reported the correlation of the *Ht* genes with the virulent and avirulent of the pathogenicity of the races whereas the race 0 was ineffective (avirulent) against all Ht genes (Ht1, Ht2, Ht3 and HtN), and the race 1 was only effective (virulent) against Ht1 gene. However, the identification of these races should be rapid under controlled conditions due to inoculated isogenic lines responses (Leath et al., 1990). Karnataka et al. (2007) who reported that the phenotype of plant disease resistance is affected greatly by environmental factors. Similarly to Abebe *et al.*, 2008 that each corn variety expressed differently in resistance level to E. turcicum due to vertical resistant gene, resistant to specific pathogen (Van der Plank, 1968). Beshir et al. (2012) reported the difference disease severity of the sorghum infected with E. turcicum in the greenhouse and the field evaluation as the effects of high humidity and moderate temperature in the greenhouse, so the higher disease severity was commonly found in the greenhouse. Furthermore, the environment can have major effects on the disease response of specific sorghum genotypes to Turcicum leaf blight. From our results, resistance level of each corn variety to 2 isolates of E. turcicum, which were isolated from different locations are also agreeable with the report of Welz and Geiger (2000) who reported that each corn variety will exhibit dissimilarity in resistance level when tested in different locations. In addition, Lipps and Mills (2002) and Abebe and Singburaudom (2006) found that the responsiveness was dependent on the intensity of the disease in each area. New races of the pathogen were present and could overcome some sources of the monogenic resistance. Polygenic resistance is manifested through fewer and smaller lesions somewhat resembling a susceptible reaction (Frederikson and Franklin 1980). However, polygenic resistance is stable and effective against all known races of the pathogen (Muiru et al., 2010).

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