Received: 11 February 2024 | Revised: 4 June 2024 | Accepted: 17 June 2024

DOI: 10.1111/jph.13350

Journal of Phytopathology WILEY

ORIGINAL ARTICLE

Maize insensitivity to culture filtrates from *Exserohilum turcicum* is related to quantitative resistance

Carlos Muñoz-Zavala[1,2] | Paul H. Goodwin[3] | Santos G. Leyva-Mir[2] | Alexander Loladze[1] | Mateo Vargas-Hernández[4] | Elizabeth García-León[5] | Juan M. Tovar-Pedraza[6] | Félix M. San Vicente-García[1] | Amos Alakonya[1]

[1]International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico

[2]Departamento de Parasitología Agrícola, Universidad Autónoma Chapingo, Texcoco, Mexico

[3]School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

[4]Departamento de Suelos, Universidad Autónoma Chapingo, Texcoco, Mexico

[5]Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Valle del Fuerte, Guasave, Mexico

[6]Laboratorio de Fitopatología, Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, Culiacán, Mexico

Correspondence
Amos Alakonya, International Maize and Wheat Improvement Center (CIMMYT), Texcoco 56237, Estado de México, Mexico.
Email: a.alakonya@cgiar.org

Abstract

Turcicum leaf blight (TLB) of maize is caused by *Exserohilum turcicum*. The TLB resistance is mainly associated with qualitative race-specific resistance that is linked to several *Ht* genes namely *Ht1*, *Ht2*, *Ht3*, *HtM*, *HtN*, *HtNB* and *HtP*. However, quantitative TLB resistance also occurs, but its mechanisms are poorly understood. In this study, tolerance or insensitivity to chlorosis and necrosis of *E. turcicum* culture filtrate was associated with quantitative TLB resistance. A novel detached maize seedling assay was developed for *E. turcicum* culture filtrate using methanol-treated modified Fries medium. Screening of *E. turcicum* isolates of races 2, 3, 23, 3 N, 23 N and 123 N against 61 maize inbred lines with diverse levels of resistance to TLB was conducted in the greenhouse by fungal inoculation and the detached seedling culture filtrate assay. Using an isolate of race 123 N, which can overcome all the qualitative resistance genes, a simple linear regression model ($R^2 = 0.68$, $p = .90$) for the 61 inbred lines was established between disease severity from foliar inoculations and culture filtrate symptom rating of the detached seedling assay. Two maize lines CML474 and CML483 had relatively high culture filtrate symptom ratings but very low disease severity with foliar inoculation indicating that these sources of quantitative TLB resistance may be unrelated to tolerance to *E. turcicum* culture filtrates, whose effect on seedlings could be due to the toxin known as HT-toxin or monocerin.

KEYWORDS
HT-toxin, in vitro bioassays, monocerin, northern corn leaf blight, *Setosphaeria turcica*, turcicum leaf blight

1 | INTRODUCTION

Turcicum leaf blight (TLB) or Northern corn leaf blight (NCLB) is caused by the hemibiotrophic fungus *Exserohilum turcicum* (syn. *Helminthosporium turcicum*), which affects maize (*Zea mays* L.) and sorghum (*Sorghum* spp.) worldwide (Jakhar et al., 2017). Foliar symptoms are 5–20 cm long cinnamon-brown cigar-shaped lesions, which expand until the plant is partially or completely blighted. Severe leaf infections can cause wilting, but most of the yield loss is due to reduction of photosynthetic ability (Hooda et al., 2017). Yield losses of 10–30% have been reported, but losses of up to 70% can occur when favourable disease conditions and infections continue post-pollination stage (Hooda et al., 2017). In recent years, losses caused by *E. turcicum* have increased in Mexico, mainly in Sinaloa

state, the largest maize production state in Mexico (Félix-Gastélum et al., 2018).

Crop rotation, resistant varieties and chemical fungicides are the most common management strategies; however, the pathogen can evolve into diverse physiological races and infect previously resistant maize (Hooda et al., 2017). Qualitative (race-specific/vertical) resistance to *E. turcicum* is based on race-specific *Ht* genes: *Ht1*, *Ht2*, *Ht3*, *HtM*, *HtN*, *HtNB* and *HtP* (Ahangar et al., 2022). However, *Ht2*, *Ht3* and *HtN* (=*Htn1*) have been shown to be allelic, which has important implications for resistance breeding (Yang et al., 2021). There is also quantitative (race-non-specific/horizontal) resistance to *E. turcicum* controlled by many genes with small effects (Levy & Pataky, 1992). Quantitative disease resistance typically confers visually less obvious resistance than qualitative resistance, but the protection is durable as the pathogen cannot easily overcome non-race-specific resistance genes (Lindhout, 2002). However, compared with qualitative resistance, quantitative disease resistance is controlled by many QTLs and is strongly influenced by environmental conditions, making it more challenging to identify and introgress into elite germplasm. For TLB, many farmers still apply fungicides if they observe disease symptoms in cultivars having quantitative resistance, even if resistance is sufficient to prevent significant yield losses. TLB varietal resistance can be beneficial as it can be more stable and durable against different races of the pathogen due to the buffering effect of polygenic resistance (Galiano-Carneiro & Miedaner, 2017).

Some isolates of *E. turcicum* secrete the non-specific HT-toxin, also known as monocerin, which is a dihydroisocoumarin and polyketide metabolite (Bashan et al., 1995). There is evidence suggesting that HT-toxin is an important virulence factor, and promotes appressoria formation and conidia production of *E. turcicum* (Bashan & Levy, 1992). At the cellular level, HT-toxin disrupts membranes, particularly cytoplasmic membranes, inhibits maize chlorophyll synthesis and elicits defence responses, such as expression of maize PR proteins (Kotze et al., 2019). Due to the importance of HT-toxin in *E. turcicum* virulence, several in vitro validation bioassays have been developed. The most common assay involves applying cell-free culture filtrate onto the surface of attached or detached leaves and then monitoring for chlorosis and necrosis (Bashan et al., 1995; Bashan & Levy, 1992; Cuq et al., 1993; Gu et al., 2014; Zhang et al., 2012). A detached seedling assay, however, would allow for the use of the same tissue in greenhouse assays for TLB resistance. Also, the plants can be inoculated with known races in the greenhouse, whereas mixed races may occur under natural infection in the field. The TLB resistance of seedling and adult plants has been positively correlated, in previous studies with13 maize breeding lines with physiological races 0, 1, 23 and 23N over 3 years (Adipala et al., 1993), and 133 maize breeding lines with physiological race 123N for 1 year (Yang et al., 2017).

Other aspects affecting phytotoxin bioassays are the type of media used for microbial growth and the solvent used to extract the culture filtrate. Phytotoxin production for in vitro bioassays can be greatly affected by the type of culture media. The media most often used for the growth of *E. turcicum* to produce HT-toxin is Fries medium (Gu et al., 2014; Li et al., 2016; Zhang et al., 2007, 2012), but also potato dextrose broth (Wathaneeyawech et al., 2015) and sterilized rice broth have been used (Pinheiro et al., 2016). However, no suitability comparisons of different media in in-vitro bioassays have been conducted. HT-toxin is soluble in organic solvents, such as methanol, chloroform, ether or ethyl acetate (Gu et al., 2014; Li et al., 2016; Zhang et al., 2007, 2012). The most popular HT-toxin solvent used in culture filtrate assays is methanol (Bashan et al., 1995; Bashan & Levy, 1992; Cuq et al., 1993; Gu et al., 2014; Zhang et al., 2007, 2012). Treatment with methanol can remove compounds produced in *E. turcicum* cultures that can interfere with bioassays (Zhang et al., 2007). However, some negative control-treated leaves (treated with methanol alone) occasionally showed similar leaf necrosis symptoms as those treated with methanol-treated HT-toxin (Li et al., 2016).

Many sources of quantitative TLB resistance have been identified and the resistance is highly heritable and controlled by many genes, some of which have significant effects. Still, it can also be controlled by a few genes (Levy & Pataky, 1992). No reports yet compare differences among maize genotypes with TLB resistance and their sensitivity to culture filtrate, where HT-toxin may be found. Instead, studies using in vitro HT-toxin bioassays have been done to examine differences among isolates to relate HT-toxin production with *E. turcicum* virulence (Bashan et al., 1995; Bashan & Levy, 1992; Cuq et al., 1993; Gu et al., 2014; Wathaneeyawech et al., 2015; Zhang et al., 2007, 2012). The aim of this study was to develop a novel *E. turcicum* culture filtrate bioassay using detached maize seedlings. A modified culture media, which alone did not cause similar symptoms as HT-toxin, was used to produce a methanol-treated *E. turcicum* culture filtrate that caused similar symptoms as HT-toxin. In addition, this study determined that a collection of maize inbred lines developed by the International Maize and Wheat Improvement Center (more commonly known by its Spanish acronym CIMMYT for Centro Internacional de Mejoramiento de Maíz y Trigo) showed differences in qualitative and/or quantitative TLB resistance. Finally, the levels of quantitative resistance and sensitivity to the culture filtrate, presumably containing HT-toxin, were compared to the level of resistance to determine if tolerance or insensitivity to culture filtrate was related to quantitative resistance.

2 | MATERIALS AND METHODS

2.1 | Biological materials

Inbred maize lines (Table 1) were obtained from the CIMMYT germplasm collection with grain colour, maturity, climate adaptation and reaction to TLB reported in several growing environments at CIMMYT research stations in Mexico, Colombia and Kenya (https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10246, accessed January 2024). For a susceptible control, a maize inbred line A619ht4 was obtained from the Maize Genetics Cooperation Stock Center (USDA/ARS), University of Illinois, Urbana-Champaign. A619ht4 is highly susceptible to *E. turcicum* and lacks *Ht1*, *Ht2*, *Ht3* and *HtN*

TABLE 1 Code, pedigree, grain colour, climate adaptation and TLB field resistance of 61 maize inbred lines obtained from the CIMMYT germplasm collection used in this study.

Inbred line code[a]	Pedigree information	Grain colour	Maturity	Adaptation[c]	Reaction to *E. Turcicum* in the field
CML423	G18-C19-HS100-4-1-1-B	Yellow	Early	Tropical	Susceptible
CML498	CML265/CL00303/CML264	White	Intermediate	Tropical	Resistant
CML503	CML176/CML264	White	Intermediate	Tropical	Resistant
CML576	CLFAWW11/CML494	White	Intermediate	Tropical	Resistant
CML596	CL04325/CML401	White	Intermediate	Tropical	Resistant
CML600	CLRCW88/CLRCW96	White	Intermediate	Tropical	Resistant
LPSF96	La Posta Seq C7-F96	White	Intermediate	Tropical	Susceptible
CML474	SW92145-2EV	Yellow	Intermediate	Tropical	Resistant
CML497	CL00331/V-3-B-3-2-1-B	Yellow	Intermediate	Tropical	Resistant
CML501	CL02709/V-B*3–1-1-B	Yellow	Intermediate	Tropical	Resistant
CML577	CML454/CML451	Yellow	Intermediate	Tropical	Resistant
CML597	CML285/CL00356	Yellow	Intermediate	Tropical	Resistant
CML598	CML413/CML287	Yellow	Intermediate	Tropical	Resistant
CML599	CLRCY041=P390AM	Yellow	Intermediate	Tropical	Resistant
CML154	EV8762SR-17-1-B	White	Late	Tropical	Susceptible
CML491	6207QB/6207QA	White	Late	Tropical	Resistant
CML495	PNVABCOSD/NPH	White	Late	Tropical	Resistant
CML549	CML498/CLRCW36	White	Late	Tropical	Resistant
CML554	CML491/CLQRCWQ13	White	Late	Tropical	Resistant
CML573	CML495/CML401	White	Late	Tropical	Resistant
CML601	CLRCW79/CLRCW98	White	Late	Tropical	Resistant
CML451	NPH-28-1/G25	Yellow	Late	Tropical	Resistant
CML602	CLRCY040/CML451	Yellow	Late	Tropical	Resistant
CML540	INTA-F2-192–2-1-1-1-B	White	Early	Subtropical	Resistant
CML545	CML312/CML445/Tuxp	White	Early	Subtropical	Resistant
89LMBR	89(L/LMBR)11-F2	White	Intermediate	Subtropical	Resistant
CML370	MBRETW-F2-14-3-1-3-B	White	Intermediate	Subtropical	Resistant
CML517	CML312/IR(BC3)F2-	White	Intermediate	Subtropical	Resistant
CML544	CML395/CML444/Tuxp	White	Intermediate	Subtropical	Resistant
CML547	DRB-F2-60-1-1-1-B	White	Intermediate	Subtropical	Resistant
CML585	KILIMAST94/M37W/ZM607	White	Intermediate	Subtropical	Resistant
CML326	P45-C6-F6-2-1-1-B	Yellow	Intermediate	Subtropical	Susceptible
DTMA85	200-6xGUAT189xP84c1	Yellow	Intermediate	Subtropical	Susceptible
CML202	ZSR923-B*4–5-1-B	White	Late	Subtropical	Resistant
CML384	P502-C1-771-2-2-1-3-B	White	Late	Subtropical	Resistant
CML483	P502-C2-16–2-2-1-3-B-1-B	White	Late	Subtropical	Resistant
CML484	MBRETW-C1-F139-2-1-B	White	Late	Subtropical	Resistant
CML513	CML202/IR76(BC3)F2-2-3-B	White	Late	Subtropical	Resistant
CML514	CML204/IR34(BC3)F2-1-1-B	White	Late	Subtropical	Resistant
CML519	CML384/IR(BC3)F2-B-B-B	White	Late	Subtropical	Resistant
CML543	(CML202/CML395)-B-B-2-1	White	Late	Subtropical	Resistant
CML240	P85-C3-FS50-4-5-1-1-B	White	Early	Temperate	Susceptible
CML349	P36-HTBA89-136	White	Early	Temperate	Susceptible
CML524	CML242/CML176QPM	White	Early	Temperate	Resistant

(Continues)

TABLE 1 (Continued)

Inbred line code[a]	Pedigree information	Grain colour	Maturity	Adaptation[c]	Reaction to *E. Turcicum* in the field
CML525	CML244/CML176QPM	White	Early	Temperate	Resistant
CML526	CML246/CML176QPM	White	Early	Temperate	Resistant
CML528	CML352/CML176QPM	White	Early	Temperate	Resistant
CML529	CML354/CML176QPM	White	Early	Temperate	Resistant
A619ht4[b]	[(A171 X Oh43) Oh43]	Yellow	Early	Temperate	Susceptible
CML245	P86-C0-FS77-21-2-1-2-1	Yellow	Early	Temperate	Susceptible
CML594	CHYE140=P86-C5	Yellow	Early	Temperate	Resistant
CHWTI23	B.I.Z.T.R.L.PR93	White	Intermediate	Temperate	Resistant
CHWTI59	G31*G32/CML-349	White	Intermediate	Temperate	Resistant
CML457	P87-C5-F95-24-1-1-2-1-B	White	Intermediate	Temperate	Resistant
CML459	(P940-C3-F31-1/P87-C4-F17	White	Intermediate	Temperate	Resistant
CML561	CETL08001=G9A-C7/SR	White	Intermediate	Temperate	Resistant
CML583	CEL07002=G9A-C7/SR	White	Intermediate	Temperate	Resistant
CML463	G9A-C6-RL6-1P-1P-1P-2P	White	Late	Temperate	Resistant
CML464	G9A-C6-HS3-1-3-1-1-2P	White	Late	Temperate	Resistant
CML558	CETL08003=G9A-C7/SR	White	Late	Temperate	Resistant
CML595	CHYL10=P88-C3	Yellow	Late	Temperate	Resistant

[a]Inbred lines previously characterized for grain colour, maturity, adaptation and reaction to *E. turcicum* in the field by CIMMYT (https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10246 last consultation in January 2024).

[b]All lines obtained from the CIMMYT germplasm collection, except A619ht4, which was obtained from the Maize Genetics Cooperation Stock Center (USDA/ARS), University of Illinois, Urbana-Champaign.

[c]Temperate adaptation refers to mid-latitude subtropical and highland tropical environments, not to high-latitude and low-latitude temperate environments.

(Carson, 1995). As a resistant control, the inbred line CML495 was obtained from the CIMMYT germplasm collection, which has been reported to be highly resistant to *E. turcicum* (Ding et al., 2015).

Isolates of *E. turcicum*, JAL19-05, GUA19-05, BA19-01, TAM19-05, AF19-03 and SIN19-04 used in this study were collected in 2019 from infected leaves showing typical cinnamon-brown cigar-shaped lesions on plants in temperate, subtropical and tropical regions of Mexico (Muñoz-Zavala et al., 2023). These isolates were previously characterized as races 2, 3, 23, 3N, 23N and 123N with virulence on the differential lines A619Ht2 (*Ht2*), A619Ht3 (*Ht3*), A619Ht2/A619Ht3 (*Ht2/Ht3*), A619Ht3/B68HtN (*Ht3/HtN*), A619Ht2/A619Ht3/B68HtN (*Ht2/Ht3/HtN*) and A619Ht1/A619Ht2/A619Ht3/B68HtN (*Ht1/Ht2/Ht3/HtN*), respectively (Table 2).

2.2 | Media compositions

The culture broths tested were: Potato dextrose (Becton Dickinson), Czapek and Czapek-Dox (Sigma-Aldrich), Richards (HiMedia Laboratories), SNA (JT Baker) and Leung (glucose/yeast extract/peptone medium) (Leung et al., 2006). In addition, maize leaf broth composed of 10g of 3-week-old greenhouse seedling leaves of A619ht4, dissected into 1cm pieces and autoclaved for 20min in 1L dH_2O was also tested. Maize spike broth contained 10g of field-collected spikes of TLB-susceptible breeding line A619ht4 at the reproductive stage R1, dissected into 1cm pieces and autoclaved for 20min in 1L dH_2O. Modified Fries medium was composed of 10g sucrose, 1g dibasic ammonium tartrate $(NH_4)_2C_4H_4O_6$, 1g ammonium nitrate (NH_4NO_3), 1g monobasic potassium phosphate (KH_2P0_4), 0.5g magnesium sulphate ($MgS0_47H_20$), 0.1g sodium chloride (NaCl), 0.1g calcium chloride dihydrate ($CaCl_2$ $2H_2O$) and 0.5g yeast extract (Difco) per L dH_2O.

2.3 | Culture filtrate preparation

Isolate SIN19-04 (race 123N) of *E. turcicum* was grown on lactose-casein agar at 25°C with 12h light/12h dark cycles to produce inoculum for HT-toxin production (Tuite, 1969). At 10days, three agar discs (5mm in diameter) were transferred to 500mL flasks containing 300mL of modified Fries medium. After 21days at 25°C in the dark without shaking, the 300mL *E. turcicum*-inoculated broths were first filtered through four layers of cotton gauze to remove mycelium and then passed through Whatman No. 1 paper with a vacuum pump to remove conidia. The crude filtrate was frozen at −80°C for 24h and then freeze-dried in a freeze dryer (Labconco Freezone Triad Freeze Dry Systems) for 72h until it reached 10% of the starting volume. To create methanol-treated culture filtrate, 20mL of 100% methanol was added, mixed gently for 10min and incubated overnight at 4°C. The solution was then centrifuged at 2000rpm for 10min and the

TABLE 2 Code, race, location and climate adaptation of six isolates of *Exserohilum turcicum* collected in 2019 and used in this study.

Isolate code[a]	Race	Virulence on the differential lines	Location in Mexico	Adaptation[b]
JAL19-05	2	A619*Ht2*	Jalisco	Subtropical
GUA19-05	3	A619*Ht3*	Guanajuato	Subtropical
BA19-01	23	A619*Ht2*/A619*Ht3*	Estado de México	Temperate
TAM19-05	3N	A619*Ht3*/B68*HtN*	Tamaulipas	Tropical
AF19-03	23N	A619*Ht2*/A619*Ht3*/B68*HtN*	Veracruz	Tropical
SIN19-04	123N	A619*Ht1*/A619*Ht2*/A619*Ht3*/B68*HtN*	Sinaloa	Tropical

[a]Isolates collected in 2019 from symptomatic leaves by Muñoz-Zavala et al. (2023) and stored in 30% sterile glycerol at −80°C in the CIMMYT fungal culture collection.

[b]Temperate adaptation refers to mid-latitude subtropical and highland tropical environments, not to high-latitude and low-latitude temperate environments.

supernatant was removed. This was repeated, and then the supernatant was incubated at 45°C until the methanol evaporated. The final volume was adjusted to 50 mL with sterile distilled water (sdH_2O), vortexed for 10 s and then added to the tube containing the maize seedling prepared as described below. SdH_2O was included as an additional control. The tests were performed on V4 stage detached maize seedling of 61 lines, including susceptible and qualitative resistance control lines.

2.4 | Detached seedling assay

Maize seedlings were grown for 3 weeks in potting soil in the greenhouse at 25°C with 16 h sunlight until they reached vegetative stage V4. The seedlings were excised 1 cm above the crown, incubated in 70% ethanol for 30 s, and then transferred to sdH_2O, where a second cut was made under the sdH_2O, removing 1 cm from the excised stem base. The seedlings were then transferred to 15 mL borosilicate glass tubes (Corning Pyrex #9820-16XX, Corning) filled with either sdH_2O, non-inoculated media, crude *E. turcicum*-inoculated culture filtrate or methanol-treated *E. turcicum*-inoculated culture filtrate. The tubes with detached seedlings were incubated in a controlled environment room at 25°C under continuous light for 96 h. Liquid uptake and seedling culture filtrate symptom severity were assessed every 24 h. The culture filtrate symptom severity scale was 0 = no symptoms, 1 = 10–30% chlorosis with no necrosis, 2 = 31–50% chlorosis with no necrosis, 3 = >51% chlorosis with 10–30% leaf tip necrosis, 4 = 31–50% necrosis and 5 = >51% necrosis. The experiment was repeated three times with nine tubes each time and each tube containing one seedling with 15 mL of solution incubated under continuous light at 25°C.

2.5 | Seedling disease assay

The inoculation procedure was performed as described above (Muñoz-Zavala et al., 2023). Three maize seeds were sown in 5 × 5 cm plastic pots and allowed to grow for 14 days or until they attained vegetative stage V3. Three days before inoculation, the plants were thinned to one plant per pot. Ultrasol 18-18-18 (N-P-K) (SQM) was added to the potting soil at planting and 14 days post-planting. Isolates JAL19-05 (race 2), GUA19-05 (race 3), BA19-01 (race 23), TAM19-05 (race 3N), AF19-03 (race 23N) and SIN19-04 (race 123N) of *E. turcicum* were grown on lactose-casein agar at 25°C with 12 h light/12 h dark for 10 days, and then the plates were flooded with 15 mL sdH_2O plus 10 μL L^{-1} Tween 20 to collect the conidia. The conidial concentration was adjusted using a Neubauer chamber to 2×10^4 conidia mL^{-1}. A conidial suspension (200 μL) was placed on the leaf whorls of 14-days-old maize seedlings using a micropipette, and the inoculated plants were incubated in a humidity chamber for 24 h. Day temperature was maintained at 24–25°C and night temperature was kept at 16–18°C with shading during the day to maintain 100–200 W m^{-2}. The plants were then grown in the greenhouse at 27°C during the day and 18°C at night at 600–800 W m^{-2} and watered every 7 days. TLB disease and pathogen disease severity ratings were performed at 14 days post inoculation (28 days post planting) on the inoculated leaf using a modified version of the visual scale of 0–5 where 0 = no symptoms, 1 = 0.1–0.2 cm^2 chlorotic area without necrosis, 2 = 0.2–5 cm^2 chlorotic area without necrosis, 3 = >5 cm^2 chlorotic area with <5 cm^2 necrosis but no sporulation, 4 = >5 cm^2 necrosis with sporulation, 5 = necrosis of entire leaf with sporulation (Muiru et al., 2011). The experiment was repeated three times with 12 pots each time and one seedling per pot.

2.6 | Data analysis

The statistical model considered nine biological replicates of 11 non-inoculated broths and 12 replications of the inoculated culture filtrates, including the methanol-treated culture filtrate, as random effects, while the water and modified Fries control were considered as fixed effects. The qualitative resistance to *E. turcicum* in the 61 lines was determined based on three repetitions with 12 pots/plants of each line. Data were subjected to analyses of variance (ANOVA) and a comparison of means with Tukey's

honestly significant difference method (HSD) was performed at a significance level of 5% using PROC GLM in SAS (version 9.3; SAS Institute, Cary). Regression analysis was also performed using PROC GLM in SAS.

3 | RESULTS

3.1 | Non-inoculated media seedling assay

A culture filtrate assay was tested using detached V4 seedlings with surface sterilized stems of a susceptible genotype, A619ht4, that were recut under water and then placed in a solution. Cut seedlings placed in non-inoculated standard Fries medium with 30 g L^{-1} sucrose (Zhang et al., 2007) had significantly less solution uptake compared to water (data not shown). However, the uptake of non-inoculated modified Fries medium with 10 g L^{-1} sucrose was not significantly different ($p > .05$) from water for solution uptake by seedlings at 24 h along with Czapek-Dox and Richards media among the 11 media tested (Figure 1a). At 48 h, solution uptake by non-inoculated modified Fries medium was significantly greater than water, and only solution uptake by non-inoculated Richards medium was not significantly different from water. At 72 h, solution uptake by non-inoculated modified Fries, Richards and SNA media were all not significantly different from water, but at 96 h, only non-inoculated modified Fries medium showed solution uptake that was not significantly different from water.

At 24 h culture filtrate symptom ratings of detached seedlings were not significantly different between water and non-inoculated modified Fries, Czapek, Czapek-Dox, Richards, SNA and Leung media (Figure 1b). At 48 h, only the chlorosis and necrosis with non-inoculated Richards and SNA media were not significantly different from water. Except for the modified Fries medium at 96 h, the symptoms due to all non-inoculated media were significantly greater than those of water at 72 and 96 h. The chlorosis and necrosis due to the culture filtrate appeared similar to that reported for HT-toxin. A comparison between solution uptake and culture filtrate symptoms of seedlings at 96 h was made using a simple linear regression model ($R^2 = 0.855$, $p = .03$; Figure 2). Except for water non-inoculated modified Fries medium had the highest solution uptake and lowest levels of chlorosis and necrosis to seedlings. Modified Fries medium was thus used to produce *E. turcicum* culture filtrate in the rest of the study.

3.2 | Crude and methanol-treated culture filtrate seedling assay

The solution uptake of inoculated crude culture filtrate, inoculated methanol-treated culture filtrate, non-inoculated medium and water was measured at 24–96 h showing that it was very similar for detached seedlings of the susceptible and resistant maize lines A619ht4 and CML495 at all time points (Figure 3). The least solution uptake was observed with inoculated crude culture filtrate, which was always significantly lower than that of inoculated methanol-treated culture filtrate. Solution uptake with inoculated methanol-treated culture filtrate was not significantly different between non-inoculated medium and/or water at 48 and 72 h post submergence of detached seedlings for both maize lines.

Chlorosis and necrosis of detached seedlings of the susceptible maize line A619ht4 were significantly higher with the inoculated crude culture filtrate compared to inoculated methanol-treated culture filtrate, except at 96 h when they were not significantly different (Figure 4a). However, chlorosis and necrosis with non-inoculated medium and water were always significantly lower than with inoculated crude or methanol-treated media. At 72 h, the symptom ratings with inoculated crude and methanol-treated culture filtrates were 5.0 and 4.0, respectively, whereas at 96 h, they were 5.0 and 4.8, respectively. For the resistant maize line CML495, the symptom rating with inoculated crude culture filtrate was also significantly higher than methanol-treated culture filtrates at all time points. Both were significantly higher than non-inoculated medium or water (Figure 4b). At 72 h, the symptom ratings with inoculated crude and methanol-treated culture filtrate were 4.0 and 1.5, respectively, and were 5.0 and 2.0, respectively, at 96 h. A key difference between the two types of inoculated culture filtrates was that with inoculated crude culture filtrate, the symptom ratings of A619ht4 and CML495 were not significantly different from each other at 72 and 96 h, whereas with inoculated methanol-treated culture filtrate the symptom rating of A619ht4 was considerably higher than that of CML495 at same time points (Figure 5).

3.3 | Qualitative resistance to *E. turcicum*

Inoculation of the 61 inbred lines including susceptible and qualitative resistance control lines with isolates of races 2, 3, 23, 3N, 23N and 123N showed that only lines CML474, CML483, CML495, CML543, CML583, CML540, CML547, CML491, CML549 and CML517 showed that there was strong qualitative resistance (Table 3). This included the 10 lines with qualitative resistance that contained a combination of *Ht1*, *Ht2*, *Ht3* and *HtN*. *Some of these resistance genes have been cloned recently, and it has been suggested that Ht2 and Ht3 are identical* (Yang et al., 2021), *which is consistent with this study where race 2 and race 3 showed identical reactions in 24 lines and similar reactions in 37 lines*.

3.4 | Disease ratings and comparison to culture filtrate symptom ratings

Seedlings of the inbred lines had culture filtrate symptom ratings ranging from 2.3 to 5.0 with *E. turcicum* isolate SIN19-04 (race 123N). The disease ratings observed with the same isolate ranged from 1.5 to 5.0. It has to be noted that the race 123N is virulent for all race-specific single genes used in the current study. Therefore,

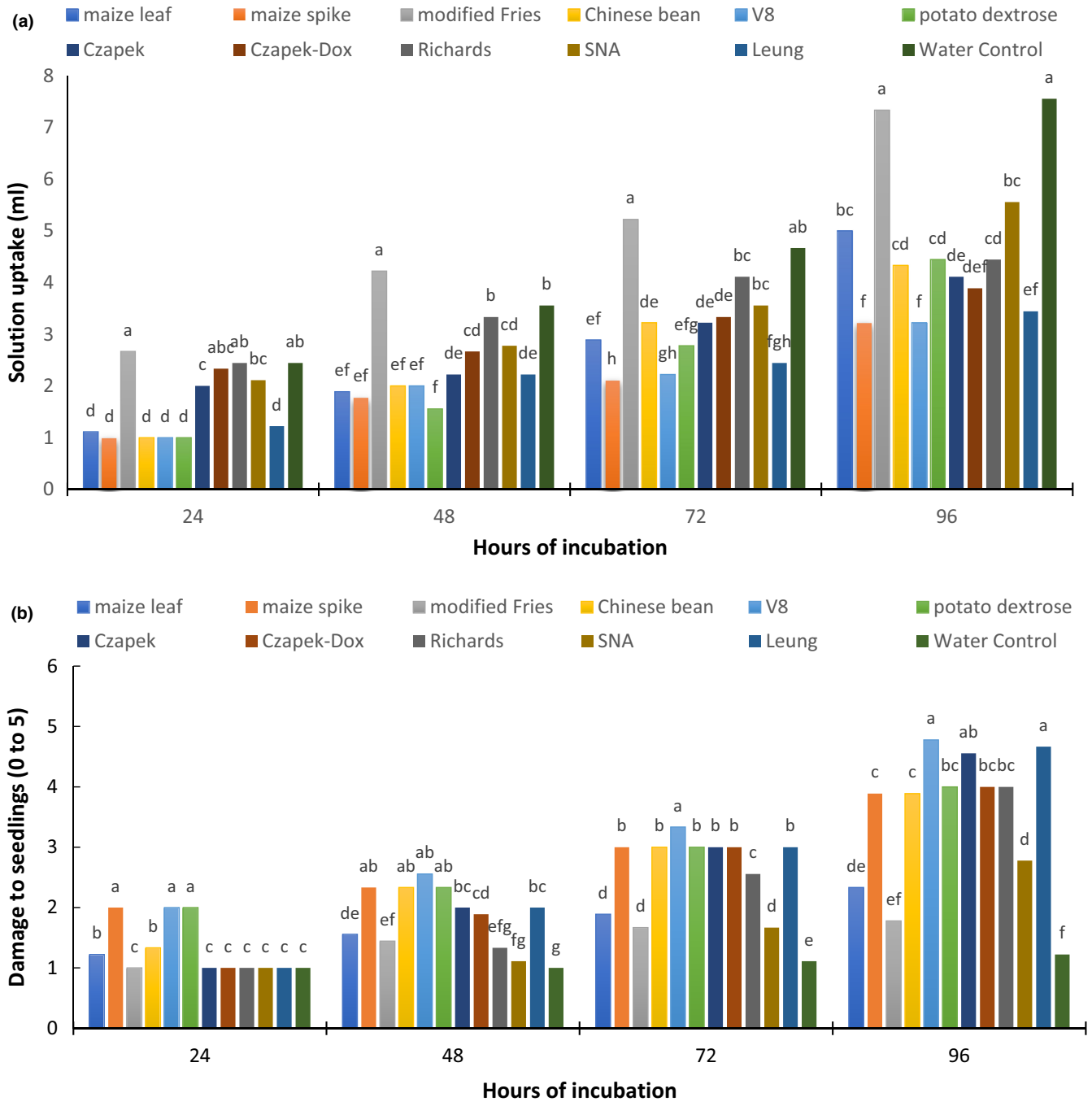


FIGURE 1 Average solution uptake and damage at V4 growth stage of TLB susceptible maize line A619ht4 seedlings assayed with 11 non-inoculated media broths or water. (a) Solution uptake by detached seedlings; (b) Seedling injury rating on a scale of 0–5. Means are from three independent experiments with nine biological replications per treatment containing one seedling per tube with 15 mL of liquid incubated under continuous light at 25°C. Means with the same letter for each time point are not statistically different based on Tukey's test (LSD, $p = .05$).

all the resistant reactions observed in the plants were likely contributed by the quantitative non-race specific resistance (Table 4). The line A619ht4, which was used as the susceptible control line as well as the line in the development of the culture filtrate assay, was the only one to have the highest rating of 5.0 for both disease severity and culture filtrate symptoms. Compared to the resistant control line, CML495, only CML474 (intermediate, tropical) showed significantly lower disease ratings, while no inbred lines showed significantly lower culture filtrate symptom ratings.

A comparison of the culture filtrate symptom rating and disease rating of each line showed that a simple linear regression model could be produced ($R^2 = 0.68$, $p = .90$; Figure 6). Two maize lines that were notable outliers were CML474 and CML483, which showed relatively high sensitivity to the culture filtrate but had the

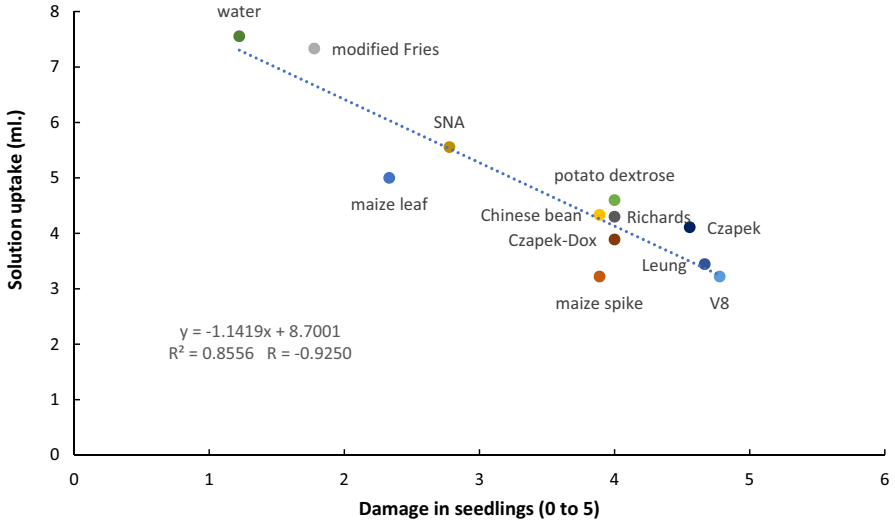


FIGURE 2 Correlation between solution uptake and seedling damage at V4 stage seedlings of TLB susceptible maize line A619ht4 seedlings assayed with 11 non-inoculated broths or water. Scatter plot is shown with each type of non-inoculated broth indicated and the correlation shown using a simple linear regression model ($R^2 = 0.86$, $p = .03$) between solution uptake and seedling injury.

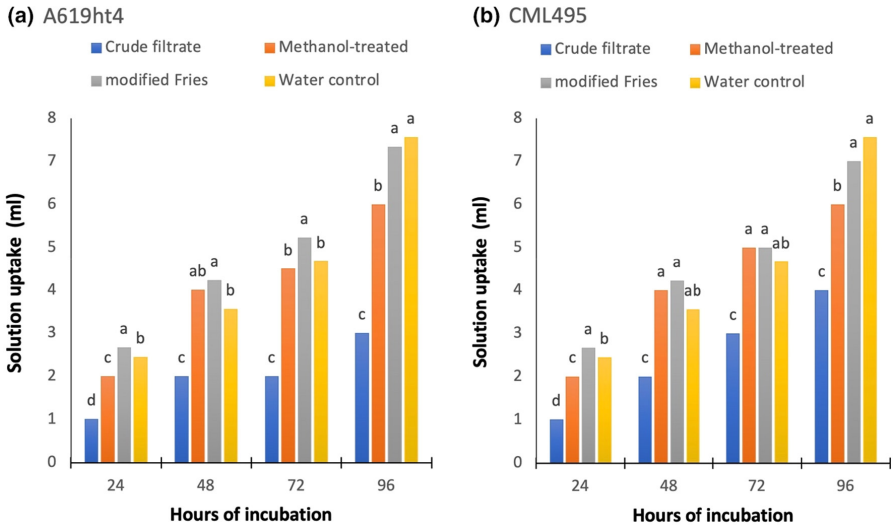


FIGURE 3 Uptake of the culture filtrate using the detached maize seedling assay. Detached seedlings of TLB susceptible maize line A619ht4 (a) and qualitative TLB resistant maize line CML495 (b) were incubated with either sdH_2O, non-inoculated modified Fries medium, crude *E. turcicum*-inoculated modified Fries culture filtrate or methanol-treated *E. turcicum*-inoculated modified Fries culture filtrate for 24, 48, 72 and 96 h. Means are from three independent experiments with nine replications per experiment containing one seedling per tube with 15 mL of liquid incubated under continuous light at 25°C. Means with the same letter for each time point are not statistically different based on Tukey's test (LSD, $p = .05$).

highest resistance to the pathogen. Another notable feature was that lines developed for temperate environment tended to be more susceptible to the pathogen (the disease rating of 15 out of 23 lines ranged from 4.3 to 5.0), whereas most lines developed for tropical environment were intermediate susceptible to the pathogen (with the disease ratings of 12 out of 17 lines ranging from 3.0 to 3.8). The range of disease reaction scores varied widely between the subtropical lines. Lines with different maturities also ranged widely in their sensitivity to the culture filtrate and pathogen. While 9 of the 10 lines with the lowest disease rating had white grain colour, both lines with white or yellow grain colour were widely distributed in their sensitivity to the culture filtrate and susceptibility to the pathogen.

4 | DISCUSSION

Among the lines tested in this study, the lines from temperate environment tended to have quantitative resistance that was less effective than those from the tropical environment, whereas subtropical lines tended to have more effective quantitative resistance against race 123N. It has been previously reported that tropical *E. turcicum* isolates from South America had greater aggressiveness compared to isolates from temperate European environments (Navarro et al., 2021). Thus, one possibility is that selection for quantitative TLB resistance in temperate regions may have been less successful in breeding programs because of lower disease pressure than in tropical regions. On the other hand, there was no relationship

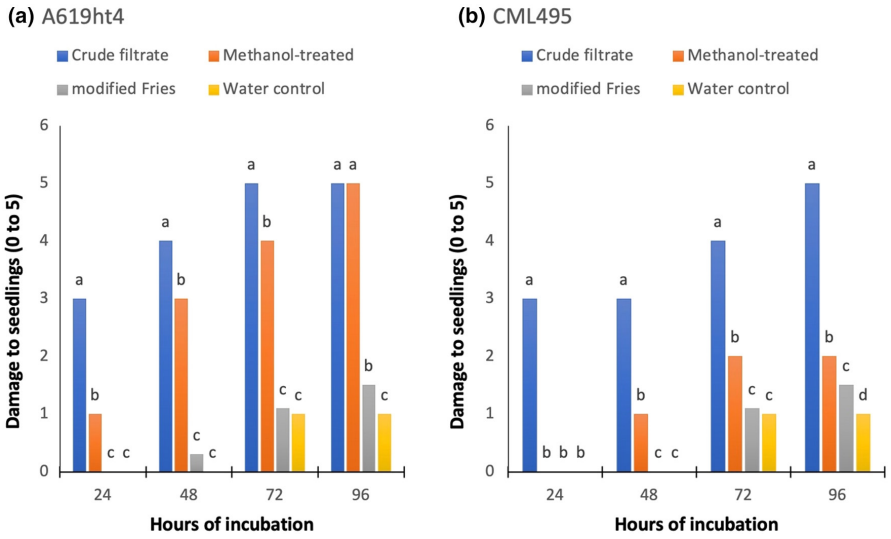
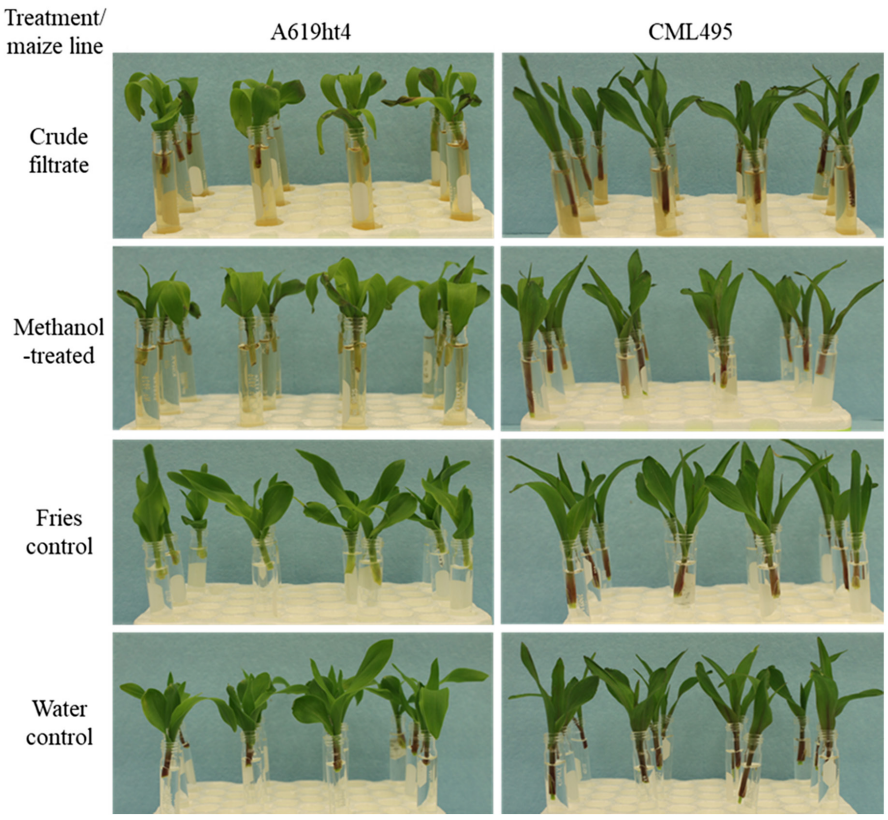


FIGURE 4 Severity rating of damage of detached seedlings using the culture filtrate assay. Detached seedlings of TLB susceptible maize line A619ht4 (a) and qualitative TLB resistant maize line CML495 (b) were incubated with either sdH_2O, non-inoculated modified Fries medium, crude *E. turcicum*-inoculated modified Fries culture filtrate or methanol-treated *E. turcicum*-inoculated modified Fries culture filtrate for 24, 48, 72 and 96 h. Means are from three experiments with nine replications per experiment containing one seedling per tube with 15 mL of liquid incubated under continuous light at 25°C. Means with the same letter for each time point are not statistically different based on Tukey's test (LSD, $p = .05$).

FIGURE 5 Damage of detached seedlings of TLB susceptible maize line A619ht4 (A) and qualitative TLB resistant maize line CML495 following incubation with either sdH_2O, non-inoculated modified Fries medium, crude *E. turcicum*-inoculated modified Fries culture filtrate or methanol-treated *E. turcicum*-inoculated modified Fries culture filtrate at 24 h of incubation. Treatments with crude culture filtrate, methanol-treated culture filtrate, non-inoculated Modified Fries medium and water control had damage ratings of 4, 3, 1 and 1, respectively, for A619ht4, and damage ratings of 3, 1, 1 and 1, respectively, for CML495.



between quantitative TLB resistance and maize maturity. Therefore, deciphering a relationship between early or late maturity and TLB resistance may depend on the parental materials and the mechanism mostly responsible for quantitative resistance.

In this study, no relationship was identified between quantitative TLB resistance and grain colour, although most of the lines with the highest quantitative resistance had white grain colour. This likely is an artefact of the CIMMYT breeding program. White maize is preferred for human consumption in southern and eastern Africa, Central America and Mexico, which are regions where CIMMYT emphasizes its efforts in maize breeding for food security (Xia et al., 2005). Therefore, evaluation of lines with white grain is

TABLE 3 Inbred lines identified with qualitative resistance to *Exserohilum turcicum* by inoculation with isolates of races 2, 3, 23, 3n, 23n and 123n.

	Reaction to *E. turcicum*[a]						
Inbred line code	Race 2	Race 3	Race 23	Race 3n	Race 23n	Race 123n	*Ht* genes
CML474	1	1	2	2	2	2	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML483	1	1	2	2	2	2	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML495	2	2	2	2	3	3	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML543	3	2	3	2	3	2	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML583	3	2	3	2	3	2	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML540	2	3	3	3	2	3	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML547	3	3	3	2	3	2	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML491	2	2	3	3	3	3	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML549	2	3	2	3	3	3	*Ht1*, *Ht2*, *Ht3* and *HtN*
CML517	3	3	3	3	3	3	*Ht1*, *Ht2*, *Ht3* and *HtN*
A619ht4[b]	5	5	5	5	5	5	No *Ht* genes

[a]Mean disease rating scale: 0 = no symptoms, 1 = 0.1–0.2 cm^2 chlorotic area without necrosis, 2 = 0.2–5 cm^2 chlorotic area without necrosis, 3 = >5 cm^2 chlorotic area with <5 cm^2 necrosis but no sporulation, 4 = >5 cm^2 necrosis with sporulation, 5 = necrosis of entire leaf with sporulation.
[b]Line A619ht4 was included as a susceptible control with no known *Ht* genes.

preferred, and only 17 lines, including the susceptible control, out of all the lines tested had yellow grain in this study. While grain colour has never been linked to TLB susceptibility, future work could examine if there is any linkage between high quantitative resistance to TLB, low sensitivity to culture filtrate and grain colour.

Phytotoxins can have an important role in disease development, and they can be used as markers for early screening of resistant genotypes as well as for in vitro selection (Buitatti & Ingram, 1991). Genetic correlations have been found between disease resistance and tolerance to host-selective toxins of 13 pathogens and to tolerance to non-host selective toxins of 12 pathogens (Buitatti & Ingram, 1991). A review of 30 plant species using in vitro selection for tolerance to culture filtrates, toxins and elicitors from approx. 40 plant pathogens showed that such selection resulted in increased disease resistance (Švábová & Lebeda, 2005). The mechanisms of increased phytotoxin resistance following in vitro selection are believed to be primarily a result of enzymatic detoxification or stimulation of plasma-membrane-localized H+-ATPase activity to avoid toxin uptake (Švábová & Lebeda, 2005).

The 61 lines, including the susceptible and qualitative resistance control lines showed a relatively high correlation between sensitivity to the culture filtrate and resistance to fungal inoculation. While this indicates that culture filtrate tolerance is related to quantitative TLB resistance, this is still only a correlation and does not prove causation. Future research would need to establish a cause-and-effect relationship, such as making crosses to determine the inheritance of this trait as well as the possible number of genes involved and identifying associated QTL (Swaminathan et al., 2016). However, the correlation between quantitative TLB resistance and tolerance to the culture filtrate was not universal as lines CML474 and CML483 in this study had relatively high sensitivity to the culture filtrate even though they had very low disease severity. This indicates that quantitative resistance to TLB could be due to several factors with tolerance to HT-toxin being one of them. Tolerance to toxins is only one of a number of different mechanisms that have been associated with quantitative plant disease resistance and other mechanisms should be investigated for CML474 and CML483.

Greenhouse tests for TLB quantitative resistance using seedlings has the advantage of controlled experimental conditions to ensure that they are consistently conducive to TLB (Mushtaq & Gul, 2015). In addition, phenotyping for quantitative TLB resistance can be more reliably done in the greenhouse using smaller plants and examining only inoculated tissues, which has been positively correlated with adult plant TLB resistance (Adipala et al., 1993; Yang et al., 2017). Another advantage of greenhouse tests is the avoidance of the confounding effects of other foliar diseases that may be present in the field and interfere with the TLB scoring. Also, *E. turcicum* is so widespread in maize-growing regions of Mexico to the extent that field evaluation for TLB resistance at CIMMYT is reliably conducted under natural infections as opposed to artificial inoculations with known strains. However, it is preferable to screen for quantitative TLB resistance with known races that carry virulence against the maximum number of resistance genes in order to ensure that that no known major and race-specific *Ht* genes will interfere with disease evaluation outcomes.

Due to the advantages of using inoculated seedlings in the greenhouse to study TLB resistance, a novel seedling assay was also developed for the culture filtrate so that the same type of plant material could be used for both culture filtrate and disease ratings. Compared to the more traditional technique of applying a droplet of purified HT-toxin or *E. turcicum* culture filtrate on a wounded leaf, such as 10 µL droplets placed on punctured leaf pieces by Cuq et al. (1993), the seedling assay in this study allowed for a much larger amount of culture filtrate to be taken up by the plant (approx. 7 mL) and more

TABLE 4 Disease ratings and culture filtrate injury ratings for the 61 CIMMYT inbred lines with *Exserohilum turcicum* isolate SIN19-04 (race 123N).

Inbred line code	Disease rating (0–5)[a]	Culture filtrate rating (0–5)[b]
A619ht4[c]	5.0 a	5.0 a
CML326	5.0 a	4.9 ab
LPSF96	5.0 a	4.5 abcde
CML154	4.9 ab	4.9 ab
CML423	4.9 ab	4.8 abc
CML526	4.8 abc	4.8 abc
CHWTI59	4.8 abc	4.2 abcdefgh
CML513	4.7 abcd	4.5 abcde
CML245	4.6 abcde	4.6 abcd
CML558	4.6 abcde	4.0 cdefghi
CML240	4.5 abcdef	4.5 abcde
CHWTI23	4.5 abcdef	4.3 abcdefg
CML525	4.5 abcdef	4.0 cdefghi
CML349	4.4 abcdefg	4.8 abc
DTMA85	4.4 abcdefg	4.6 abcd
CML595	4.4 abcdefg	4.4 abcdef
CML528	4.4 abcdefg	4.1 bcdefghi
CML459	4.4 abcdefg	4.0 cdefghi
CML594	4.3 abcdefgh	4.3 abcdefg
CML463	4.3 abcdefgh	4.1 bcdefghi
CML524	4.3 abcdefgh	4.1 bcdefghi
CML601	4.3 abcdefgh	3.8 defghi
CML503	4.3 abcdefgh	3.4 hijkl
CML561	4.2 abcdefghi	4.4 abcdef
CML585	4.2 abcdefghi	4.2 abcdefgh
CML519	4.2 abcdefghi	4.1 bcdefghi
CML457	4.2 abcdefghi	4.1 bcdefghi
CML484	4.2 abcdefghi	4.0 cdefghi
CML545	4.1 bcdefghij	4.3 abcdefg
CML514	4.1 bcdefghij	4.1 bcdefghi
CML384	4.1 bcdefghij	4.0 cdefghi
CML370	4.0 cdefghijk	4.1 bcdefghi
CML202	4.0 cdefghijk	4.0 cdefghi
CML602	3.9 defghijk	4.0 cdefghi
CML600	3.9 defghijk	3.9 defghi
CML599	3.8 efghijkl	3.9 defghi
CML498	3.8 efghijkl	3.8 defghi
CML596	3.8 efghijkl	3.8 defghi
CML597	3.7 fghijkl	4.0 cdefghi
CML554	3.7 fghijkl	3.9 defghi
CML501	3.7 fghijkl	3.8 defghi
CML576	3.7 fghijkl	3.8 defghi
CML573	3.7 fghijkl	3.7 efghi
CML451	3.7 fghijkl	3.6 fghij
CML577	3.7 fghijkl	3.4 hijkl
CML497	3.6 ghijklm	3.6 fghij
CML529	3.5 hijklm	3.5 ghijk
CML464	3.5 hijklm	3.3 ijklm
CML544	3.4 ijklmn	3.3 ijklm
89LMBR	3.3 jklmno	4.0 cdefghi
CML598	3.2 klmnop	3.4 hijkl
CML517	3.0 lmnopq	3.5 ghijk
CML491	2.8 mnopqr	2.8 jklmn
CML547	2.6 nopqr	2.6 lmn
CML549	2.6 nopqr	2.5 mn
CML495[d]	2.5 opqr	2.7 klmn
CML540	2.5 opqr	2.5 mn
CML583	2.4 pqr	2.4 n
CML543	2.3 qr	2.3 n
CML483	1.8 rs	3.5 ghijk
CML474	1.5 s	3.8 defghi

[a]Mean disease rating scale: 0 = no symptoms, 1 = 0.1–0.2 cm^2 chlorotic area without necrosis, 2 = 0.2–5 cm^2 chlorotic area without necrosis, 3 = >5 cm^2 chlorotic area with <5 cm^2 necrosis but no sporulation, 4 = >5 cm^2 necrosis with sporulation, 5 = necrosis of entire leaf with sporulation.

[b]Mean injury rating scale: 0 = no symptoms, 1 = 10–30% chlorosis with no necrosis, 2 = 31–50% chlorosis with no necrosis, 3 = >51% chlorosis with 10–30% leaf tip necrosis, 4 = 31–50% necrosis and 5 = >51% necrosis.

[c]Line A619ht4 (lacking *Ht* genes) was included as a susceptible control.

[d]Line CML495 was included as a resistant control.

tissue (entire seedlings) to be examined compared to only the size of a puncture hole of a detached leaf. Further, this technique allows the natural plant resistance mechanisms to play a role in the evaluation results.

There are many potential mechanisms of quantitative resistance, such as components downstream of pathogen perception, including transcription factors, kinases, signalling peptides, salicylic acid/ethylene/jasmonic acid defence signalling pathways, as well as further downstream factors, including vesicle-associated movement of defence compounds, enzymes for secondary antimicrobial metabolites, and antimicrobial compounds, such as defensins and pathogenesis-related proteins (Corwin & Kliebenstein, 2017). Also, there are factors that directly suppress pathogens like chitinases and β-1-3-glucanases, inactivate virulence factors like phenols, flavonoids, terpenes, fatty acids and alkaloids, and factors that reinforce plant cell walls like hydroxyproline-rich glycoproteins and lignin monomers (Kushalappa et al., 2016). In addition, there are antimicrobial compounds like peroxidases, cysteine proteases and catalases as well as morphological mechanisms of quantitative resistance, such as making it more difficult for pathogens

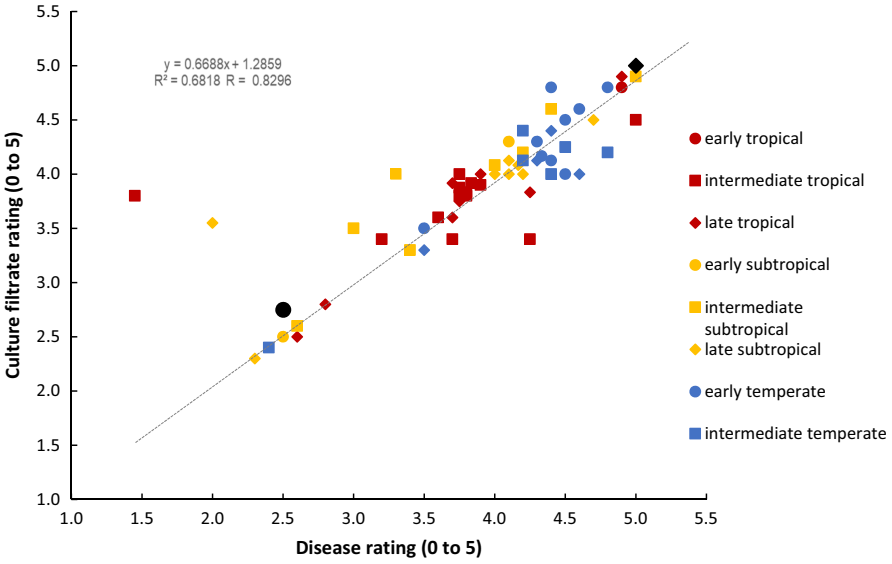


FIGURE 6 Relationship between disease rating in the seedling disease assay and culture filtrate rating in the culture filtrate assay and methanol treatment at 96 h with the detached seedling assay for the 61 inbred lines included susceptible A619ht4 (black diamond) and resistant CML495 (black circle) control lines. Scatter plot is shown with the maturity and environment of each line indicated, and a correlation is shown using a simple linear regression model ($R^2 = 0.68$, $p = .90$). Means of culture filtrate ratings are from three experiments with 12 replications per experiment containing one seedling per tube.

to penetrate stomata with excessive cuticular wax cover (Niks et al., 2015).

Several genes have now been cloned from maize related to quantitative resistance. These include the genes *ZmWAK-RLK1* and *ZmWAK* for a cell wall-associated receptor kinase, *Rcg1*, *RppC* and *RppK* for NLR proteins, *ZmMM1* for a transcription factor, *ZmFBL41* for a component of the ubiquitin–proteosome system, *qMdr9.02* for lignin production, *qRfg2* for auxin regulation, *qRfg1* for epigenetic regulation, *qMrdd1* for vesicle trafficking and *Scmv1* and *Scmv2* for molecular chaperones (Gou et al., 2023). The innate immune system of plants is complex requiring many elements (Miller et al., 2017), and thus it is not surprising that quantitative resistance will involve a wide variety of factors that could be constitutive as well as induced following pathogen infection.

Breeding for quantitative resistance to plant diseases is difficult because of the need for intensive phenotyping across locations, large populations segregating for resistance and multiple evaluation years (Galiano-Carneiro & Miedaner, 2017). Therefore, improving selection methods for quantitative resistance is needed. Augmenting natural infection in the field using known isolates of races that carry virulence against the maximum resistance genes is cost-effective for breeding programs when carried out in favourable environments for disease development. Natural infection does not guarantee disease pressure is always sufficient to select within segregating breeding populations. Screening multiple segregating breeding populations under greenhouse conditions would require large amounts of space and culture filtrate. A seedling assay, either through direct inoculation or culture filtrate bioassays, can be a more cost-effective way to address this.

This study showed that tolerance or insensitivity to culture filtrate can be a good marker for TLB resistance in existing inbred breeding lines. One limitation of this study is that only culture filtrate was used rather than purified HT-toxin ([2S,3aR,9bR]-6-hydroxy-7,8-dimethoxy-2-propyl-2,3,3a,9b-tetrahydro-5H-furo[3,2-c] isochromen-5-one). Although the extraction process for the HT-toxin followed previous studies (Bashan et al., 1995; Bashan & Levy, 1992; Cuq et al., 1993; Gu et al., 2014; Li et al., 2016; Wathaneeyawech et al., 2015; Zhang et al., 2007, 2012), the concentration of HT-toxin in the culture filtrate was not determined. Thus, the toxin symptoms observed could have been partially due to other compounds in the culture filtrate. Despite this limitation, this study showed a strong correlation (but not a cause-and-effect relationship) between symptom ratings of seedlings with the fungal culture filtrate and quantitative TLB resistance, indicating that the response to compounds in the filtrate may be related to the level of resistance. However, future studies would need to utilize the sources of resistance (CML474, CML483, CML495, CML543, CML583, CML540, CML547, CML491, CML549 and CML517) identified in this study in a breeding program to assess whether selecting for tolerance to the culture filtrate would result in greater quantitative TLB resistance in the field. All the maize lines used in this study are freely available from CIMMYT (https://www.cimmyt.org/resources/seed-request/) for breeding programs and genetic studies. Since other maize foliar pathogens produce phytotoxins, the relationship between quantitative resistance and tolerance to their toxins is a topic that requires further investigations to produce more durably resistant high-yielding maize.

AUTHOR CONTRIBUTIONS

CMZ, PHG, SGLM, AL and MVE conceived the work. CMZ, SGLM and AL carried out the lab work. MVH and EGL performed the statistical analyses. CMZ, PHG, EGL, JMTP, AA and FMSVG wrote the initial version of the manuscript. All authors reviewed the manuscript and agreed with the publication of the paper.

ACKNOWLEDGEMENTS

We thank the germplasm bank, the global maize program of the International Maize and Wheat Improvement Center (CIMMYT) and the Maize Genetics Cooperation Stock Center (USDA/ARS) for

providing the seeds of the maize inbred lines. We also appreciate the reviewer and editor comments which improved the final version of the manuscript.

FUNDING INFORMATION

Funding for this work and open access publication fees was made possible by the generous support of the Cultivos para Mexico project funded by Mexico's Secretariat of Agriculture and Rural Development (SADER) and the CGIAR Research Program on MAIZE. Any opinions, findings, conclusion or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the CGIAR Research Program on MAIZE or its funders.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.13350.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Carlos Muñoz-Zavala https://orcid.org/0000-0002-5466-5337
Paul H. Goodwin https://orcid.org/0000-0002-2808-9495
Santos G. Leyva-Mir https://orcid.org/0000-0003-1831-2806
Alexander Loladze https://orcid.org/0000-0001-6806-4295
Mateo Vargas-Hernández https://orcid.org/0000-0002-0735-3242
Elizabeth García-León https://orcid.org/0000-0001-9034-3733
Juan M. Tovar-Pedraza https://orcid.org/0000-0002-8560-959X
Félix M. San Vicente-García https://orcid.org/0000-0002-5988-3114
Amos Alakonya https://orcid.org/0000-0001-7710-499X

REFERENCES

Adipala, E., Lipps, P. E., & Madden, L. V. (1993). Reaction of maize cultivars from Uganda to *Exserohilum turcicum*. *Phytopathology*, *83*(2), 223–271.

Ahangar, M., Wani, S., Dar, Z., Roohi, J., Mohiddin, F., Bansal, M., Choudhary, M., Aggarwal, S., Waza, S., Dar, K., El Sabagh, A., Barutçular, C., Konuskan, O., & Hossain, M. A. (2022). Distribution, etiology, molecular genetics and management perspectives of northern corn leaf blight of maize (*Zea mays* L.). *Phyton*, *91*, 1–23. https://doi.org/10.32604/phyton.2022.020721

Bashan, B., Levy, R. S., Cojocaru, M., & Levy, Y. (1995). Purification and structural determination of a phytotoxic substance from *Exserohilum turcicum*. *Physiological and Molecular Plant Pathology*, *47*(4), 225–235. https://doi.org/10.1006/pmpp.1995.1054

Bashan, B., & Levy, Y. (1992). Differential response of sweet corn cultivars to phytotoxic water-soluble compounds from culture filtrates of *Exserohilum turcicum*. *Plant Disease*, *76*(5), 451–454. https://doi.org/10.1094/PD-76-0451

Buitatti, M., & Ingram, D. S. (1991). Phytotoxins as tools in breeding and selection of disease-resistant plants. *Experientia*, *47*, 811–819.

Carson, M. L. (1995). A new gene in maize conferring the "chlorotic halo" reaction to infection by *Exserohilum turcicum*. *Plant Disease*, *79*, 717–720. https://doi.org/10.1094/PD-79-0717

Corwin, J. A., & Kliebenstein, D. J. (2017). Quantitative resistance: More than just perception of a pathogen. *The Plant Cell*, *29*(4), 655–665. https://doi.org/10.1105/tpc.16.00915

Cuq, F., Petitprez, M., Herrmann-Gorline, S., Klaebe, A., & Rossignol, M. (1993). Monocerin in *Exserohilum turcicum* isolates from maize and a study of its phytotoxicity. *Phytochemistry*, *34*(5), 1265–1270. https://doi.org/10.1016/0031-9422(91)80013-Q

Ding, J., Ali, F., Chen, G., Li, H., Mahuku, G., Yang, N., Narro, L., Magorokosho, C., Makumbi, D., & Yan, J. (2015). Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. *BMC Plant Biology*, *15*(1), 1–11. https://doi.org/10.1186/s12870-015-0589-z

Félix-Gastélum, R., Lizárraga-Sánchez, G. J., Maldonado-Mendoza, I. E., Leyva-Madrigal, K. Y., Herrera-Rodríguez, G., Espinoza-Matías, S., Félix-Gastélum, R., Lizárraga-Sánchez, G. J., Maldonado-Mendoza, I. E., Leyva-Madrigal, K. Y., Herrera-Rodríguez, G., & Espinoza-Matías, S. (2018). Confirmation of the identity of *Exserohilum turcicum*, causal agent of maize leaf blight in Sinaloa. *Revista Mexicana de Fitopatología*, *36*(3), 468–478. https://doi.org/10.18781/r.mex.fit.1803-1

Galiano-Carneiro, A. L., & Miedaner, T. (2017). Genetics of resistance and pathogenicity in the maize/*Setosphaeria turcica* pathosystem and implications for breeding. *Frontiers in Plant Science*, *8*, 1490. https://doi.org/10.3389/fpls.2017.01490

Gou, M., Balint-Kurti, P., Xu, M., & Yang, Q. (2023). Quantitative disease resistance: Multifaceted players in plant defense. *Journal of Integrative Plant Biology*, *65*(2), 594–610. https://doi.org/10.1111/jipb.13419

Gu, S., Li, P., Wu, M., Hao, Z., Gong, X., Zhang, X., Tian, L., Zhang, P., Wang, Y., Cao, Z., Fan, Y., Han, J., & Dong, J. (2014). *StSTE12* is required for the pathogenicity of *Setosphaeria turcica* by regulating appressorium development and penetration. *Microbiological Research*, *169*(11), 817–823. https://doi.org/10.1016/j.micres.2014.04.001

Hooda, K. S., Khokhar, M. K., Shekhar, M., Karjagi, C. G., Kumar, B., Mallikarjuna, N., Devlash, R. K., Chandrashekara, C., & Yadav, O. P. (2017). Turcicum leaf blight—Sustainable management of a re-emerging maize disease. *Journal of Plant Diseases and Protection*, *124*(2), 101–113. https://doi.org/10.1007/s41348-016-0054-8

Jakhar, D., Singh, R., Singh, S., & Ojha, V. (2017). Turcicum leaf blight: A ubiquitous foliar disease of maize (*Zea mays* L.). *International Journal of Current Microbiology and Applied Sciences*, *6*, 825–831. https://doi.org/10.20546/ijcmas.2017.603.097

Kotze, R. G., van der Merwe, C. F., Crampton, B. G., & Kritzinger, Q. (2019). A histological assessment of the infection strategy of *Exserohilum turcicum* in maize. *Plant Pathology*, *68*(3), 504–512. https://doi.org/10.1111/ppa.12961

Kushalappa, A. C., Yogendra, K. N., & Karre, S. (2016). Plant innate immune response: Qualitative and quantitative resistance. *Critical Reviews in Plant Sciences*, *35*(1), 38–55. https://doi.org/10.1080/07352689.2016.1148980

Leung, P. H., Zhang, Q. X., & Wu, J. Y. (2006). Mycelium cultivation, chemical composition and antitumour activity of a *Tolypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. *Journal of Applied Microbiology*, *101*(2), 275–283.

Levy, Y., & Pataky, J. K. (1992). Epidemiology of northern leaf blight on sweet corn. *Phytoparasitica*, *20*(1), 53–66. https://doi.org/10.1007/BF02995636

Li, P., Gong, X., Jia, H., Fan, Y., Zhang, Y., Cao, Z., Hao, Z., Han, J., Gu, S., & Dong, J. (2016). MAP kinase gene STK1 is required for hyphal,

conidial, and appressorial development, toxin biosynthesis, pathogenicity, and hypertonic stress response in the plant pathogenic fungus *Setosphaeria turcica*. *Journal of Integrative Agriculture*, *15*(12), 2786–2794. https://doi.org/10.1016/S2095-3119(16)61472-7

Lindhout, P. (2002). The perspectives of polygenic resistance in breeding for durable disease resistance. *Euphytica*, *124*(2), 217–226. https://doi.org/10.1023/A:1015686601404

Miller, R. N. G., Costa Alves, G. S., & Van Sluys, M.-A. (2017). Plant immunity: Unravelling the complexity of plant responses to biotic stresses. *Annals of Botany*, *119*(5), 681–687. https://doi.org/10.1093/aob/mcw284

Muiru, W. M., Koopmann, B., Tiedemann, A. V., Mutitu, E. W., & Kimenju, J. W. (2011). Race typing and evaluation of aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *World Journal of Agricultural Sciences*, *7*(3), 333–340.

Muñoz-Zavala, C., Loladze, A., Vargas-Hernández, M., García-León, E., Alakonya, A. E., Tovar-Pedraza, J. M., Goodwin, P. H., & Leyva-Mir, S. G. (2023). Occurrence and distribution of physiological races of *Exserohilum turcicum* in maize-growing regions of Mexico. *Plant Disease*, *107*(4), 1054–1059. https://doi.org/10.1094/PDIS-03-22-0626-RE

Mushtaq, A., & Gul, Z. (2015). Screening of maize inbred lines under artificial epiphytotic conditions for Turcicum leaf blight (*Excerohilum turcicum*). *African Journal of Microbiology Research*, *9*(7), 481–483.

Navarro, B. L., Campos, R. Gasparoto, M. C. & von Tiedemann, A. (2021). In vitro and in planta studies on temperature adaptation of *Exserohilum turcicum* isolates from maize in Europe and South America. *Pathogens*, *10*(2), 154. https://doi.org/10.3390/pathogens10020154

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative resistance to biotrophic filamentous plant pathogens: Concepts, misconceptions, and mechanisms. *Annual Review of Phytopathology*, *53*(1), 445–470. https://doi.org/10.1146/annurev-phyto-080614-115928

Pinheiro, E. A. A., Borges, F. C., Pina, J. R. S., Ferreira, L. R. S., Cordeiro, J. S., Carvalho, J. M., Feitosa, A. O., Campos, F. R., Barison, A., Souza, A. D. L., Marinho, P. S. B., & Marinho, A. M. R. (2016). Annularins I and J: New metabolites isolated from endophytic fungus *Exserohilum rostratum*. *Journal of the Brazilian Chemical Society*, *27*, 1432–1436. https://doi.org/10.5935/0103-5053.20160140

Švábová, L., & Lebeda, A. (2005). *In vitro* selection for improved plant resistance to toxin-producing pathogens. *Journal of Phytopathology*, *153*(1), 52–64. https://doi.org/10.1111/j.1439-0434.2004.00928.x

Swaminathan, S., Abeysekara, N. S., Liu, M., Cianzio, S. R., & Bhattacharyya, M. K. (2016). Quantitative trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause foliar sudden death syndrome. *Theoretical and Applied Genetics*, *129*(3), 495–506. https://doi.org/10.1007/s00122-015-2643-5

Tuite, J. (1969). Plant pathological methods. In *Fungi and Bacteria* (pp. 239). Burgess Publishing company.

Wathaneeyawech, S., Sirithunya, P., & Smitamana, P. (2015). Study of the host range of northern corn leaf blight disease and effect of *Exserohilum turcicum* toxin on sweet corn. *International Journal of Agricultural Technology*, *11*(4), 953–963.

Xia, X. C., Reif, J. C., Melchinger, A. E., Frisch, M., Hoisington, D. A., Beck, D., Pixley, K., & Warburton, M. L. (2005). Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical Midaltitude, and Highland maize inbred lines and their relationships with elite U.S. and European maize. *Crop Science*, *45*(6), 2573–2582. https://doi.org/10.2135/cropsci2005.0246

Yang, P., Herren, G., Krattinger, S., & Keller, B. (2017). Large-scale maize seedling infection with *Exserohilum turcicum* in the greenhouse. *Bio-Protocol*, *7*(19), e2567. https://doi.org/10.21769/BioProtoc.2567

Yang, P., Scheuermann, D., Kessel, B., Koller, T., Greenwood, J. R., Hurni, S., Herren, G., Zhou, S., Marande, W., Wicker, T., Krattinger, S. G., Ouzunova, M., & Keller, B. (2021). Alleles of a wall-associated kinase gene account for three of the major northern corn leaf blight resistance loci in maize. *The Plant Journal*, *106*(2), 526–535. https://doi.org/10.1111/tpj.15183

Zhang, L., Dong, J., Wang, C., & Li, Z. (2007). Purification and structural analysis of a selective toxin fraction produced by the plant pathogen *Setosphaeria turcica*. *Agricultural Sciences in China*, *6*(4), 452–457. https://doi.org/10.1016/S1671-2927(07)60069-8

Zhang, S.-R., Hao, Z.-M., Wang, L.-H., Shen, S., Cao, Z.-Y., Xin, Y.-Y., Hou, M.-L., Gu, S.-Q., Han, J.-M., & Dong, J.-G. (2012). *StRas2* regulates morphogenesis, conidiation and appressorium development in *Setosphaeria turcica*. *Microbiological Research*, *167*(8), 478–486. https://doi.org/10.1016/j.micres.2012.02.009

How to cite this article: Muñoz-Zavala, C., Goodwin, P. H., Leyva-Mir, S. G., Loladze, A., Vargas-Hernández, M., García-León, E., Tovar-Pedraza, J. M., San Vicente-García, F. M., & Alakonya, A. (2024). Maize insensitivity to culture filtrates from *Exserohilum turcicum* is related to quantitative resistance. *Journal of Phytopathology*, *172*, e13350. https://doi.org/10.1111/jph.13350