

Short Communication

CHARACTERIZATION OF UGANDAN ISOLATES OF *EXSEROHILUM TURCICUM* FROM MAIZE

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ABSTRACT

Four representative isolates of *Exserohilum turcicum* from four major maize growing districts in Uganda were assessed with respect to their cultural variability and pathogenicity towards an isogenic maize series containing the *Ht* genes for resistance. Two other isolates, one from Zimbabwe and a race 2N isolate from Hawaii, USA, were included for comparative purposes. The isogenic line (H4460), without the *Ht* gene, developed typical necrotic susceptible lesion type following inoculation with all isolates, whilst H4460Ht1, H4460Ht2 and H4460Ht3 exhibited the resistant chlorotic lesion type. These results indicated that the Ugandan isolates comprised race 0. *In vitro* studies showed that radial growth rates differed significantly among the isolates, the Ugandan isolates tending to have higher temperature optima than the Zimbabwean one.

Key Words: *Exserohilum turcicum*, northern leaf blight, race, Uganda.

INTRODUCTION

Northern leaf blight (NLB), caused by *Exserohilum turcicum* (Pass.) (teleomorph = *Setosphaeria turcica* (Luttrell) Leonard and Suggs), has become a disease of great concern on maize in Uganda. Cultivars which had shown some levels of tolerance, for example, Kawanda Composite A, have become susceptible.

Recently Leonard *et al.* (8) described the structure of races of *E. turcicum* (0: *Ht*1, *Ht*2,

*Ht*3, *Ht*N; 1: *Ht*2, *Ht*3, *Ht*N/*Ht*1; 23: *Ht*1, *Ht*N/*Ht*2, *Ht*3; 2N: *Ht*1, *Ht*3/*Ht*2, *Ht*N). Race 0 is widely distributed in many parts of the world (1), race 1 is now believed to be widespread throughout the USA corn growing regions (6), and race 23 appears restricted to the continent of America (9). Race 2N was first reported in 1991 in Hawaii (12) but has subsequently been observed in Texas (10). Race 0 has so far been reported from Uganda (2) but the disease has not been clearly characterized. The objectives of

this study were to establish race(s) present, and to study the *in vitro* characteristics of some isolates from the major maize growing regions of the country.

MATERIALS AND METHODS

Pathogen isolates. Four isolates of *E. turcicum* were collected from four maize growing regions in Uganda, namely Kasese, Masindi, Mpigi and Iganga. Additionally, two isolates were included for comparative purposes, one from Henderson Research Station, Zimbabwe, and the other, race 2N, from Hawaii, USA. The USA isolate was provided by J.H. Windes, University of Illinois, Urbana-Champaign.

Preparation of single-spore cultures. Lesions of NLB were excised, surface sterilized in 1% sodium hypochlorite solution for one minute, rinsed in sterile distilled water (SDW) for three minutes, and transferred to moist filter paper in glass petri dishes. Cultures were incubated for three days at $(25^{\circ}\text{C} \pm 1)$ to induce sporulation. Single conidia were then transferred aseptically onto plates of lactose casein hydrolysate agar (LCH) and incubated at $25^{\circ}\text{C} \pm 1$ with a 12-hour light/dark regime. Conidia suspensions were prepared by washing 14-day old cultures with SDW and filtering the suspension through muslin cloth. Spore concentrations were then adjusted to 4×10^4 spores/ml using a hemocytometer, and used to inoculate test plants.

Host plants. Five isogenic differential maize lines, (H4460, H4460*Ht*1, H4460*Ht*2, H4460*Ht*3 and A697(W22*Ht*N)), with and without *Ht* resistance genes, provided by C. De Leon, CIMMYT Asian Regional Maize Programme, Thailand, were grown in a heated glasshouse (22–25°C) with no additional lighting. Three seeds of each line were sown in 13 cm diameter plastic pots containing peat based multi-purpose compost. Following emergence, seedlings were thinned to two per pot. Thirty-day old plants were inoculated by pipetting 1 ml spore suspension (4×10^4 spores/ml) into the whorls of each seedling. Inoculation was carried out in the evening to provide favorable conditions for establishment of infection. For each isogenic

host, there were five replicate pots arranged in a randomized complete block design.

Disease assessment. Qualitative assessments were conducted 21, 25 and 29 days after inoculation (DAI). Lesions were classified on the basis of visual symptoms: R⁺ = chlorotic lesions without necrosis; R = chlorotic lesions with some necrosis; R⁻ = chlorotic lesion with considerable necrosis; and S = wilted and necrotic lesion without chlorosis (3).

Growth rate. An *in vitro* experiment was conducted to determine the growth rate of three isolates, Iganga and Kasese from Uganda and the Zimbabwean isolate. Radial growth rates were assessed on LCH and Potato Dextrose Agar (PDA) media at four temperatures 15°, 20°, 25°, and 30°C.

Eight-millimetre plugs of each isolate were placed mycelium side down, in the centre of 9 cm diameter plastic petri dishes containing the different media. Three replicate plates for each treatment were incubated in continuous light at the four temperatures. Colony diameters were measured daily starting from the second day of seeding, except for cultures grown at 15°C whose diameters were measured at two-day intervals starting from the fourth day of seeding. Radial growth rates were derived from the means of two diameters taken at right angles to each other. The diameter of the inoculum plug was subtracted from the total diameter recorded in each case. The growth rate (mm/day) considered was for measurements taken in the exponential phase of growth only, and the lag period was assumed to have lasted two days from seeding.

RESULTS AND DISCUSSION

Three of the isogenic differential lines: H4460*Ht*1, H4460*Ht*2 and H4460*Ht*3 developed chlorotic lesions, after inoculation with all isolates, rather than typical blight lesions. The classic necrotic lesions were observed for line H4460 (without gene *Ht* or *Ht*N), against all isolates, and in A697(W22*Ht*N) inoculated with the USA isolate (Table 1).

Failure of Ugandan and Zimbabwean isolates to form necrotic lesions on isolines with

TABLE 1. Reaction of five isogenic maize lines with and without the *Ht* genes to six isolates of *Exserohilum turcicum*

| Isolate | Maize lines | | | | |
|----------|-------------|----------------|----------------|----------------|----------------|
| | H4460 | H4460Ht1 | H4460Ht2 | H4460Ht3 | A697 (W22HtN) |
| Masindi | S | R ⁺ | R ⁺ | R ⁺ | R ⁺ |
| Kasese | S | R ⁺ | R ⁺ | R ⁺ | R ⁻ |
| Iganga | S | R ⁺ | R ⁺ | R ⁺ | R ⁺ |
| Mpigi | S | R ⁺ | R ⁺ | R ⁺ | R ⁺ |
| Zimbabwe | S | R ⁺ | R ⁺ | R ⁺ | R ⁺ |
| 2N (USA) | S | R ⁺ | R ⁺ | R ⁺ | S |

R⁺ = chlorotic lesions without necrosis ; R = chlorotic lesion with some necrosis; R⁻ = chlorotic lesion with considerable necrosis; and S = wilted and necrotic lesion without chlorosis.

TABLE 2. Mean radial growth rates of three isolates of *Exserohilum turcicum* grown at four temperatures on two media

| Isolate | Growth rate (mm/day) | | | |
|---------------------------------|----------------------|-------|-------|--------|
| | 15°C | 20°C | 25°C | 30°C |
| Lactose casein hydrolysate agar | | | | |
| Iganga | 6.93 | 22.90 | 24.67 | 22.33 |
| Kasese | 4.27 | 22.40 | 23.37 | 24.97 |
| Zimbabwe | 9.47 | 21.57 | 22.67 | 19.60 |
| Potato dextrose agar | | | | |
| Iganga | 9.27 | 33.90 | 32.67 | 33.77 |
| Kasese | 9.10 | 33.10 | 33.60 | 36.40 |
| Zimbabwe | 12.50 | 34.77 | 32.27 | 28.37 |
| LSD ₅ | 2.80 | 5.51 | 5.83 | 4.62 |
| CV(%) | 20.00 | 13.30 | 12.50 | 110.30 |

the *Ht* and *HtN* genes suggest that the Ugandan and Zimbabwean isolates were race 0 (8). Our results are consistent with earlier reports that race 0 was probably the only biotype of *E. turcicum* in Uganda (2). Thus, a program utilizing monogenic resistance could successfully incorporate genes *Ht1*, *Ht2*, *Ht3* and *HtN*. However, since selection for novel races with virulence to single *Ht* genes may cause breakdown of resistance under field conditions, inclusion of polygenic resistance is important.

Differences in radial growth rates between the two Ugandan and one Zimbabwean isolates were significant on both media tested (Table 2). Radial growth rates were faster on PDA than on

LCH. The Zimbabwean isolate grew significantly faster (Table 2) than the two Ugandan isolates (Iganga; Kasese) at 20°C (PDA) and 15°C (PDA and LCH), indicating a lower temperature optima. This may have been related to the origin of the isolate; Henderson has a temperature range of 8–26°C which is generally cooler than Kasese and Iganga, sources of the Ugandan isolates. The Kasese isolate grew faster than the other two isolates at 25°C and 30°C, probably also indicating a higher temperature optima for the isolate. Kasese is one of the hottest districts in Uganda, with a temperature range of 18–32°C.

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