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Fungi Associated with Tomato Wilt in the Tropical Humid Lowlands of Southeastern Nigeria and Preliminary Evaluation for Disease Tolerance in the Crop.

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ABSTRACT

Isolations on potato dextrose agar (PDA) showed that the following fungi were frequently associated with natura infected, wilted tomato plants in Umudike: *Fusarium oxysporum, f.sp. lycopersici, F.solani, F.equiseti, F.acuminatur Trichoderma viride, T.harzianum, Mucor hiemalis, Sclerotium rolfsii, Phoma lycopersici* and *Rhizopus microsporu* Evaluation of 10 tomato cultivars for disease tolerance indicated that *Fusarium* wilt, incited by *Fusarium oxysporum f.s lycopersici,* with an incidence of 80-100% and a severity index of 4.0-5.0, was the most important fungal disease of fie grown tomato in the area. Pathogencity tests, employing the direct soil inoculation technique, showed that only isolate of *F. oxysporum f.sp. lycopersici* induced wilting in all tomato cultivars evaluated.

KEYWORDS: *Fusarium* wilt, tomato cultivars, tomato wilt, disease tolerance, tropical humid lowlands.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill), which is perhaps the most important vegetable crop in the world (Phene, 1989) and also an important constituent of the daily diet of most Nigerians, is not grown commercially in south-eastern Nigeria. According to FAO (1984) estimates, Nigeria produces about 600,000 metric tonnes of tomato fruits annually, with the northern savanna zones as the main production centre. The factors which limit large scale tomato production in the humid tropical lowlands of south-eastern Nigeria can be traced to inadequate information on the production of the crop. Others include high relative humidities (51-87%), temperatures (20-33°C) and high rainfall, averaging 2,162.7mm annually.
(Agromet Division, NRCRI, 1998). High incidence of diseases caused by fungi and other pathogens as well as inadequate facilities for plant disease control. have contributed to making south-eastern Nigeria unfavourable for large scale tomato cultivation.

Some major diseases of tomato in Nigeria are: *Fusarium* wilt, incited by *Fusarium oxysporum f.sp. lycopersici* (Sacc.) Snyder and Hansen; early blight, caused by *Alternaria solani* (Ell. And Mart.) (L.R. Jones and Grout); *Septoria* leaf spot induced by *Septoria lycopersici* Speg.; late blight caused by *Phytophthora infestans* (Mont.) DBY; leaf mould incited by *Cladosporium fulvum* Cooke; basal stem rot, caused by *Sclerotium rolfsii* (Sacc.); tomato leaf curl induced by viruses; root galls caused by root knot nematodes (*Meloidogyne spp.)* and bacterial wilt incited by *Pseudomonas solanacearum* (Smith) Smith (Bailey, 1966; Quinn,1971, 1975, Huntinton *et al* 1976; Erinle, 1977, 1976; Adelana and Simons, 1980; Wokocha, 1984; Osuinde and Ikediugwu, 1996). These reports come almost entirely from the northern savanna and the rainforest zones of the south-western Nigeria. Reports of tomato diseases in the humid tropics of south-eastern Nigeria are scantly and little research has been conducted in this area on the subject.

The aim of this investigation was to screen some tomato cultivars, under rainfed conditions for tolerance to major fungal diseases in Abia State, Nigeria.

MATERIALS AND METHODS

Source of seeds and seed bed preparation

Seed of 10 tomato cultivars obtained from the National Institute of Horticulture (NIHORT), Ibadan, were used in this investigation. These were: DT 95/133^{B}, DT 95/146, Local B, DT 95/433, DT 95/30, DT 95/237, DT 95/43, DT 95/257, DT 95/140^{A} and DT 95/207^{A}. The seeds were surface – disinfected in 5% sodium hypochlorite solution for 3 minutes, rinsed in several changes of sterile distilled water and sown in drills, in heat-sterilized soil in seedling trays 90 x 60 x 60 cm in dimension. The trays were kept in the green house at 22-27°C and watered twice daily.

Field plots and experimental design

Field experiments were conducted during the rainy season at the western farm of the Michael Okpara University of Agriculture, Umudike (5°29' and 7°33'E; altitude 122m.above mean sea level (AMSL) in the centre of the humid lowland forest zone of south-eastern Nigeria. Soil at the experimental site was a sandy-clay loam of pH 5.6, 82% organic matter, 1.05% total C and 0.11% total N. The experimental design was a randomized complete block design, replicated three times, with a one – meter path between the blocks. Tomato seedlings (3-to 4-weeks-old) were transplanted (in the first week of April) into plots of beds 4x2m. in size and 60cm apart. Each tomato cultivar was transplanted per bed and constituted a treatment and each bed a replicate. Beds were covered with grass mulch. Transplants were arranged in two rows of eight seedlings each at a spacing of 60 x 45cm, to give a total of 480 seedlings.

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Three weeks after transplanting, NPK (20:10:10) fertilizer was applied as a side dressing at the rate of 300kg/ha. The field was hand-weeded three times during crop life. Disease development was assessed at weekly intervals beginning from two weeks after transplanting. Assessment was made on every second plant in a row (totaling 240 plants). Disease scores were based on a 1-5 point scale similar to that used by Wokocha (2000) as follows:

1 = no visible wilt symptom
2 = 1-3 leaves wilted
3 = 4-6 leaves wilted
4 = 7-9 leaves wilted
5 = >10 leaves wilted

Vertical sections of wilted plants were made, using cool, flame-sterilized scapels, and then examined using a hand lens.

Isolation of fungi from wilted tomato plants

Isolation of fungi was made, separately, from the stems (4-5cm above soil level) and roots of partially as well as completely wilted tomato plants obtained from the field. Five millimeter segments were cut from these materials to include the interface between healthy and necrotic tissues. The segments were surface disinfected in 10% sodium hypochlorite solution for 3 minutes and rinsed in three changes of sterile distilled water, dried between sheets of sterile filter paper before plating out on freshly prepared potato dextrose agar (PDA) medium in 9.00cm Petri dishes (five segments per plate). The plates were incubated at $28^0 \pm 1^0$C for 7 days, during which fungi growing out of the inocula were isolated and identified.

The frequency of occurrence of each organism isolated was recorded. The identification of the *Fusarium spp.* was confirmed by the International Mycological Institute, Kew, England, while other fungal isolates were identified by reference to Barnett (1960) and Funder (1961).

Pathogenicity test

Three fungal isolates, namely; *Fusarium oxysporum f. sp. lycopersici* (IMI No. 238285), *F.solani* (Mart.) Sacc. (IMI No. 254460), and *F.equiseti* (Corda) Sacc. (IMI No. 238284) which occurred at high frequency in the stems, roots and fruits of wilted, naturally infected tomato plants in Umudike were employed in pathogenicity tests on ten tomato cultivars earlier evaluated in the field.

Inocula of the three *Fusarium spp.* Were prepared separately by placing 4-5 mycelial discs (5cm in diameter) taken from the margins of 5-day-old pure cultures of each fungus on PDA, in one litre Erlenmayer flasks, each containing 500ml of potato dextrose broth. The flasks were incubated for 7-10 days at room temperature. The contents of each flask were filtered off in a large funnel, using 18cm filter paper. The resulting fungal mat was washed in several changes of sterile distilled water to remove traces of stalling material. The fungal mat was then placed in 250ml of sterile distilled water containing 5% glucose solution in a Warring blendor and homogenized for one minute at low speed. The concentration of each inoculum suspension was adjusted to 10^7 conidia/ml. Heat sterilized sandy clay warm soil of pH 5.6 in 13cm diameter plastic pots was inoculated by mixing separately 20ml of each inoculum suspension/300g. of soil. Three-week-old tomato seedlings were transplanted into inoculated soil at the rate of one seedling per pot. Twenty seedlings of each cultivar were used. Control seedlings were similarly transplanted into uninoculated soil in pots. All seedlings were watered immediately and kept in the green house at temperatures of $22\text{-}27^0$C.

Observations were recorded over a period of 3-4 weeks, following inoculation.

RESULTS AND DISCUSSIONS

The results of screening 10 tomato cultivars for disease tolerance in Umudike, Abia State, indicated that wilt by *F.oxysporum f.sp. lycopersici* was the most economically important fungal disease of field grown tomato in the area. The incidence of *Fusarium* wilt on cultivar Local B (80.5%) was significantly (P = 0.05) lower than on the other cultivars (Table 1). Severity of the wilt disease was high (4.0 – 5.0) but not significantly (P = 0.05) different among the various cultivars including cultivar Local B.

The *Fusarium* wilt disease was most severe on tomato between flowering and the on-set of fruit. This is the first report of the disease in the rainforest agro-ecological zone of south-eastern Nigeria. Erinle (1977, 1989) had earlier reported the wide spread occurrence of tomato wilt disease incited by two races (1 and 2) of *F.oxysporum f.sp. lycopersici* in the Zaria area of the northern savanna zone of Nigeria. Similar occurrences of the disease have been reported in Ibadan. (Adelana and Simon, 1980) and Ishan (Osuinde and Ikediugwu, 1996) areas of the south-western forest zone of the country. The results of the present investigation fill a gap in our knowledge of the tomato wilt disease in the south-eastern Nigeria. It also showed that the *Fusarium* wilt, an economically important disease of tomato in Nigeria (Erinle, 1989) was wide spread throughout all the agro-ecological zones of the country and not limited to the drier parts alone as suggested by Erinle (1977) and Adelana and Simons (1980).

Table 1: Incidence and severity of *Fusarum* wilt in different tomato cultivars under rainfed conditions in the humid tropical lowlands of Umudike in south-eastern Nigeria.

Tomato	Disease	
Cultivar	Incidence(%)	Severity Index
DT 95/146	100.0a	4.5a
Local B	80.5b	4.0a
DT 95/433	100.0a	5.0a
DT 95/38	95.0a	4.7a
DT 95/133[B]	100.0a	5.0a
DT 95/237	100.0a	4.6a
DT 95/43	100.0a	5.0a
DT 95/257	90.7a	4.5a
DT 95/140[A]	97.6a	4.3a
DT 95/207[A]	100.0a	5.0a

Means in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

Table 2: Fungi isolated from wilted, naturally – infected tomato plants in Umudike in the south-eastern rain forest zone of Nigeria

Fungal isolates	Frequency occurrence (%)
Fusarum oxysporum	
f.sp. lycopersici	75.0
F. solani	48.5
F. acuminatum Ell.& Ev.	30.3
F. equiseti	52.1
Aspergillus flavus	
Link ex Friers	32.0
Sclertium rolfsii	18.5
A. niger vanTieghem	39.0
Trichoderma viride Pers.	
Ex S.F. Gray	25.2
T. harzianum Rifai	17.6
Mucor hiemalis Wehmer	10.6
Rhizopus microsporus	
Van Tieghem	7.0
Phoma lycopersici Cooke	2.5

Recognition of the *Fusarium* wilt disease in the field during study was based on the occurrence of wilted leaves or leaflets usually accompanied by epinasty, yellowing and the development of adventitious roots along the stem. These symptoms were similar to those observed by Erinle (1977). Vertical sections of wilted plants revealed a progressive dark-brown colouration of the pith and xylem tissues, most of which had disintegrated. Wilted plants showed extensive damage of the tap root and lateral root systems. However, no slimy, viscous drops were observed when stems of wilted tomato plants were cut across and squeezed or placed in water. This was an indication that *Pseudomonas solanacearum* (Bailey, 1966; Osuinde and Ikediugwu, 1996) was not implicated in the present tomato wilt.

Results of isolations on potato dextrose agar (PDA, Table 2), showed that *F. oxysporum f.sp. lycopersici* was the most frequently isolated fungus from naturally infected and wilted tomato plants in Umudike. *Fusaria spp.* With a frequency of occurrence ranging from 30.3-52.1% were: *F.solani, F.equseti* and *F.acuminatum*. Other fungal species isolated included *Aspergillus niger, A.flavus, Trichoderma viride, T.harzianum, Mucor hiemalis, Rhizopus microsporus, Sclerotium rolfsii* and *Phoma lycopersici*. These were the most important fungi associated with the tomato wilt disease, under rainfed conditions in Umudike. These records agree with observations made by Osuinde and Ikediugwu (1996) in the Ishan area of the rain forest zone of south-western Nigeria.

In pathogenicity tests, disease symptoms appeared 3-5 days after inoculation and 80-100% of infected seedlings in all cultivars died within the 25-day period of observation. This indicated a highly virulent strain of *F.oxysporum f.sp. lycopersici* in Umudike, similar to that reported by Osuinde and Ikediugwu (1996). The search for tomato cultivars with a high degree of tolerance to *F. oxysporum f.sp. lycopersici* is in progress at Umudike.

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