IN VITRO RESPONSE AND MORPHOLOGICAL VARIABILITY OF FOUR EXOTIC TOMATO VARIETIES (*Solanum lycopersicum L.*) TO TOXIN FILTRATES OF TOMATO WILT FUNGI

*Okogbaa, J.I., Terna, T.P., and Ogah, J.

Department of Botany, Federal University Lafia. P.M.B 146 Lafia, Nasarawa State, Nigeria.

Correspondence Email: jamesokogbaa@yahoo.com.

Received 11/04/2017  Accepted: 12/10/2017  Published: December 2017

ABSTRACT
A study was carried out to evaluate morphological variability and response of four exotic tomato varieties, namely; Copernic f1, Lindo f1, Cobra f1 and Kiara f1 to fungal wilt in Lafia, Nasarawa State, Nigeria. Healthy leaves collected from 8 weeks old tomato seedlings were inoculated with 21-day old culture filtrates of *Colletotrichum gloeosporiodes*, *Pythium* spp. and *Fusarium oxysporum*, and observed for 3 days for the development of leaf necrosis and yellowing. All four varieties were susceptible to disease initiation by crude toxin filtrates of the wilt fungi. Lindo f1 was the least susceptible with 33.33% leaf necrosis and 29.17% leaf yellowing. Leaves of Cobra f1 were the most susceptible to necrotic symptoms (72.92%), while the highest incidence of leaf yellowing (79.17%) was observed in filtrate-inoculated leaves of the Kiara f1 tomato variety. Differences in mean disease incidence among the evaluated tomato varieties were significant (P ≤ 0.05). *Fusarium oxysporum* was the least pathogenic, inducing 30.00% leaf necrosis and 36.25% leaf yellowing. *Pythium* spp. induced the highest incidence of leaf necrosis and leaf yellowing (56.00% respectively). Kiara f1 had the highest plant height (34.40cm), leaf area (40.70cm²) and stem girth (6.20mm). Copernic f1 had the highest number of leaves (91), number of fruits (4.40) and least plant height (23.3cm). Lindof1 had the least leaf area (20.00cm²) and stem girth (4.40mm), while Kiara f1 had the least number of fruits (0.40). Differences in morphological variations were significant (P ≤ 0.05) except in plant height. Lindo f1 was the most disease resistant of the tomato varieties and could be selected for enhanced yield and productivity.

Keywords: Exotic tomato varieties, Morphological variations, Wilt fungi, Toxin filtrates, Disease incidence, Disease resistance
INTRODUCTION
Tomato (Solanum lycopersicum L.) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetables in the world (Cox, 2000). Tomatoes have, undoubtedly assumed the status of a food with functional properties, considering the epidemiological evidence of reducing the risk of certain types of cancer (Golia, 2008). Consumption might be beneficial for reducing cardiovascular risk associated with type 2 diabetes (Shidfar et al., 2011). It contains many nutrients, anti-oxidants and secondary metabolites such as vitamin C and E, B-Carotene, flavoniodes, organic acids, phenolics, and chlorophyll II which are important for human health (Giovianelli and Paradisco, 2002; Radwan and Salama, 2006; Demirbas, 2010).

With a total cultivated area of one million hectares and annual yield of about 2.5 million tons, Nigeria is ranked the second largest producer of tomato in Africa and 13th in the world (Adegbola et al., 2012). In spite of this huge annual production capacity, Nigeria currently ranks as the largest importer of tomato paste from China and Italy, largely because of frequent crop losses to disease and other production challenges in the tomato value chain.

Several studies have been carried out by a number of researchers on the pathogenicity of various fungal pathogens on local and improved varieties of tomato in the North-Central Region of Nigeria (Bem, 2009; Terna et al., 2015), but none has focused on the role of fungal toxins in the development of disease symptoms. The purpose of this work is to determine the susceptibility of four exotic tomato varieties to disease initiation by secondary metabolites of selected wilt fungi. The findings of this study shall serve as veritable means of selecting better resistance in tomato varieties against fungal pathogens that cause huge annual yield losses in the study area.

MATERIALS AND METHODS
Certified seeds of four exotic tomato varieties (Copernic f1, Lindof1, Cobra f1 and Kiara f1) were purchased from Da-ALLGREEN SEEDS LIMITED, Kaduna State, Nigeria. The tomato wilt fungi used in the study (Colletotrichum gloeosporiodes, Pythium spp and Fusarium oxysporum) were obtained from the culture bank of the Plant Pathology Unit of the Department of Botany, Federal University Lafia, Nasarawa State.

Five kilograms each of sandy-loamy soil in metal drum, were sterilized at 180-200°F (82.2-93.3°C) for 45min and dispensed into sterile polyethylene bags and allowed to cool. Sterilized soils were moistened with water and incorporated with healthy tomato seeds at the rate of about 30 seeds per pot, and 10 pots for each variety (4 varieties in total). The potted plants were placed under a shade and watered twice daily (morning and evening) to ensure seedling growth.

Four weeks old seedlings of tomato were transplanted onto fresh sterile potted soils at the rate of 2 seedlings per pot. Transplanting was done in the evening to prevent excessive loss of water and wilting due to the desiccating effects of the heat of the sun.

Measurement of morphological parameters such as number of leaves, stem height, stem girth and leaf area using of the tomato varieties were carried out six (6) weeks after transplanting. Leaf area was determined as reported by Osei-Yeboah et al.,(1983) thus:

\[ \text{Leaf Area} = L \times W \times 2.325 \]  

(The Correction factor).

The method of (Zheng et al., 2010) was used for Fungal cultures and toxin production as follows: Cultures of 3 wilt fungi of tomato (Colletotrichum gloeosporiodes, Pythium spp and Fusarium oxysporum) were grown on Potato Dextrose Agar (PDA) plates at 25°C for 7 days and then stored at 4°C. Four 6-cm-diameter mycelial plugs were obtained from cultures of test fungi using a sterile cork borer, and transferred into separate conical flasks each containing 200 ml of sterile Potato Sucrose Broth (PSB). Inoculated broths were incubated at 25°C in the laboratory under continuous diffused light for 21 days, and culture filtrates obtained by passing the liquid through four layers of cheesecloth and Whatman no. 1 filter paper.

The biological activities of culture filtrates of the wilt fungi were determined by leaf necrosis assay using the methods of Zheng et al., (2009). Petioles of excised tomato leaves of the different varieties were separately wrapped in sterile cotton wool, moistened with 2ml of fungal filtrates in sterile Petri dishes, and observed for the appearance of disease symptoms for a duration of 3 days. Tomato leaf petioles wrapped in sterile cotton wool and moistened in 2ml sterile PSB served as controls.

The treatments were laid out in Completely Randomized Design (CRD) with 3 replicates in a 3x4x4 layout. Data obtained from assessment of disease incidence and morphological variations were subjected to analysis of variance (ANOVA) at 5% level of probability.
RESULTS AND DISCUSSION

Variations were observed in the mean number of leaves of the four evaluated tomato varieties in the field after transplanting (Fig.1.). Number of leaves increased steadily in all four varieties, from the 1st week and peaked at the 6th week after transplanting. Copernic f1 had the highest mean number of leaves (91), followed by Cobra f1 (69.6), Kiara f1 (65.4) and Lindo f1 (49.6).

Variations were observed in the mean leaf area of the four evaluated tomato varieties (Fig.2.). Kiara f1 had the highest mean leaf area (40.70m²) followed by Copernic f1 (35.90m²), Cobra f1 (33.40m²) and Lindo f1 (20.00m²).

The stem height increased steadily from the 1st week after transplanting and peaked at the 6th week, in all four tomato varieties (Fig. 3). Copernic f1 had the highest mean stem height of (34.4cm) followed by Cobra f1 (32.2cm), Lindo f1 (25.8cm) and Kiara f1 (23.4cm).

Variations in the mean stem girth among the four tomato varieties are presented in Figure 3. Kiara f1 had the highest mean stem girth of (6.2mm) followed by Cobra f1 (5.7mm), Copernic f1 (5.5mm) and Lindo f1 (4.4mm). All evaluated tomato varieties showed the highest stem girth at the 6th week after transplanting.

Fig. 4. Variations in Number of Fruits among the Evaluated Tomato Varieties.
Note: Columns with same alphabets are not significantly different (P≤0.05)

Variations in number of fruits produced by the evaluated tomato varieties are presented in Figure 4 and Plate 4. Copernic f1yielded the highest mean number of fruits (4.40) followed by Cobra f1(2.20), Lindo f1(2.00) and Kiara f1(0.40). Differences in mean number of fruits were significant between Kiara f1 and the other studied varieties (P≤0.05).
Plate 4. Copernic f1 (A), Lindo f1 (B), Cobra f1 (C) and Kiara f1 (D) Tomato Varieties Showing Variations in Fruit Yield 10 Weeks after Transplanting.

Table 1. Comparative Disease Susceptibility of Tomato Varieties to Inoculated Fungal Pathogens

<table>
<thead>
<tr>
<th>Variety</th>
<th>Necrosis (%)</th>
<th>Yellowing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copernic f1</td>
<td>45.83b</td>
<td>53.33c</td>
</tr>
<tr>
<td>Lindo f1</td>
<td>33.33b</td>
<td>29.17b</td>
</tr>
<tr>
<td>Cobra f1</td>
<td>72.92c</td>
<td>77.08d</td>
</tr>
<tr>
<td>Kiara f1</td>
<td>50.00b</td>
<td>79.17d</td>
</tr>
<tr>
<td>Control</td>
<td>0.00a</td>
<td>0.00a</td>
</tr>
</tbody>
</table>

Means followed by same superscripts within same column are significantly different (P≤0.05).

Varying degrees of leaf necrosis and yellowing were observed in vitro on tomato leaves inoculated with toxin filtrates of fungal pathogens (Table 1 and Plates 1-3). Cobra f1 had the highest incidence of leaf necrosis (72.92%) followed by Kiara f1 (50.00%), Copernic f1 (45.83%) and Lindo f1 (33.33%). Differences in mean disease incidences were significantly different (P≤0.05). The highest incidence of leaf yellowing was observed on leaves of Kiara f1 (79.17%), followed by Cobra f1 (77.08%), Copernic f1 (53.33%), and Lindo f1 (29.17%). Differences in incidence of foliar disease symptoms among the evaluated tomato varieties were significant (P≤0.05).

Plate 1: Lindo f1 Leaves Showing Symptoms of Necrosis and Yellowing, 3 Days after Inoculation with Culture Filtrates of Fusarium oxysporum (A); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (B).

Plate 2: Cobra f1 Leaves Showing Necrotic Lesions and Yellowing 3 Days after Inoculation with Culture Filtrates of Colletotrichum gloeosporiodes(C); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (D).
Plate 3: Kiara f1 Leaves Showing Various Degrees of Necrosis and Yellowing 3 Days after Inoculation with Culture Filtrates of Pythium spp (E); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (F).

Table 2. Overall Pathogenicity of Fungal Pathogens on Inoculated Tomato Varieties

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease Incidence (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Colletotrichum gloeosporioides</em></td>
<td>35.00a</td>
<td>53.75b</td>
</tr>
<tr>
<td><em>Pythium spp</em></td>
<td>56.25b</td>
<td>56.25b</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>30.00a</td>
<td>36.25a</td>
</tr>
</tbody>
</table>

Means followed by same superscript within same column are significantly different (P≤ 0.05).

Results of overall pathogenicity of fungal pathogens on inoculated tomato varieties (Table 2) revealed that *Pythium* spp induced the highest incidence of both leaf necrosis (56.25%) and leaf yellowing (56.25%). Tomato leaves challenged with culture filtrates of *Fusarium oxysporum* showed the least incidence of necrosis (30.00%) and yellowing (36.25%). Differences in mean total diseases induced by culture filtrates of the test fungi were significant (P≤ 0.05).

CONCLUSION

The evaluated tomato varieties showed significant variations in their pre-and post- infection growth and yield attributes. Lindo f1 was the most disease tolerant of the four tomato varieties and could be selected for enhanced productivity and crop yield.

REFERENCES


Ploetz, R.C. (2006). Fusarium wilt of Banana is caused by several pathogens referred to as *Fusarium oxysporum f. sp.cubense*. *Phytopathology, 96:* 653-656.


