Susceptibility of Tomato Genotypes to a Fungal Pathogen

Eboigbe Lugard[1a] and Eshikhogie Liberty Eshiozemogie[1b]

1a. Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, PMB 1154, Benin City, Edo State Nigeria.

1b. Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, PMB 1154, Benin City, Edo State Nigeria.

ABSTRACT

A pathogen classified as Oidium neolycopersici was isolated from tomato genotypes prepared for characterization. This fungus which appears to be new in this region poses a great threat to the cultivation of tomato if not controlled. If allowed in the field with just contact and creeping conditions fulfilled can devastate tomato plant no matter how big is the hectares. Eleven tomato genotypes were evaluated in this experiment and all were full susceptible to the pathogen. The mode of infection of the pathogen is yet to be cleared, though the mode of transmission seem to be on contact. Attempt was made to identify the fungus, however due to the present conflicting morphological characteristics confirmed by literature, genomic sequencing is needed to identify the fungus genetically.

Key words: Susceptibility, tomato Genotypes, Pathogen

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated crop worldwide. It is also widely consumed both in the raw and processed form. It well known as fruit vegetable that contains vitamin A and C, lycopene (a natural antioxidant). This nutritional value of tomato has given it a very wide publicity. Lycopene in one instance is claimed to be an antioxidant which is important in human health, as it reduces the risks of nervous system disorder, heart disease, cancer and obesity (Dufera, 2013). Another feature that may have

informed the worldwide cultivation and consumption of tomato, is it adaptablilty to varied climatic conditions (Abdussamee and Hussain *et al.,*2014). In Nigeria, a total area of one million hectares is used for the cultivation of about 2.5 million tons of tomato annually at 25 tons/hectare. Nigeria is ranked the second largest producer of tomato in Africa and 13th in the world. Despite this fact, Nigeria is the largest importers of tomato paste from China and Italy due to various limitations in tomato production in Nigeria. However, tomato breeding has received a great deal of attention and the major focus presently is in fruit size, shape total solids lycopene, B-carotene, firmness, nutritional quality, flavor and other important fruit quality characteristics including pH, titratible acidity and vitamin content (Akhtar and Harza, 2013).

With the growing needs in human health, it is expected that breeding for tomato with higher quality will remain in focus. Unfortunately, tomato cultivation has been greatly hampered by disease incidence all over the world. Presently, it is known that there are more than 200 pathogens that affect tomato of which the effects of the majority of the pathogens are very devastating in the field. Among the pathogens, those of fungal origin seem to be more prominent (personal observation). Here we report on a fungal pathogen that ravaged tomato planted for preliminary evaluation and characterization. After the Koch postulate, we suggested that this fungus could be very devastating if time and chance give the right conditions for the organism to thrive.

MATERIALS AND METHODS

Collection of Plant Materials

Tomato genotypes NG/SA/01/01/002, NG/OA/07/10/003, NG/RM/Jan/10/001, L00169, NG/AA/sep/09/037, NG/MR/May/09/006 and NG/DE/May/09/019 were collected from National Centre for Genetic Resources and Biotechnology(NAGRAB) Ibadan , Nigeria. Genotypes NHGB/09/120 and NHGB/09/113) were collected from Nigeria Institute of Hoticulture (NIHOT) while genotypes Gabon, Asempete (as locally called) were collected from Igbodo farm settlement Delta State Nigeria.

Isolation Pathogen

The pathogen under investigation was isolated from tomato following the procedure of Morid, Hajmansoor and Kakvan,(2012). The tomato genotypes were meant for preliminary characterization and evaluation. Eleven genotypes of tomato were seeded out in the screen house of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. Based on the observation of clear symptoms, a portion of the leave affected was aseptically transferred to a Petri dish containing 4% hypochlorite solution and was left for 10min. Thereafter it was transferred aseptically to sterile water in order to complete the rinsing process. After the cleansing, it was inoculated on a potato dextrose agar plate and was incubated for 36hrs. This procedure was repeated thrice. The pathogen that consistently appeared on the was sub cultured into a fresh plate containing the same medium.

Koch Postulate: Re-inoculation of Pathogen via Root Dip

In the characterization of tomato genotypes, natural infection was permitted by using untreated soil for the growth of the tomato both at the nursery and after transplanting. In the second stage of the work, the tomato plants were re-inoculated with the fungal pathogen isolated from tomato during the characterization process. The tomato were infected using root dip method following the procedure of Tzima *et al.*,(2010). Four weeks' old tomato seedlings were root dipped into liquid concentrate of the fungal pathogen for 20min, before transplanting. Seedlings were between the height of 10-16cm.After the treatment, transplants were transferred into prepared sterilized soils for growth till maturity. All other cultural practices were fully followed.

Measurement of Disease Incidence

Disease ratings were plotted over time and the area under the disease progress curve (AUDPC) was calculated using the trapezoidal integration method (Campell and Maden, 1990). The disease severity therefore, was expressed as a percentage of the maximum

possible AUDPC for the period of experiment and it is referred to as relative AUDPC (Korolev *et al*.,2001).

Statistical Analysis

IBM SPSS Statistics version 22 Software package was employed for all statistical analysis done in this work. Data was analyzed by analysis of variance (ANOVA) and the significance of differences within treatments were separated by using LSD test at probability levels P= 0.05(Steel *et al*., 1997)

RESULTS

The results from this experiment show the very devastating effect of this fungal pathogen in tomato genotypes. All the genotypes evaluated in this experiment were all heavily affected. As a part of our observation, this is the first time we are encountering this pathogen in Edo state, Nigeria to the best of our knowledge. The pathogen was first noticed in the screen house during the first characterization of the tomato genotypes we collected. Eleven tomato genotypes were seeded out after four weeks' nursery. Since our interest was on developing resistant genotypes, we aimed at maintaining the tomato at the worst condition i.e without the treatment of the soil. This mean, any pathogen present in the soil is free to attack the tomato plant. Under this regiment, we expected that any resistant variety as a part of the characterization procedure would become a potential tool for us to develop a better variety in tomato. Interestingly, among the symptoms expressed by the tomato, all pointed to a particular pathogen which is described below. Isolation of this pathogen was repeated thrice as the first attempt to identifying it. The various ways the pathogen presented itself is shown in plate 1,2 and 3.

Plate1: The earliest stage of the disease condition. The symptom showed basically on the leaves with dark or sclerotial-like substances sporting the leaves, which when inoculated on Potato Dextrose Agar plate, produce fungal growth. It appears to spread from leaf to leaves. The mode of infection is not yet understood

Plate 2: Showed a more advanced stage of infection on the leaves. In addition to the black sclerotial-like substance, the pathogen also developed white powdery substance (powdery mildew) which might probably be the means of disseminating the spores of the pathogen to other leaves

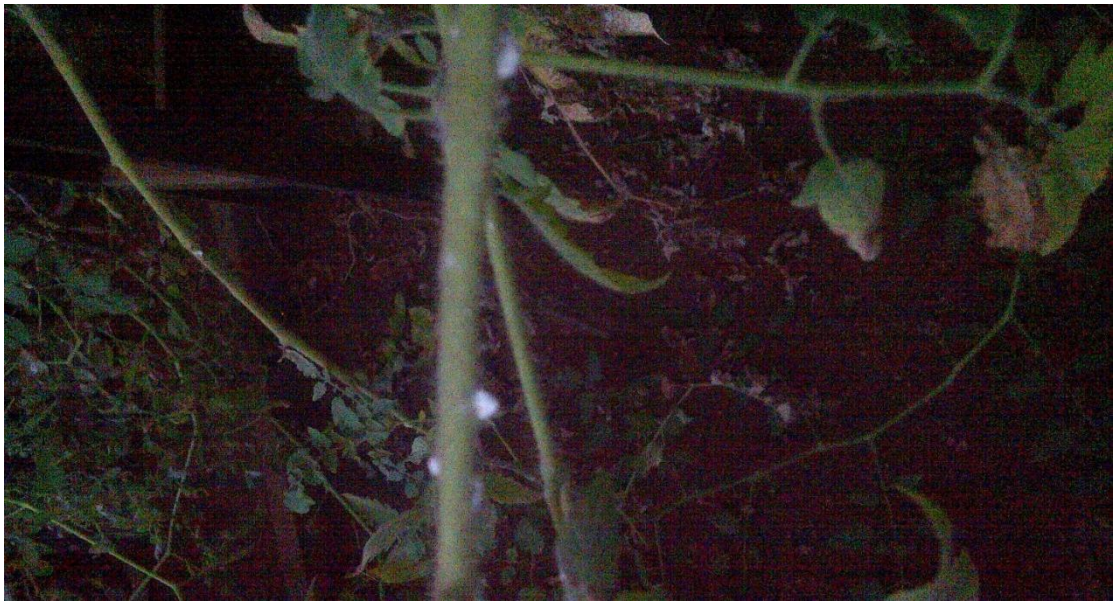

Plate 3: Pathogen appearance on stem of the tomato. Here it appears in form of a white woolly substance. This is also the early stage of its effect on stem.

At the earliest part of the infection, the pathogen is seen as a dark sport-like sclerotial on the upper surface of the leave which spread gradually over the surface of the leaves. Multiple leaves from the same branch could be affected with varying degree. At an advanced stage, the pathogen produce white powdery substances containing the dark sclerotial(powdery mildew). Instances in which we inoculated Potato Dextrose Agar(PDA) medium separately with the white powdery substances or the sclerotial, it produced the same morphology of the pathogen suggesting that we were dealing with the same pathogen. At a later stage, the same white substance also appeared on the stem (plate 3), however it appears woolly in nature. We also inoculated from this and found it to be the same with the former isolates.

At an advanced stage of infection the pathogen run through the length of the stem, thus this infection is known to run through the whole length of the tomato plant from the top to the soil level.

Assessment through the AUDPC

The disease incidence was fully assessed using initially the number of the leaves affected by the pathogen. This was to compare the reaction of each tomato genotype to the attack by the pathogen. The result of the AUDPC showed that there was no significant difference in the reaction of the tomato genotypes screened for resistance to the pathogen (fig.1and2).

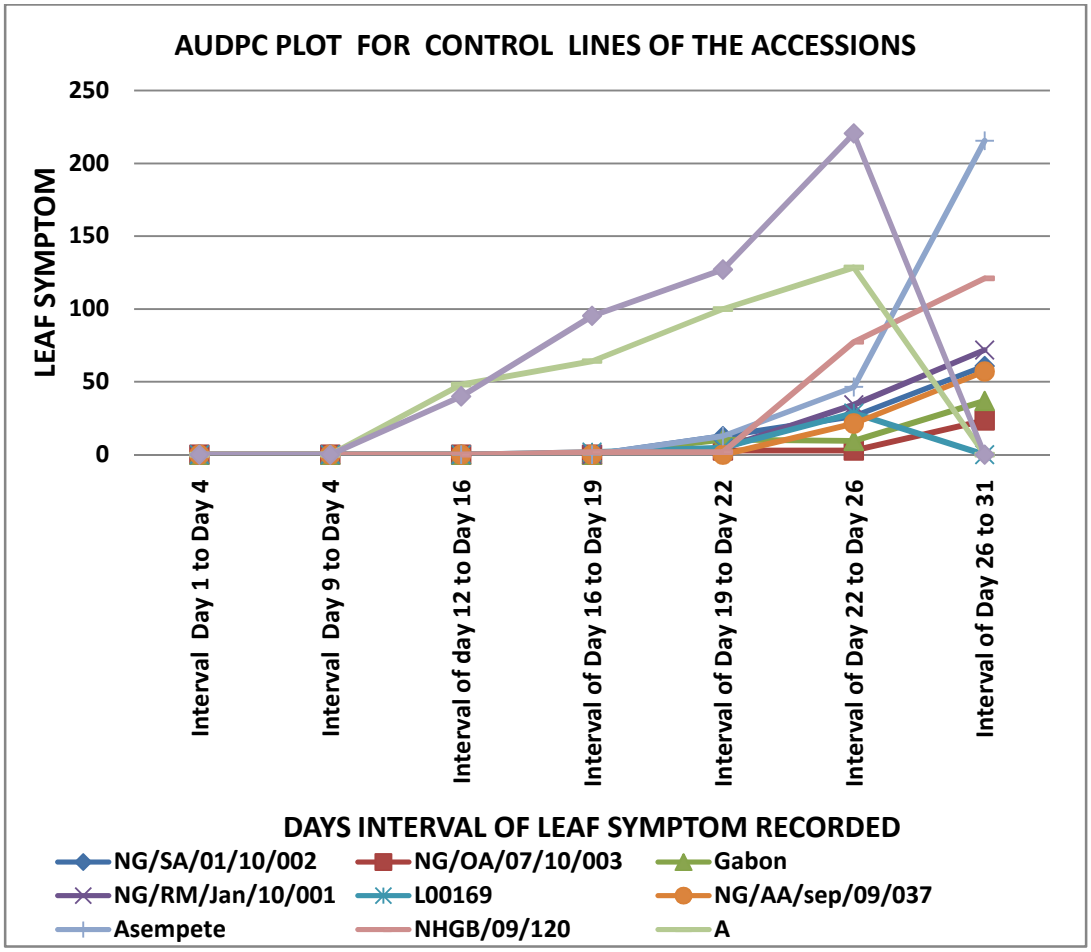

Fig.1: The level of symptoms recorded for the control experiment showed that there was a delay in the appearance of symptoms as shown. The only time symptoms began to appear was when the leaves of the control plant touched that of the infected plants. The tomato plant that first responded (Gabon and GN/RM/JAN10/001) were also the one that first came in contact with the infected plant.

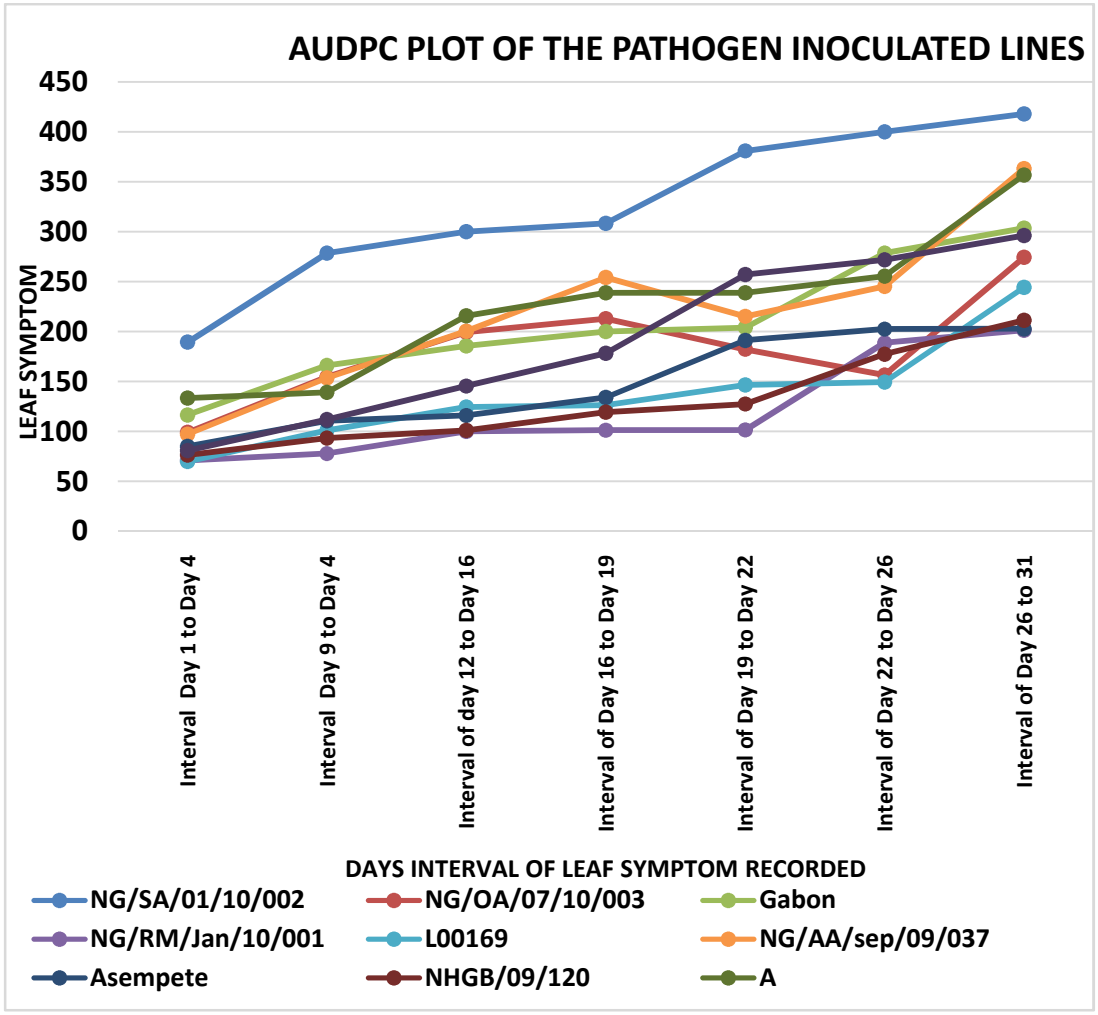

Fig. 2: The levels of fungal symptoms in the tomato genotypes evaluated. Symptoms were observed in plants after three weeks of inoculation. From that point, there was a progressive increase until the death of the plants.

All the tomato genotypes screened were all susceptible to the pathogen. This was further confirmed by statistical analysis using one way ANOVA. Considering the disease progress index, the level of susceptibility to this pathogen was very high. Moreover the pathogen overwhelm the plant within a short period. At the first incidence of the disease, all the plants were destroyed and could not fruit. Those that manage to flower aborted. The only genotype that fruited(NG/RM/Jan/10/001) could not continue to fruit. The fruiting of the genotype was thus greatly affected. The re-inoculation of the tomato produced the same results; there was

no fruiting in the plant inoculated. The only record of fruiting at the second experiment were in those plants which were not inoculated. Since it was a potted experiment and plants were together, by contact with the plants inoculated, the control experiment started showing symptoms of the disease. However, the disease incidence occurred much later in the control experimental plants(fig.1), consequently the number of fruits were affected.

Identification of Pathogen

As a powdery mildew, the morphology of the pathogen as viewed under the light microscope suggested *Oidium neolycopersici*; as there were spores or conidia which were ellipsoidal in shape. Furthermore, there were fruiting bodies, branched hypha which are consistent with the *O. neolycopersici.* The pathogen is observed majorly as mass of mycelia; the hypha strand branched and sometimes demarcated into cellular compartments(plate 4). We are yet to study the life cycle of the pathogen. On plate, it presents a strand-like growth with scanty hair-like growth on the surface of the mycelia mass. The mycelia is dirty brown in color, sometimes producing melanin heavily below the agar block. The production of this melanin has not been associated with the previously identified *O. neolycopersici*, hence we first consider this fungus as belonging to *Verticillium Sp.* Presently there is a lot of confusion on the real identity of this fungus.

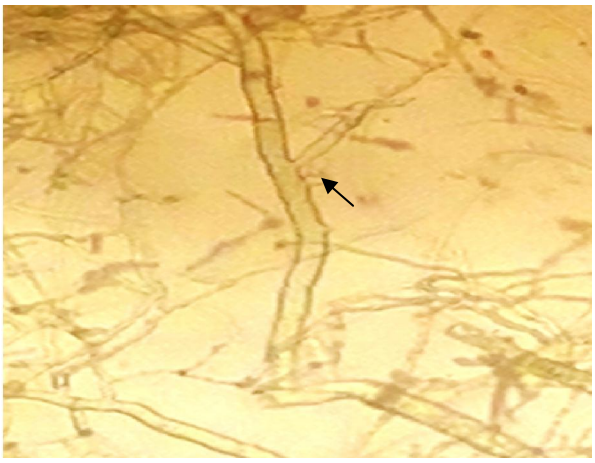

Plate 4: Cultural morphology of the pathogen on PDA plate. Magnification(X40) . The arrow shows the fruiting body.

DISCUSSIONS

In this experiment a fungal pathogen identified as *Oidium neolycopersici* was isolated from tomato genotypes prepared for characterization. The symptoms exhibited by the pathogen on tomato appear to be new at least to the best of our knowledge. The effect of this pathogen on tomato appears to be very devastating. If such a pathogen comes in contact with a tomato plant in the field, despite the stage in growth, the progress of the plant would be arrested and the purpose of cultivation will be defeated. The fungus appears to spread fast through contact, since by contact alone the control experiment was also affected. However, we could not ascertain the mode of infection of the fungus as the deliberate inoculation of the pathogen on the leaves did not produce any visible symptom on the leaves. Furthermore, we inoculated each plant with root dip method, the disease appeared on the plant after three weeks from the date of inoculation. We were sure that the reaction of the tomato germplasm was as a result of the deliberate inoculation, since at the time we started recording the disease incidence, the control was yet to be infected. The symptoms on the control only came on board when there was contact with the other plants inoculated. This suggested that the disease spread by contact. The question that is yet to be answered is how the disease got to the leaves considering the method of infection

In an attempt to identify this pathogen, we viewed the different cultural characteristics under the microscope. The morphology of the pathogen implicated that of *O. neolycopersici,* in that there was the presence of conidia,branched hypha and fruiting bodies(plate 4) which are typical of the fungus. However, the presence of melanin created a dilemma yet to be resolved. The presence of melanin in growth of the fungus as secondary metabolite suggested *Verticillium sp.* In literature the different morphological appearance of this organism have made the true identity of the fungus complicated. This fungus has bore different names from the past (Jones, Whipps and Gurr 2001). From the characteristics of our isolates it could be a different race or all together a new powdery mildew.

From our investigation, we have realized that what predisposes tomato plant to infection is the growth habit. According to Sajjad *et al*.,(2011), tomato like other vegetables is more prone to disease mainly due to its tenderness and softness. It is not possible to stake all the tomatoes growing in vast hectares of land. Based on the stem girth, there is no tomato that can stand when heavily fruited. Breeders are interested in high yielding genotypes. The components of yield traits are in number, size and weight of fruits. These facts made it almost impossible for the tomato not to creep on the soil. Since contact with one another and creeping on the ground are unavoidable, tomato plants are highly vulnerable to a lot of pathogens in general and fungal in particular. Breeders have tried to breed for resistance varieties to many fungal pathogens. This goal has been challenged by pathogens coming up with new prototypes and the growth habits of tomato which expose such resistant variety to other pathogens. The implication of this is the known genetic shift in pathogen population which make previous resistant variety to become susceptible (Akram *et al.,*2014). This pathogen which appears to be new so to say poses a very high threat to tomato cultivation if not controlled. As a result of the above, we have decided to focus on the fungus rather than screening further for genetic resistance to this fungus. Our further research is to sequence the whole genome of the fungus in search of all possible pathogenicity genes present in the organism. This will serve as a base in identifying the pathogen genetically.

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