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Isolation and Identification of Fungal Pathogens Associated with Tomato Genotypes/Lines in Nsukka South-Eastern Nigeria

Agriculture

KEYWORDS : Tomatoes, disease pathogens, Fusarium oxysporum and Rhizopus stolonifer

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ABSTRACT

Field studies were conducted in 2011 and 2012 rainy seasons to evaluate seven genotypes/lines of tomato for fungal disease problems. Tomato seedlings raised in the nursery and later transplanted to the field were observed for fungal disease symptoms. The design of the experiment was randomized complete block design (RCBD) with four replications. The tomato plants with symptoms were taken to the laboratory for isolation and identification. The results showed that Fusarium oxysporum and Rhizopus stolonifer were the predominant fungal pathogens isolated from tomato lines. Therefore, they were considered as the most endemic fungal pathogen affecting tomato in Nsukka south eastern Nigeria.

INTRODUCTION

Tomato is one of the major fruit vegetable crops in Nigeria. Hardly is there any household that does not eat this fruit vegetable at least once in every 3 days in Nigeria. It is second most important vegetable crop after potato in the world (Panthee and Chen, 2010) Tomato commands high commercial value because of its high nutritive value (Ensminger et al., 1995). However, due to the unfavourable environmental conditions (Olaniyi et al., 2010) in southern Nigeria, the fruit quality and quantity are severely affected. This is because microbial infection is rapid and severe especially in areas where high humidity favours rapid microbial growth. The increasing cost of synthetic fungicides, non-availability when needed and lack of technical skills by local farmers limits the usage of these chemicals. Besides, there is a growing awareness on the dangers associated with the usage of some of these chemicals because they have high mammalian toxicity and are considered not safe even on the environment (Eze and Eze, 2010).

Therefore, the use of resistant varieties is one of the safest, economical, environmentally sound methods to manage these disease causing fungal organisms. In the present investigation, genotypes/lines of tomatoes were evaluated to identify major fungal disease pathogens in an effort to be specific in ensuring proper breeding and development of resistant lines.

MATERIALS AND METHODS

A two years experiment was conducted at the Department of Crop Science Research Farm and laboratory unit, University of Nigeria, Nsukka. Located in the derived savannah zone (latitude 06^{o} 52 ^{i}N, longitudes 07^{0} 24 ^{i}E and 447.26m above sea level), the soil was a well-drained sandy clay loam classified as ultisol belonging to Nkpologu series (Nwadialo, 1989).

Tomato seeds were obtained from the Department of Crop Science, University of Nigeria Nsukka tomato improvement programme. The planting materials for the experiment comprised two tomato species *Solanum lycopersicum* (cultivated varieties i.e. Roma vf and Tropica) and *Solanum pimpinellifolium* (wild) with their advanced generations of the interspecific hybrids (S2S, S3S, S4S and S1E) obtained from crosses between them.

Seven nursery boxes were provided, for the experiment since it involved seven different lines. The seedlings were raised in the different nursery boxes filled with sterilized soil mixture of top soil, well-cured poultry manure and river sand at a ratio of 3:2:1 by volume. The seeds were planted 5 cm apart. The spacing was to permit the removal of seedlings during transplanting with some balls of earth. After planting the boxes were watered, covered with board and placed under a shade where it remained undisturbed until emergence. At 3 weeks after planting the seedlings were hardened before transplanting to the experimental farm.

The experimental farmland was cleared, ploughed, harrowed and made into ridges with the help of a tractor and laid out in randomised complete block design (RCBD) with four replications. The blocks were separated 1 m apart. Each block was divided into seven plots with each treatment assigned to a plot. Each of the plots measured 6m x 3m. The plots were separated from each by a pathway of 0.5m. Plant spacing of 1m x 0.5m was used giving 30 stands per plot. Weeding was done manually as and when necessary. Harvesting was done by hand-picking at full maturity. Data were collected on percentage disease incidence, severity and fruit yield

Procedures for isolation and identification of fungal pathogens associated with diseased tomato plants

Samples from plants with symptoms were obtained from the established experimental field, and the following processes were used to isolate and identify the fungi pathogens. Leaves and roots from the diseased plants were cut into 5 mm pieces washed and surface sterilized with 0.1% mercury chloride. The pieces were dried with sterilized filter paper and plated (3 pieces per plate) on fresh water agar and observed for fungal growth for 3 day. Thereafter, the fungal growth was transferred on fresh potato dextrose agar (PDA) medium impregnated with streptomycin and incubated for 7 days at 28^{o} C.

Pure cultures were obtained by sub-culturing three times. Pure cultures of the final isolate were maintained on PDA slant in test tube. The isolates in the slants were viewed under binocular

microscope to observe the mycelia structure and conidia. The spore head identification of the isolated fungal was carried out based on the cultural, chemical and morphological characteristics with the help of identification scheme of Barnett and Hunter (1972).

RESULTS AND DISCUSSIONS

Plants grown in the 2011 planting season produced fruits with higher fruit length, fruit girth and single fruit weight, while those grown in 2012 planting season produced higher number of fruits and total weight of fruits per plant. On the fruit length, fruit girth and single fruit weight, the hybrids were significantly ($P<0.05$) higher than that of the wild genotype (Table 1). The hybrids also gave significantly ($P<0.05$) higher total weight of fruits per plant in the both years. This could have resulted from the gene of resistance against microbial infection in the hybrids. However, the wild tomato genotype had highest number of fruits per plant. The hybrids had moderately sized fruits that were significantly ($P<0.05$) higher in number per plant than those produced by the cultivated parents in the two years (Table 2).Table 3 shows the mean value of the disease incidence percentage of the different tomato genotypes in 2012 planting season. At 90 days after planting (DAP) more than 85% of each cultivated parents plants population were diseased, while the wild and the hybrids had less than 37% population of the plants diseased and were all statistically lower than the exotic parents at $P<0.05$. The same trend repeated at 100 and 110 DAP where the wild genotype and the hybrids had no significance difference ($P<0.05$) among each other, but were all significantly ($P<0.05$) lower than cultivated parents which were almost all diseased.

All diseased tomato genotypes showed signs of fungal infection (Ballisager, 2007), the yellowing of leaves, downward curving and apparent dropping of flowers were pronounced at fruiting stage. The fruits that eventually stayed to ripening became rotten at harvest. Subsequently wilting of plants was common Figure 2. Laboratory investigation on the infected parts of the tomato plants revealed that *Rizopus solonifer* and *Fusarium oxysporum* were the most prevalent fungal organisms isolated from all the genotypes. This finding was in line with the research carried out by Michielse and Rep (2009), and Nishijima (1993) on the micro-organisms that attack tomatoes.

The microphotographs of the isolated organisms from the tomato samples which were associated with soft rot in tomato fruits are shown in Plates 1 and 2. *Rhizopus stolonifer* (Plate 1) showed white cottony colonies on potato-dextrose-agar (PDA) at 25°C. There was presence of sporangia and then brownish black in age, spreading rapidly by means of stolons fixed at various points to the substrate by rhizoids. The sporangia were somewhat flattened at base, white at first, then black and have many spores. The columella was flattened, light brownish grey, and is umbrella-shaped when dehisced. The apophysis was present and visible below young columellae. The rhizoids and stolons are transparent to dark brown. The brownish-black sporangiospores were irregular round, oval, elongate or angular in shape (Lunn, 1974).

Fusarium oxysporum (Plate 2) showed a rapidly growth on PDA. This according to Edmunds and Pottorff (2012) could be because of voracious and destructive nature of *F. oxysporum* Conidiophores were short, arrayed in densely branched clusters. Micro conidia were abundant, never in chains, mostly non-septute ellipsoidal to cylindrical curved. Chlamydospores were terminal and hynaline rough-welled (Andrew and Andrew, 2011).

CONCLUSION

Greater percentage of the tomato yield in south eastern Nigeria is loss to fungal infection. The present challenge is therefore to isolate and identify the actual fungal organisms responsible for the noticed diseases and consequently make efforts to develop a resistant tomato genotype. These when achieved will raise the standard of living and help recover the self-respect of tomato growers in this region. Probably, this will encourage more people to go into its production; hence helping the nation to attain food security. The important of this research cannot be over emphasized.

Heavy rainfall which characterized this experimental period had been reported to result in greater vegetative growth and increased disease incidence (Pangey *et al*., 2006). Fungal pathogenic organisms, *Fusarium oxysporum* and *Rhizopus stolonifer* were found in all the diseased tomato plants. These organisms were all pathogenic and could probably be the cause of the observed tomato disease problems and fruit rots encountered in the experiment. Therefore, there is an urgent need to breed tomato cultivars that will tolerate and resist the effect of these organisms that are prevalent in the region.

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Table 1: Effects of planting year on the fruit girth, fruit length (cm) and single fruit weight (g) of the tomatoes lines.

Planting	Genotypes							
Year	Roma	S2S	S3S	S4S	S1E	Tropica	Wild	Mean
fruit girth (cm)								
2011	12.00	8.75	8.50	7.00	9.25	12.25	4.00	8.82
2012	8.93	7.55	7.03	6.35	7.70	11.65	4.40	7.66
Mean	10.46	8.15	7.76	6.68	8.48	11.95	4.20	
fruit length (cm)								
2011	6.30	4.47	4.52	3.95	4.95	6.55	2.27	4.71
2012	5.95	4.12	3.77	3.12	4.32	7.27	2.15	4.38
Mean	6.12	4.30	4.15	3.53	4.63	6.91	2.21	
single fruit weight (g)								
2011	28.20	10.50	11.35	7.05	18.05	31.60	1.50	15.46
2012	23.60	8.85	6.75	4.52	18.12	26.00	1.90	12.82
Mean	25.90	9.67	9.05	5.79	18.09	28.80	1.70	

	fruit girth (cm)	fruit length (cm)	single fruit weight (g)
$LSD_{0.05}$ for year	0.518	0.2820	1.663
$LSD_{0.05}$ for genotype	0.969	0.5276	3.112
$LSD_{0.05}$ for year x genotype	1.370	0.7462	4.40

Table 2: Effects of planting year and genotypes on the number of fruits and total weight of fruits (g) per tomato plant

Planting	Genotypes							
Year	Roma	S2S	S3S	S4S	S1E	Tropica	Wild	Mean
number fruits/plant								
2011	5.5	61.3	89.0	158.8	36.5	6.8	325.5	97.6
2012	11.3	149.5	181.2	192.8	72.5	8.3	424.8	148.6
Mean	8.4	105.4	135.1	175.8	54.5	7.5	375.0	
total weight of fruits /plant (g)								
2011	147.0	639.0	985.0	1148.0	661.0	214.0	473.0	610.0
2012	263.0	1319.0	1230.0	877.0	1317.0	220.0	809.0	862.0
Mean	205.0	979.0	1107.0	1013.0	989.0	217.0	641.0	

	number fruits/plant	total weight of fruits /plant (g)
$LSD_{0.05}$ for year	26.66	122.1
$LSD_{0.05}$ for genotype	49.88	228.4
$LSD_{0.05}$ for year x genotype	70.54	323.1

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Table 3: Disease incidence percentage of the tomato genotypes for 2012 planting season

Genotypes	D 90	D 100	D 110
SIE	36.50	48.20	54.00
S2S	33.80	44.20	52.50
S3S	33.00	40.80	47.00
S4S	34.50	41.80	48.20
ROMA	85.80	87.00	93.50
TROPICA	85.00	95.50	97.80
WILD	33.50	42.00	47.80
F-LSD	11.25	10.51	10.77

D90 = percentage disease incidence at 90 days after planting, D100 = percentage disease incidence at 100 days after planting, D110 = percentage disease incidence at 110 days after planting.

Figure 1a: Rhizopus stolonifer

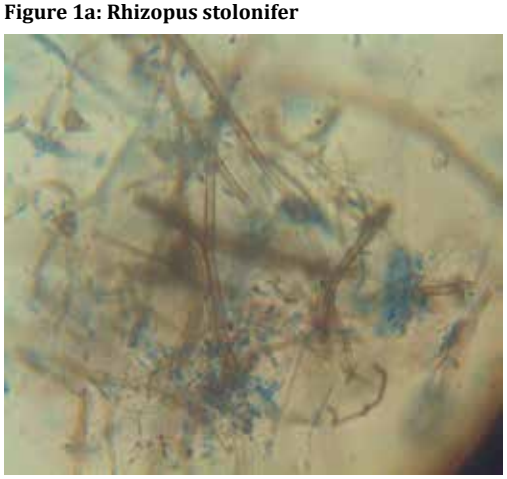

Figure 1b: Rhizopus stolonifer

Figure 2a: Fusarium oxysporum

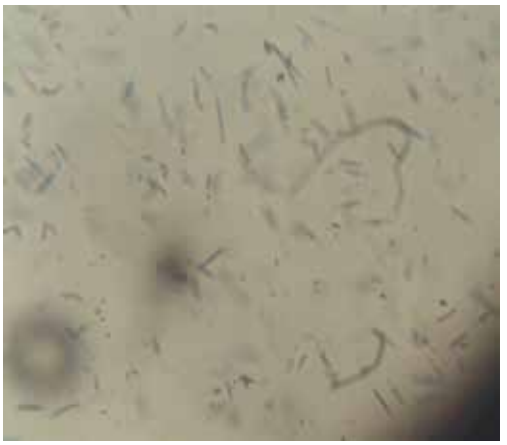

Figure 2b: Fusarium oxysporum

Figure 2: Tomato plants in the experimental farm with signs of fungal infection

Experimental farm in 2011 season

Experimental farm in 2012 season

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