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Detached Leaf Assay: A Sustainable Method for Screening Yam Genotypes for Resistance to Yam Anthracnose Disease Caused By *Colletotrichum Gloeosporioides* (Penz and Sacc)

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Abstract
The use of resistance cultivars is the most economical and effective means of control of anthracnose disease in water yam (Dioscorea alata) caused by Colletotrichum gloeosporioides. Screening for resistance genotypes among many lines is normally carried out in the field or screenhouse under natural environmental conditions. However, the use of detached leaf assay (DLA) technique has become popular among different pathosystems with its numerous advantages. Three different crosses between resistance males- 01/00061, 11/00246 and 11/00010 with a common susceptible female- 99/00240 from germplasms maintained at NRCRI, Umudike were made. Their F_1 progenies were screened for resistance to C. gloeosporioides infection. Three months old surfaced sterilized leaves of test water yam genotypes were inoculated with 30µl drop of the pathogen -Fast growing gray (FGG) strain of C. gloeosporioides spore suspension of the concentration of 10^6 spores ml^{-1} on the abaxial surface. The leaves were placed inside a transparent tray lined with plies of moistened blotter papers. Incubation in the laboratory was at 28 2 and evaluation of disease severity on a 1-5 scale for 21 days was carried out. The F_1 progenies of the 3 sets varied significantly ($P<0.05$) in their disease severities. More populations were highly resistant, fewer susceptible and none highly susceptible to anthracnose diseases. Based on the numerous advantages of Detached Leaf Assay (DLA) techniques (rapid, cheap and ability to work under controlled environment), there are potentials for advance researches in breeding, epidemiology etc over the whole plant assay.

Keywords: *D. alata, anthracnose, detached leaf assay, resistance, genotypes, C. gloeosporioides*

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I. Introduction

Yams (*Dioscorea spp*) are important staple tuber crops cultivated in the tropical and sub tropical zones of the world. There are more than 3,000 species. Among the ten (10) listed most important yam species, D. alata commonly called greater yam or water yam is the most adaptable and cultivated (Lebot 2009). The edible tuber has an average crude protein content of 7.4%, starch content of up to 84% and vitamin C up to 24mg/100g (Muzac-Tucker *et al*. 1993). The production of *D. alata* especially in Nigeria is hampered by many field diseases especially yam anthracnose disease (YAD) caused by the fungus- *Colletotrichum gloeosporioides* resulting in yield loss up to 80% (Nwankiti and Ene, 1984, Onyeka *et al*,*2006,* Abang *et al,* 2006). *Colletotrichum gloeosporioides* Penz (Telemorph: *Glomerrala cingulata* (Stonen)Spauld and Schsenk), a pleomorphic pathogen induces leaf necrosis and shoot die back resulting in the reduction of the photosynthetic surfaces of the crop and the ultimate death of the plant. The use of cultural practices as well as fungicides is common in the management of yam anthracnose disease, However, frequent use of these synthetic chemicals lead to the development of fungicides resistance *Colletotrichum* strains (Smith *et al,* 2013, Forcelini *et al,* 2016). The use of resistance cultivars has been found to be the most durable way of controlling anthracnose diseases of crops even yam (Agrios 2005 Abang *et al,* 2006). There is evidence of natural resistance to anthracnose among cultivars of *D. alata* (Nwankiti and Ene 1984, 11TA 1993, Abang 1997) For a sustainable yam breeding program, a precise and reproducible disease screening protocol is needed to differentiate between susceptible and resistance lines. Three common methods are usually employed - field screening, screen house/green house screening with whole plant and excised or detached leaf assays (Osoria *et al*, 2014, Smith 2008). The detached leaf assay has successfully been used and reported for screening common been cultivars for resistance against anthracnose (Tu, 1986, Perria *et al,* 2013 and Kolade *et al,* 2018). Foolad *et al,* (2015) successfully reported the determination of late blight resistance among tomato germplasms, apple lines were screened for resistance to Alternaria blotch (Abe *et al*, 2010). The advantages of detached leaf assay are many and include increased replications, the ability to test and maintain many susceptible lines (Browne and Cook, 2004), economy in

labour, plant material, space and inoculum (Nwadili *et al*, 2017, Dhingra and Sinclair 1995). Detached leaf assay can be done under controllable environmental conditions such as humidity, light and contaminations. The aim of this study was to assess the responses of three mapping populations of F_1 progenies of *D. alata* genotypes with strains of *Colletotrichum gloeosporioides* using the detached leaf assay.

II. Materials And Methods

Location of Experiment

Sampling and collection of diseased yam leaves were conducted at the research farms of the National Root Crops Research Institute (NRCRI) headquarters in Umudike Umuahia, Abia State between 2017-2019, Leaves showing evidence of anthracnose were randomly picked from ridges that fall into the Z pattern according to Dhingra *et al*, Sinclair (1995). The leaf samples were put into a sterile polythene bag and taken to the laboratory for further analysis.

Isolation and Identification of Causal Organisms.

infected *D. alata* leaves were first washed under running tap water, surface sterilized in 20% Sodium hypochlorite solution for one minute and rinsed in four changes of sterile distilled water separately in beakers and finally blotter dried between sterile blotter papers. The leaves were asceptically cut into 5cm size towards the advancing edges and plated on freshly prepared Potato Dextrose Agar medium. Incubation was done for 5-7 days at 28, 70-95% relative humidity. Afterwards, with sterile inoculation loop, spores of the fungal organisms were reisolated and reinoculated onto fresh PDA medium and incubated for 5 days for confirmation of pathogenicity using the morphological characteristics of *Colletotriclum gloeosporioides*.

Preparation of Inoculum Suspension

A 7 day old culture plate of *Colletotriclum gloeosporioide*s was flooded with sterile distilled water and with the aid of sterile inoculation loop, the conidia were gently scraped off the plate, The spore suspension from several plates were pooled together, filtered through three layers of cheese cloth and concentration adjusted to 10^6 spores ml^{-1}.

Detached Leaf Assay (DLA)

The detached leaf assay was carried out using the F_1 populations of *D. alata* genotype of the three sets of crosses between resistant males- TDa 01/00061, TDa 11/00246 and TDa 11/00010 series with a common susceptible female- TDa 99/00240 from germplasms maintained in the NRCRI fields in Umudike showing varying anthracnose resistance levels. All samples of visibly healthy young leaves of *D. alata* (about 3 months old) were first surface sterilized using 20% Sodium hypochlorite solution for 1 minute then gently rinsed in 3 changes of sterile distilled water and placed inside a sterile transparent plastic plate lined with moistened blotter papers. The leaves were inoculated with 30µL drop of the inoculum suspension at the concentration of 10^6 spores ml^{-1} on the abaxial surface. Two controls were established a positive control comprising of healthy *D. alata* leaves inoculated with 30µL drop of sterile distilled water and a negative control comprising of leaves not infected at all (Plain). The experimental design was a completely randomized design (CRD) replicated three times. Incubation in the laboratory at was at 28 ± 2^0c and observation made for 21 days for development of lesions.

The resistance of *D. alata* genotypes was evaluated based on the severity of anthracnose infection observed on a 1-5 points scale thus:

1= 0% symptom seen= Highly resistant
2= 1 -25% area with symptoms= Resistant
3= 26-50% area with symptoms = Moderately resistant
4= 51-75% area with symptoms= Susceptible
5= 76% and above upto dying = Highly susceptible

III. Results

Necrotic infections occurred on the healthy leaves inoculated with the spore suspensions of the test fungus (*C. gloeosporioides*) as against the inoculated leaves of the positive and negative controls. On the pathogen inoculated *D. alata* leaves disease severity progressed over time as diameter of the necrotic areas increased (Fig.1). Results of the three different sets of crosses (Series 1401, 1506 and 1512) of *D. alata* yielding 3 groups of F_1 mapping populations screened for resistance to yam anthracnose are shown in Fig 2-4. Out of 129 genotypes in Series 1401, 46 genotypes representing 35.66% were found to be highly resistant. Seventy one (71) genotypes (55.04%) were resistant, 8 genotypes (6.20%) moderately resistant, 4 genotypes (3.10%) were susceptible while no genotype was highly susceptible (Fig 2). Out of the 108 genotype in series 1506, 25 yam genotypes (51.02%) showed highly resistance 44 genotypes (40.74%) were resistant, 5 genotypes (4.63%) moderately resistant, 4 genotypes (3.10%) susceptible and none highly susceptible (Fig 3). In series 1512, out of 108 genotypes, 58 (58.54%) were highly resistant, 44 genotypes (40.74%) were resistant, 5 (4.63%) were moderately resistant, 4 genotype (3.10%) susceptible and none was highly susceptible (Fig 4).In summary, the

genotype TDa/1408/1086, TDa/1408/313, TDa/14018/366; TDa-EB-15-1512-78231, TDA-EB-12-TDA-EB-15-15-1512-9340 and TDA-EB-12-1512-59352; AND TDA-UM-15-1506-12065, TDA-UM-15-1506-22594 from the three series (1401, 1512 and 1506) respectively were highly resistant to *Colletotriclum gloeosporioides* infection while TDA/14018/1071, TDA-EB-15-1512-34640, and TDA-UM-15-1506-34892 were highly susceptible.

IV. Discussion

With the establishment of disease evidenced in the development of leaf necrosis on the inoculated areas, pathogenicity of *Colletotriclum gloeosporioides* isolates were confirmed by using different *D. alata* genotype according to Agrios (2005). The observed progression of disease severity with time in the detached leaf essay adds up to the list of possible alternatives for providing and investigating disease resistance in plant breeding programs in yams.Generally among the three series of the F_1 segregating progenies, more genotypes when assayed proved resistant to *D. alata* anthracnose disease as against fewer susceptible lines. This agrees with the result of other workers- Mignouna *et al,* (2001), Abang *et al,* (2006). Abang (1997) commemorated in Mignouna *et al* (2001) were of the opinion that the source of inheritance of resistance genes in segregating F_1 progenies of D. alata may be due to expression of one or more dominant R genes. These resistant genes are most cases race specific (vertical) and race non-specific (horizontal) in *D.alata* as observed by Chakraborty *et al,* (1989), Kelewu *et al,* (1996). The result of this experiment using the detached leaf assay technique are comparable to earlier experiments done in the screenhouse and field environment (Onyeka *et al* ,2006).With other pathosystems such as screening of common beans (*Phaseolus vulgarise* L) against angular leaf spot (Rezene *et al,* 2018), or against anthraenose by *Colletotrichum* species (Elliston *et al,* 1996) and Tu (1986), clear differentiations between susceptible and resistant lines in fixed liner and segregating progenies have been made.

V. Conclusion

This study has proved the detached leaf assay technique to be an effective, simple, rapid and economical means of screening for yam anthracnose disease resistance among *D. alata* genotype. It will be very useful in handling large scale evolution of genotype in yam breeding programs as well as other pathosystems. This can also be used in combination with molecular markers to facilitate progeny selection during marker assisted gene pyramiding.

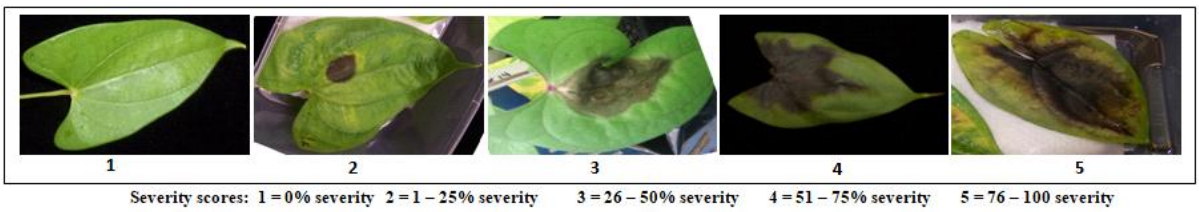

Fig 1: The various responses of F1 progeny leaves on inoculation with *C. gleosporioides*

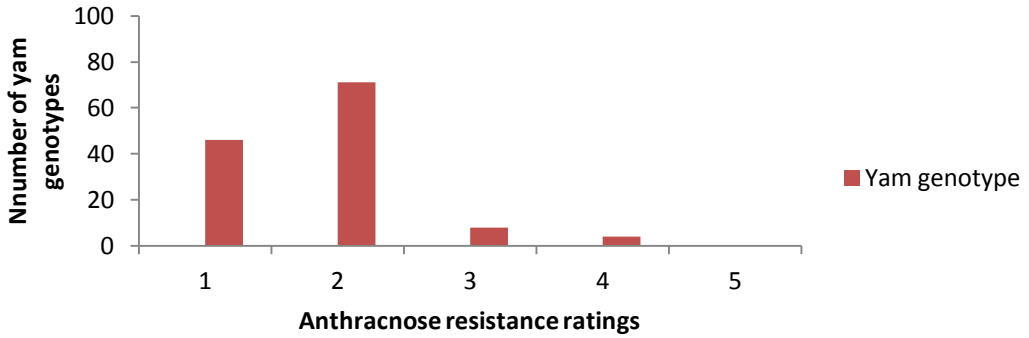

Fig 2: Responses of F1 progenies of Series 1401 of *D. alata* genotypes to *C. gleosporioides* inoculations

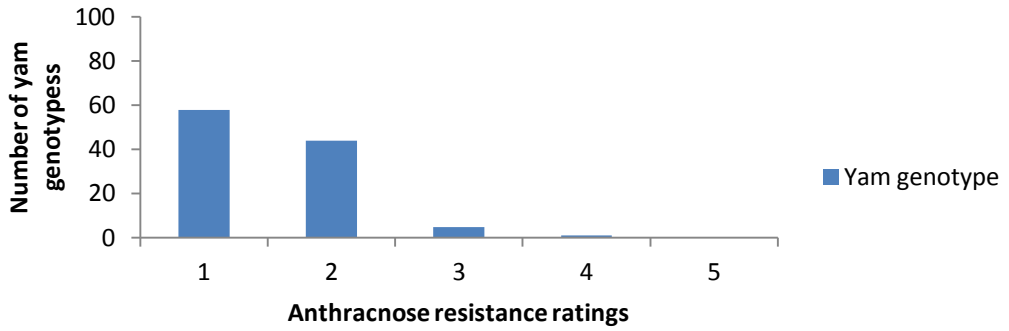

Fig 3: Responses of F1 progenies of Series 1512 of *D. alata* genotypes to *C. gleosporioides* inoculations

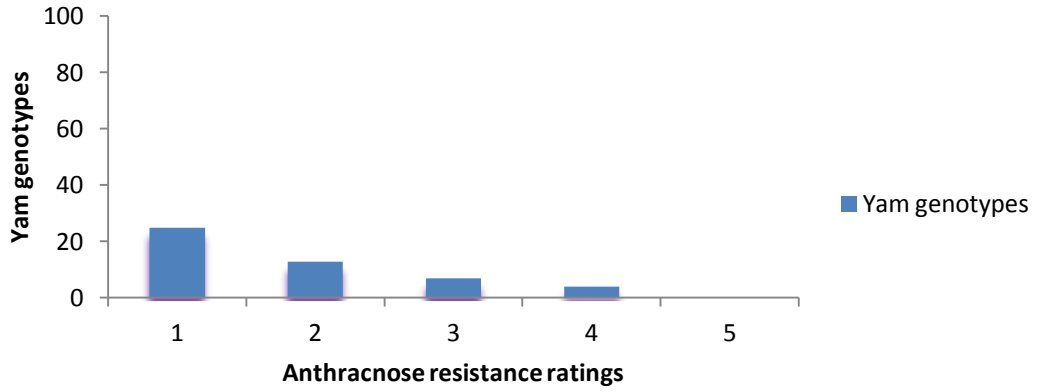

Fig 4: Responses of F1 progenies of Series 1506 of *D. alata* genotypes to *C. gleosporioides* inoculations

Conflict of Interest

The authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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