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Rapid assessment of resistance of tissue-cultured water yam (Dioscorea alata) and white guinea yam (Dioscorea rotundata) to anthracnose (Colletotrichum gloeosporioides Penz.)

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Rapid assessment of resistance of tissue-cultured water yam (*Dioscorea alata*) and white guinea yam (*Dioscorea rotundata*) to anthracnose, (*Colletotrichum gloeosporioides* Penz.)

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Yam anthracnose is caused by the pathogen *Colletotrichum gloeosporioides* Penz. and has been identified as the most important biotic constraint to yam production worldwide. Rapid assessment of the disease is vital to its effective diagnosis and management. In this study, tissue-cultured yam plantlets of five lines of *Dioscorea alata* and nine of *D. rotundata* were rapidly assessed for their reactions to two isolates of yam anthracnose. The plantlets, obtained from meristem of the nodal cuttings, were grown for 8 weeks on Murashige and Skoog (MS) basal medium, acclimatised for 3 weeks, hardened for an additional 3 weeks, arranged in screen house in completely randomised design and sprayed with spore inocula prepared from 7 day-old culture of the two strains of *Colletotrichum gloeosporioidies* Penz. The relative resistance of the different *Dioscorea* spp. was evaluated using three disease indices – severity at seventh day after inoculation, SD7; area under disease progress curve, AUDPC; and disease severity rate, Rd. A modified rank-sum classification method put TDa 1425 and TDr 2040, with rank sum of 2.0 each, as resistant. TDr 2121, TDr 2287 and TDr 2048 were susceptible with rank sum of 27.50, 25.50 and 24.50, respectively. *Dioscorea alata* TDa 1425 and *Dioscorea rotundata* TDr 2040 were recommended in areas endemic with yam anthracnose, and also as parent lines while breeding for resistance to anthracnose.

Keywords: Yam anthracnose; tissue culture; disease assessment

Introduction

Yam (*Dioscorea* spp.) belongs to the family Dioscoreaceae. It is a large genus that contains 600 species, important as food or as source of bioactive substance used in a range of applications (Mignouna and Abang 2009). Of the 600 known species of yams, only six are edible or food yams. Some of them are *Dioscorea rotundata*, *D. cayenensis, D. alata, D. dumetorum, D. esculanta* and *D. bulbifera* (Mignouna et al. 2003).

Dioscorea spp. constitutes an economically important staple food for millions of people in the tropics and sub-tropics. West Africa accounts for about 95% of world production and 93% of the total yam production area, with Nigeria topping the world in 2010 with a total yam production of 29 million metric tons (FAO 2012).

The long growth cycle of yam, lasting about 8 months or more, exposes the crop to a wide range of pests and disease among which are bacteria (*Erwinia* spp.); nematodes (*Scutellenoma bradys*, *Meloidogyne* spp.); virus (Yam mosaic virus (YMV) genus

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potyvirus); insect pest (*Crioceris livida*, *Heterolygus* spp.) and fungi (*Colletotrichum gloeosporioides*) which can be particularly damaging (Green 1998).

Yam anthracnose caused by *Colletotrichum gloeosporioides* Penz. [Teleomorph *Glomerella cingulata* (Stonem.) Spauld and Schrenk] remains a major threat to the cultivation of yam worldwide (Abang and Wanyera 2001) attacking all yam plant parts e.g. leaves tubers and seeds of yam (Abang et al. 2002).

Anthracnose disease causes leaf necrosis and shoots die-back in yams, thus, reducing the photosynthetic efficiency of the plant with resultant yield losses of over 90% in susceptible genotypes (Green and Simeon 1994). It is an important foliar disease of food yams *(Dioscorea* spp.*)* and has been reported on almost all cultivated species of yam in the humid and sub-humid tropics (Emehute et al. 1998).

Attempt to increase yam production by control of this disease using fungicide led to increased production but has adverse effects such as fungicide persistence in the environment and increased production cost. It could also lead to the development of chemically resistant strains of a pathogen (Onyeka et al. 2006b). The use of host plant resistance is a more sustainable and environmentally acceptable management strategy (Green et al. 2000; Onyeka et al. 2006b).

However, host plant resistance is hampered by uncertainties regarding the variability of the yam anthracnose pathogen and the availability of a rapid and reliable method of screening the host for disease resistance (Onyeka et al. 2006b).

This paper reported the outcome of a rapid assessment of resistance to anthracnose in tissue culture-derived plantlets of five lines of *D. alata* and nine of *D. rotundata.*

Materials and methods

Five accessions of *D. alata* and nine of *D. rotundata* were obtained from the Genetic Resources Unit (GRU) of I.I.T.A., Ibadan, Nigeria (Table 1). All the accessions were evaluated for responses to two strains of *C. gloeosporioides*, designated as Isolates A and B. The fungal isolates, also obtained from IITA, were maintained in culture tubes and were sub-cultured in Potato Dextrose Agar (PDA) for 7 days before they were used for inoculum preparation.

Tissue culture-derived plantlets were obtained from the meristem of the nodal cuttings of both *D. alata* and *D. rotundata*. The *in vitro* plantlets were grown for 8 weeks on a solid Murashige and Skoog (MS) basal medium under 16 h photoperiod at 24 ± 2 °C. These plantlets was then transferred from the tubes into polythene bags containing sterilised soil, and acclimatised in a growth room for 3 weeks at 28 °C/25 °C day/night

Table 1. Lines of *D. alata* and *D. rotundata* assessed for resistance to anthracnose.

Dioscorea alata lines	*Dioscorea rotundata* lines
TDa 1412	TDr 1438
TDa 1422	TDr 2032
TDa 1425	TDr 2040
TDa 1432	TDr 2048
TDa 1435	TDr 2105
	TDr 2110
	TDr 2121
	TDr 2191
	TDr 2287

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temperature and at 16 h photoperiod. Acclimatised plantlets were transferred into plastic pots and grown for an additional 3 weeks in the greenhouse for further hardening. Potted plants was transferred into a screenhouse and maintained at high RH (>80%) required for disease development.

Inoculum suspension was prepared from each of the two strains separately by washing off the fungal culture grown on Potato Dextrose Agar for 7 days with sterile distilled water. This was then filtered through double layer cheesecloth to separate mycelia from spore suspension and adjusted to 10^5 conidia/ml using haemocytometer.

The inoculum was sprayed on two leaves of each yam plant in three replicates. They were then kept in humidified condition for 24 h to aid disease development. Yam accession sprayed with sterile distilled water was used as the control.

The resistance of the yam varieties to *Colletotrichum gloeosporioides* was evaluated based on single-point assessment using disease severity score at the seventh day after inoculation (SD7), area under the disease progress curve (AUDPC) and disease progress rate (Rd).

The inoculated plants were scored for disease severity at 3, 5 and 7 days after inoculation (DAI) on a scale of 0–6 using the percentage whole plant area scoring method of Simeon and Green, (1994) with slight modification; 0 = 0%; 1 = 1%; 2 = 2%; 3 = 3–9%; 4 = 10–24%; 5 = 25–50% and 6 > 50% affected plant leaves (Onyeka et al. 2006b).

AUDPC was computed from the mean disease severity ratings as:-

$$\text{Area} = \sum_{\mathrm{i}=1}^{n} \left[\frac{(X_{\mathrm{i}} + X_{\mathrm{i}+1})}{2} \right] t \qquad (1)$$

where X_{i} is the severity rating at time i and $X_{\mathrm{i}+1}$ is the rating at time i + 1.

Disease severity rate (Rd) was a measure of disease severity over time.

The relative resistance of the different *Dioscorea* spp. was evaluated using a modified rank-sum classification method (Onyeka et al. 2006a) based on the mean AUDPC scores of each variety for the two pathogen isolates. Technique of Onyeka et al. (2006b) was followed while calculating rank sum. The AUDPC scores of the lines averaged across the two trials for each pathogen isolate were assigned ranks in ascending order using the rank procedure of GenStat. The rank sum was computed for each variety and compared with the grand mean of the rank sums across all varieties. Deviation of each variety from the grand mean was calculated. These varieties were classified based on deviation (d) from the grand mean as follows: −2.0 to −1.1, resistant; −1.0 to –0.1, moderately resistant; 0.0 to 1.0, moderately susceptible; and 1.1 to 2.0, susceptible (Onyeka et al. 2006a).

All statistical analyses were carried out using Genstat Statistical package release 7.2 DE3, copyright 2008, VSN International Limited.

Results

The two isolates of *Colletotrichum gloeosporioides* produced anthracnose symptoms on the five lines of water yam (*Dioscorea alata)* and nine lines of white guinea yam (*Dioscorea rotundata).* The extent of symptoms was evaluated using three disease parameters – disease score at the seventh day (SD7); area under disease progress curve (AUDPC) and disease progress rate (Rd).

These three disease parameters revealed wide variation in the response of lines of *D. alata* and *D. rotundata* (Table 2) to the two fungal isolates. TDa 1425, TDr 2040, TDr

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Table 2. SD7 and rank-sum resistance classification of *Dioscorea* spp. inoculated with two isolates of anthracnose pathogen, *Colletotrichum gloeosporioides.*

Line	of *Dioscorea* spp.	*Colletotrichum gloeosporioides* isolate A				*Colletotrichum gloeosporioides* Isolate B				Rank-sum classification		
		SD7	Rd	AUDPC 1	RANKING R1	SD 7	Rd	AUDPC 2	RANKING R2	c	d	Group
1	TDa 1412	R 3.00	0.50	5.00	7.50	R 2.00	0.25	4.00	7.50	15.00	0.01	MS
2	TDa 1422	R 4.00	0.50	7.00	10.50	R 2.00	0.50	3.00	5.50	16.00	0.13	MS
3	TDa 1425	R 0.00	0.00	0.00	1.00	R 0.00	0.00	0.00	1.00	2.00	−1.46	R
4	TDa 1432	R 4.00	0.50	8.00	12.00	R 0.00	0.00	0.00	1.00	13.00	−0.21	MR
5	TDa 1435	R 2.00	0.38	3.00	6.00	R 1.50	0.25	3.00	5.50	11.50	−0.38	MR
6	TDr 1438	S 5.00	1.00	6.00	9.00	S 5.00	0.50	9.00	13.00	22.00	0.81	MS
7	TDr 2032	R 3.00	0.25	5.00	7.50	S 5.00	0.50	9.00	13.00	20.50	0.64	MS
8	TDr 2040	R 0.00	0.00	0.00	1.00	R 0.00	0.00	0.00	1.00	2.00	−1.46	R
9	TDr 2048	R 4.00	0.25	7.00	10.50	S 5.00	0.25	10.00	14.00	24.50	1.09	S
10	TDr 2105	R 0.00	0.00	0.00	1.00	S 4.50	0.25	4.00	7.50	8.50	−0.72	MR
11	TDr 2110	R 0.00	0.00	0.00	1.00	R 3.50	0.25	6.00	9.00	10.00	−0.55	MR
12	TDr 2121	S 5.00	0.50	10.00	14.50	S 5.00	0.50	9.00	13.00	27.50	1.43	S
13	TDr 2191	S 5.00	0.50	9.00	13.00	R 4.00	0.50	7.00	10.00	23.00	0.92	MS
14	TDr 2287	S 5.00	0.50	10.00	14.50	R 4.00	0.25	8.00	11.00	25.50	1.2	S
15	CONTROL	R 0.00	0.00	0.00	1.00	R 0.00	0.000	0.00	1.00	2.00	−1.46	R
	Grand Mean, G									14.87		
	Std Dev, SD									8.83		

SD7 = Resistance assessment on the basis of a single diseases score at the seventh day after inoculation on a scale of 0–6, where scores ⩽ 4 are considered resistance reactions and scores > 4 are considered susceptible reactions.
Rd, Disease severity rate = disease severity over time.
AUDPC 1 = Cumulative area under the disease progress curve with isolate A and AUDPC 2 = Cumulative area under the disease progress curve with isolate B.
R1 = Lines of *Dioscorea* spp. ranking on the basis of AUDPC 1 and R2 = Lines of *Dioscorea* spp. ranking on the basis of AUDPC 2.
c = Rank sum (R1 + R2) for each line of *Dioscorea* spp.
d = Deviation from grand mean (G) of rank sums [d = (C – G) / SD].
R = Resistance; MR = Moderately resistance; MS = Moderately Susceptible; and S = Susceptible.

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2105 and TDr 2110 had value 0 for the three parameters, indicating resistance, to Isolate A. In Isolate B, TDa1425, TDa1432 and TDr 2040 were resistant. Based on the rank-sum classification (Table 2), 2 lines (14.3%) were resistant, 4 lines (28.6%) moderately resistant, 5 (35.7%) moderately susceptible and 3 (21.4%) susceptible.

The rank-sum classification agreed to some extent with isolate-specific SD7 ranking (Table 2). TDa 1425 and TDr 2040 showed resistance both at SD7 (for the two isolates) and at group rank-sum classification.

Types of *Dioscorea* lines significantly influenced the response to anthracnose infestation. This was indicated in two of the disease parameters, except the disease progress rate, Rd (Table 3). However, the type of isolate used became an issue only when Rd was used to evaluate the disease. The other two parameters did not ascribe significance to type of pathogen isolate. The isolate–line interaction was significant for all parameters, indicating that *Dioscorea* lines and *Colletotrichum gloeosporioides* types of isolate interacted to produce significant effect on rank-sum classification of resistance status (Table 3).

Ranking of the disease parameters in Table 4 showed that AUDPC was the most effective parameter for disease evaluation, while Rd was the least effective based on percentage variance.

Discussion

Percentage variances of the three parameters were above 50%, indicating that more than half of the sources of variations were accounted by the disease parameters. This meant that any of the three parameters could actually be used in this disease evaluation

Table 3. Summary of generalised linear mixed modelling of the reaction of tissue culture-derived whole plants of 14 lines of *Dioscorea* spp. to anthracnose disease severity evaluation.

			SD7		AUDPC		Rd	
Fixed term	df	rdf	F	P	F	P	F	P
Lines	14	28	27.61	<0.001	22.59	<0.001	49.00	0.090
Isolates	1	28	9.00	0.205	49.00	0.090	19.36	<0.001
Lines x isolate	14	28	9.00	<0.001	8.83	<0.001	5.57	<0.001

SD7 = Disease severity score at the seventh day after Inoculation.
AUDPC = Area under the disease progress curve.
Rd = disease progress rate.
Rdf = Residual degree of freedom.
F = F statistic.
P = Probability of F statistic.

Table 4. Effectiveness of indices of disease measurement as indicated by percentage variance.

Indices of disease measurement	Percentage variance	Standard error	Comment
SD7	66.4	0.147	Effective
AUDPC	96.5	0.737	Most effective
Rd	52.3	2.70	Less effective

SD7 = Single disease score at the seventh day after inoculation.
AUDPC = Area under disease progress curve.
Rd = Disease progress rate.

exercise. Area under disease progress curve (AUDPC) with percentage variance of 96.5 ranked the best of the three parameters. Some *Dioscorea* lines manifested early but slow development of disease while others showed the symptom late but the disease developed more rapidly. In view of this, and considering the view of Onyeka et al. (2006b), AUDPC as a parameter that was anchored on multiple disease score assessment would be the most preferable. Simon and Green (1994) observed that the use of quantitative multiple method of disease assessment gave a better representation of disease status of yam (*Dioscorea alata).* Sweetmore et. al. (1994) also identified disease progress curve as the most suitable tool to estimate severity, placing the emphasis on the earliest, least visible stages of the disease.

The development and utilisation of anthracnose resistant varieties represent a potential control measures that could be an important component of disease management strategies. This is considered as appropriate for the subsistent farmers in view of the high genotypic diversity of the pathogen as reported by McDonald and Linde (2002) and Amusa et al. (2003).

Abang and Wanyera (2001) proposed inoculation of tissue culture plantlets for rapid screening of yam genotypes for resistance to anthracnose under a controlled environment. This has been optimised by Onyeka et al. (2006a, 2006b), and had been applied in this study to evaluate different levels of anthracnose resistance in yam accessions.

The result obtained were in agreements with that of Onyeka et al. (2006b) where all the used *D. alata* cultivars showed no isolate specific reactions as they were all either resistant, moderately resistant, moderately susceptible or susceptible to the two *C. gloeosporioides* isolates used, indicating that variation among pathogens isolates may not be an important considerations in assessing for resistance or susceptibility in *D. alata* varieties. The use of single but highly aggressive isolate in screening for host resistance has been recommended for some plant disease systems (Arseniuk and Czembor 1999).

However, *Dioscorea rotundata* lines showed isolate-specific reactions to the *C. gloeosporioides* isolates with some varieties being resistant to Isolate 2 while others were susceptible. TDr 2040 and TDr 2121 were the only lines that were resistance or susceptible, respectively, to the two isolates using the two parameters of SD7 and rank-sum classification. Mignouna et al. (2001) confirmed that resistance to yam anthracnose could be isolate specific in *D. rotundata*. It is therefore important that *D. rotundata* varieties be subjected to different *C. gloeosporioides* isolates found in other locations in other to fully reveal the full spectrum of anthracnose resistance or susceptibility in *D. rotundata* germplasm.

Conclusion

Tissue culture-based assay has the potential to rapidly assess large number of yam germplasm for resistance to yam anthracnose. However, it is important that different isolates of *Colletotrichum gloeosporioides* and multiple disease indices be employed for the assessment in other to fully evaluate isolate host-specific reactions.

It is recommended that the use of TDa 1425 and TDr 2040 be intensified while planting in areas endemic with yam anthracnose.

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