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Inheritance of resistance in water yam (*Dioscorea alata*) to anthracnose (*Colletotrichum gloeosporioides*)

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Abstract *Colletotrichum gloeosporioides* causes anthracnose, the most severe foliar disease of field-grown water yam (*Dioscorea alata*). The inheritance of resistance to a moderately virulent (FGS) strain of the pathogen was investigated in crosses between tetraploid *D. alata* genotypes: TDa 95/00328 (resistant)×TDa 95–310 (susceptible) (cross A), and TDa 85/00257 (resistant)×TDa 92–2 (susceptible) (cross B). Segregation of F_1 progeny fitted genetic ratios of 3:1, 5:1 (crosses A and B) and 7:1 (cross A) resistant:susceptible when inoculated with the FGS strain, indicating that resistance is dominantly inherited and suggesting that more than one gene controls the inheritance of resistance to this strain in the accessions studied. When parental and progeny lines of cross A were inoculated with an aggressive (SGG) strain of the pathogen, all plants expressed a susceptible phenotype, indicating strain-specific resistance in TDa 95/00328. Screening of 20 cultivars/landraces confirmed the high susceptibility of *D. alata* accessions to the SGG strain and revealed the presence of apparent strain non-specific resistance in TDa 85/00257. TDa 85/00257 and TDa 87/01091 which were resistant to the SGG strain, will be useful both as sources of resistance and in the development of a host differential series for *D. alata*.

Keywords *Colletotrichum gloeosporioides* · Yam · Anthracnose · Screening · Inheritance · Resistance

Introduction

Yam anthracnose is caused by *Colletotrichum gloeosporioides* Penz. [teleomorph *Glomerella cingulata* (Stonem.) Spauld. & Schrenk]. This pathogen induces leaf necrosis and shoot die-back, hence reducing the effective photosynthetic surface of the crop with deleterious effects on tuber yield. The disease is particularly severe on *Dioscorea alata*, the most widely distributed species (Nwankiti and Ene 1984; Winch et al. 1984). Yield reductions of more than 80% have been reported from West Africa (Nwankiti et al. 1984) and the Caribbean region (Mignucci et al. 1988; Green 1994).

Natural resistance to anthracnose can be found in the available germplasm, mainly among landraces of *D. alata* (Degras et al. 1984; Nwankiti and Ene 1984; Nwankiti et al. 1987; IITA 1993; Abang 1997). Little is known concerning the inheritance of resistance to anthracnose in yam, due to the lack of segregating yam populations. Hybridization of yam has become feasible due to a better understanding of the reproductive biology of cultivated yam (Asiedu 1994).

Some insight has been gained into the diversity of *C. gloeosporioides* from yam, with at least two genetically distinct virulence phenotypes (SGG and FGS strains) having been reported (Thottappilly et al. 1999; Abang et al. 2001). Differential interactions have been observed between *D. alata* genotypes and isolates of *C. gloeosporioides* (Abang 1997; Green et al. 2000), but analysis of variance tests indicated that interactions contributed less to overall variation than the main effects due to isolate and cultivar. The continuous variation in anthracnose resistance observed in natural *D. alata* populations in the field (IITA 1993), and the gradation in mean cultivar responses across isolates in controlled experiments, led Abang (1997) to assume that resistance is inherited polygenically.

In this paper, we examine the inheritance of resistance in two *D. alata* parental accessions using a moderately virulent fast-growing salmon (FGS) strain and an aggressive slow-growing grey (SGG) strain of *C. gloeosporioides* from yam.

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Materials and methods

Plant materials

Two crosses were made between tetraploid yam accessions with contrasting reactions to anthracnose (TDa 95/00328×TDa 95–310, cross A, and TDa 85/00257×TDa 92–2, cross B). The susceptible parent TDa 95–310 was a landrace. The resistant parent TDa 95/00328 was a breeding line which originated from a controlled cross between the susceptible landrace TDa 92–2 and the resistant breeding line TDa 85/00257. TDa 95/00328 has consistently shown field resistance to anthracnose across locations. The parents were planted and crossed in the field. Progenies from these crosses were sown in seedling nurseries to generate minitubers. Minitubers were later planted in pots in the screenhouse for in vitro multiplication using nodal cuttings. In vitro shoot cultures were grown according to the method of Ng (1992). The in vitro plants were used in the screening experiments after a growth period of 2 weeks, at which stage they had developed 3–5 young, but fully expanded, leaves. Leaf age was determined as described by Simons and Green (1994) and Sweetmore et al. (1994).

Fungal strains

Two single-spore isolates, Cg33 (FGS) and Cg25 (SGG), were obtained from a *C. gloeosporioides* culture collection at IITA, Ibadan-Nigeria (Abang 1997). The virulence of these isolates was confirmed by inoculation of healthy yam leaves and re-isolation in pure culture. The isolates were grown on potato dextrose agar (PDA) treated with streptomycin (300 ppm) at 28°C under an alternative 12-h fluorescent light (60 µE $m^{-2}s^{-1}$)/12-h dark to limit mycelial growth and induce sporulation (Green et al. 2000). Seven-to-ten-day old plate cultures were flooded with sterile distilled water supplemented with the wetting agent Tween 80 (2% v/v) and conidia were gently scraped off the plates. Spore density was adjusted with a hemacytometer and inoculum concentration was 10^6 spores ml^{-1}.

Screening tests

Three days before inoculation the plants in the screenhouse were transferred to a containment facility (conditions: natural 12-h daylight/night cycle, 27±1°C and 98–100% RH). Leaves of all ages including shoots were inoculated; however, symptoms were scored only on the young fully developed leaves. An artist's paintbrush was used to apply newly prepared inoculum onto both leaf surfaces to the point of run-off. Inoculum was allowed to adhere on the leaves for about 30 min, after which leaves were misted twice a day with sterile distilled water using an atomizer.

The parental lines and F_1 progeny were tested with both strains in separate experiments conducted at the same time. In both experiments, the layout was a completely randomised design with two clones per genotype. Non-inoculated plants that had their leaves brushed with only sterile distilled water amended with Tween 80 (2% v/v) were used as controls. The disease was scored 7 and 14 days after inoculation with the FGS strain, and 4 and 7 days after inoculation with the SGG strain. Disease severity was evaluated using the individual leaf method of Simons and Green (1994) and Sweetmore et al. (1994), in which percentage of leaf area affected by anthracnose was scored on a 0–6 scale. Genotypes with a mean leaf area damage of 0–17.5% (corresponding to scores 0–4) were considered to be resistant, while those with a mean leaf area damage of >17.5% (corresponding to scores 5 and 6) were considered susceptible. In another experiment, 20 *D. alata* parental accessions were screened with an aggressive (SGG) *C. gloeosporioides* strain from yam, in order to identify sources of resistance to this strain.

Data analysis

The GLM procedure of SAS (SAS Institute Inc. 1989) was used to test hypotheses concerning the main effects of treatments. Mean separation was by Fisher's Least Significant Difference (F-LSD). Chi-square (χ^2) analysis were carried out to test for Pearsons goodness-of-fit to specific genetic segregation ratios.

Results

TDa 95/00328 was resistant to the FGS strain but highly susceptible to the SGG strain. TDa 85/00257, however, showed resistance to both the FGS and SGG strains, indicating that the two resistant accessions carry different genes for anthracnose resistance. Segregation into resistant and susceptible phenotypes occurred in both crosses following inoculation with the FGS strain and indicated the presence of R genes in both F_1 progeny (Table 1). There were significant differences in disease severity ($P \leq 0.01$) between the resistant and susceptible parents in both crosses. Also, the variation between F_1 individuals was significant in both crosses (LSD, $P \leq 0.01$).

Table 1 presents the number of genotypes susceptible or resistant to the FGS strain. Screening with the FGS strain showed that restricted, or no, lesions developed on resistant parental and progeny lines up to 14 days after inoculation (DAI) whereas susceptible lines were clearly

Table 1 Segregation ratios of resistant and susceptible genotypes in crosses between resistant breeding lines and susceptible landraces, after inoculation with a fast-growing salmon (FGS) strain of *C. gloeosporioides* from yam

Cross, R×S[a] (code)	Isolate (strain)	*n*	Observed (R:S)	Expected segregation[b]		
				Ratio (R:S)[c]	χ^2	*P*
TDa 95/00328	Cg33	71	58:13	3:1	1.69	*P*=0.19
×TDa 95–310	(FGS)			5:1	0.13	*P*=0.72
(A)				7:1	2.19	*P*=0.14
TDa 85/00257	(FGS)			5:1	2.87	*P*=0.09
×TDa 92–2 (B)	Cg33	37	27:10	3:1	0.08	*P*=0.77

[a] R=resistant, S=susceptible
[b] Frequencies of segregation expected assuming Mendelian inheritance of dominant R genes
[c] Inferred genetic constitution for the different segregation ratios are: 3:1=2 R genes (Ra and Rb) in simplex, 100% chromosome segregation; 5:1=1 R gene in duplex, 100% chromosome segregation; 7:1=3 R genes (Ra, Rb, and Rc) in simplex, chromosome segregation

diseased by 7 DAI. After the inoculation of 71 progeny with Cg33 (FGS), 58 resistant and 13 susceptible genotypes could be identified (Table 1). The observed segregation pattern fits almost perfectly to a 5 resistant: 1 susceptible ratio (χ^2=0.13, P=0.72), which is in agreement with the presence of a single dominant locus in a duplex configuration in TDa 95/00328. Ratios of 3:1 (P=0.19) and 7:1 (P=0.14) were also possible but gave a weaker fit. Two resistant genes may have segregated in the progeny of cross B, as the segregation pattern gave a very close fit to a ratio of 3:1 R:S (χ^2=0.08, P=0.77), which is expected for two independent R genes in a simplex status. A 5:1 ratio was also possible for cross B by the χ^2 test, suggesting the action of a single dominant gene in duplex status, but there was only a weak fit to this ratio (χ^2=2.87, P=0.09). The assumption of 7:1 was rejected by the χ^2 test (P=0.008).

Of the 20 parental accessions tested, only TDa 87/01091 and TDa 85/00257 showed resistance to the SGG strain, with a significantly ($P \leq 0.05$) lower disease severity score than the others. The resistant cultivars also suffered no leaf abscission and showed only restricted shoot lesions. In contrast, severe foliar necrosis, abundant sporulation, and abscission of inoculated leaves followed by severe die-back and premature death of plants was observed on all other accessions by 7 DAI. Symptoms first appeared on all plants 2–3 DAI, and days to appearance of the first symptoms was not a good indicator of resistance. In contrast, score-6 symptoms (>50% of leaf area infected) appeared on susceptible accessions 3–4 DAI, whereas score-5 symptoms were observed on the two resistant accessions 6–7 DAI.

Discussion

We studied the inheritance of resistance to anthracnose in *D. alata*. Segregation into resistant and susceptible individuals in progenies of crosses A and B indicated expression of one or more R genes in the resistant parents studied. The segregation towards a resistant response in both F_1 mapping populations suggests that resistance to the FGS strain has a dominant nature. Both race-specific and race non-specific resistance appear to be present in *D. alata*, which agrees with findings on the *Stylosanthes-C. gloeosporioides* pathosystem (Davies et al. 1984; Miles and Lenné 1984; Chakraborty et al. 1988; Kelemu et al. 1996).

Assuming Mendelian inheritance of dominant R genes in tetraploid *D. alata*, the almost perfect fit to a ratio of 5:1 (resistant:susceptible) for progeny of cross A suggests the action of a single dominant resistance gene in a duplex configuration in the breeding line TDa 95/00328.

The FGS strain is considered the most-widespread form of *C. gloeosporioides* occurring on yam in Nigeria (Abang 1997; Abang et al. 2001), and has been shown by random amplified polymorphic DNA (RAPD) analysis to represent a genetically heterogeneous population (Thottappilly et al. 1999). The finding that resistance in TDa 95/00328 to the FGS strain is polygenically inherited may help explain its resistance to all FGS isolates tested so far, and the consistent 'rate-reducing' field resistance of the cultivar across locations. TDa 95/00328 was susceptible to the SGG strain, indicating that the resistance of TDa 95/00328 is specific for the FGS strain. Such strain-specific resistance is in agreement with earlier studies on *D. alata* (Abang 1997; Green et al. 2000; Abang et al. 2001). The SGG strain was initially thought to be restricted to the humid forest agro-ecology (Abang 1997), but has now been isolated from severely attacked yam in Mokwa, in the southern guinea savanna of Nigeria (Abang, unpublished).

Abang (1997) observed that *D. alata* cultivars possess characteristics consistent with both strain-specific (vertical) and strain non-specific (horizontal) resistance. Segregation ratios observed with progeny of cross B indicate that one or more genes control resistance in TDa 85/00257 to the FGS strain. Further research is needed to determine the number of genes involved, but the apparent 'rate reducing,' strain-nonspecific nature of the resistance suggests that the resistance is probably quantitatively inherited. The resistance of TDa 85/00257 to the SGG strain has implications for the anthracnose resistance breeding program. TDa 85/00257 and TDa 87/01091 are, to our knowledge, the only known sources of resistance to the aggressive SGG strain.

Besides searching for new sources of anthracnose resistance, we have developed strategies for marker-assisted selection for resistance breeding in yam. The mapping populations described in the present study, together with other populations being developed, will be used to search for molecular markers closely linked to anthracnose resistance genes.

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