A PRELIMINARY EVALUATION OF CELL AND TISSUE CULTURE METHODS SUITABLE FOR SCREENING ANTHRACNOSE DISEASE REACTIONS IN TROPICAL YAMS[1]

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ABSTRACT - Effects of different temperatures (15,19,25 and 29°C), media type (PDA and Czapek Dox agar) and light on sporulation of a Caribbean pathovar of *Colletotrichum gloeosporioides* f.sp. *dioscoreae* were studied. Czapek Dox agar and temperature of 25°C were found to be the best conditions for supporting growth and sporulation of this organism. Long-term fungal cultures (2 years axenic culture) did not sporulate effectively under the above conditions unless they were first brought into contact with living tissues of *Dioscorea*. Methods for inoculation of yams with conidiospores of *C.gloeosporioides* were developed at the plant, microplant and cell levels. *In vitro* shoot culture clones showed a similar range of disease reactions to the pathovar as field-grown plants. *D.alata* showed marked sensitivity to the disease while *D.esculenta* was the most tolerant species followed by *D.composita* and *D.cayenensis*. *Dioscorea* protoplasts responded differently when cultured in the presence of mycelial culture filtrates and Czapek Dox liquid medium. Glasshouse-raised plants developed anthracnose symptoms only after wounding.

Index terms: Sporulation, *Colletotrichum gloesporioides* f.sp. *dioscoreae*, Czapek Dox agar, microplant, clones.

AVALIAÇÃO DE MÉTODOS DE CULTURA DE CÉLULAS E TECIDOS VEGETAIS PARA SELEÇÃO DE DIFERENTES GENÓTIPOS DE *DIOSCOREA* SPP. RESISTENTES OU TOLERANTES À ANTRACNOSE.

RESUMO - Foram estudados os efeitos de diferentes temperaturas (15, 19, 25 e 29°C), meios de cultura (PDA ou agar Czapek Dox) e luz na esporulação de um patovar de *Colletotrichum gloesporioides* f.sp. *dioscoreae* originário do Caribe. As melhores condições para crescimento e esporulação deste isolado foram proporcionadas em meio Czapek Dox agar a 25°C. Após 2 anos em cultura, o fungo nao esporulou satisfatoriamente a menos que exposto a tecido vegetal do hospedeiro (*Dioscorea* spp.). Foram desenvolvidos métodos de inoculaçao de plantas, plântulas e células de *Dioscorea* utilizando conidiósporos de *Colletotrichum gloesporioides*. Encontrou-se uma relação positiva entre a reação dos diferentes clones em casa de vegetação e *in vitro*. *D.alata* mostrou-se extremamente suscetível à enfermidade enquanto *D.esculenta* provou ser a espécie mais resistente, seguido de *D.composita* e *D.cayenensis*. Protoplastos de *Dioscorea* responderam diferentemente quando cultivados em presença de filtrados de cultura de micélio ou meio Czapek Dox. Plantas inoculadas em casa de vegetaçao so desenvolveram sintomas de antracnose após ferimento com agulha.

Termos para indexação: esporulação, *Colletotrichum gloeosporioides* f. sp. *dioscoreae*, Czapek Dox agar, microplanta, clones.

INTRODUCTION

Anthracnose disease is a major constraint to *Dioscorea* yam production in many parts of the world (Nwankity & Okpala, 1981; Nwankity *et al*., 1984; Winch *et al*., 1984; Degras, 1986). Resistant varieties are required and research is being carried out to breed and/or select plants less susceptible to this

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disease (Degras *et al*., 1983; Nwankity & Okpala, 1984). Micropropagation methods for yams are now well proven (Mantell *et al*., 1978; Ammirato, 1984) and as such they represent an important tool for breeding programs in that vegetative clones can be multiplied rapidly and distributed easily. The availability of *in vitro* shoot cultures raises the possibility that rapid screening of yam germplasm might be possible at the *in vitro* level. Artificial inoculation methods for studying anthracnose on greenhouse-raised yams have already been developed (Nwankity & Okpala, 1981; Nwankity & Ene, 1983; Winch *et al*., 1984). However, besides being more rapid, cell and tissue culture methods could be important for evaluation of anthracnose disease reactions in yam germplasm as a way to overcome costly and laborious field trials, enabling the selection of superior plants at early stages of a breeding programme. *In vitro* screening of plantlets has already been utilized successfully for several disease interactions such as those of banana and *Mycosphaerella fijiensis* (Mourichon *et al*., 1987) and cassava and *Colletotrichum manihotensis* (Van der Bruggen *et al*., 1986). At the cell level, Crucefix *et al.* (1987) used infection of protoplasts of lettuce with spores and an extract solution of *Botrytis lactucae*. Pullman & Rappaport (1983) used culture filtrates of *Fusarium oxysporum* to select disease resistant variants of celery. Many other examples are described in the literature and reviewed by Daub (1986).

The aims of the present study were to develop suitable cell and tissue culture methods which might be useful for screening germplasm of tropical yams (*Dioscorea* spp.) for their susceptibility to anthracnose infections. Methods are given for obtaining spores of *Colletotrichum gloeosporioides* required for the inoculum preparation.

MATERIALS AND METHODS

Culture conditions and sporulation

Colletotrichum gloeosporioides Penz f.sp. *dioscoreae* was isolated from a *D.alata* cv. Crop Lisbon crop grown at Friendship Plantation in Barbados during a severe outbreak of anthracnose in 1985. Cultures were maintained on potato dextrose agar (PDA) plates for two years in the dark at 28°C and was positively identified by the Commonwealth Mycological Institute, Kew, UK in 1987. From the stock cultures, mycelia were collected and recultured for use in the inoculation studies. Disks (8 mm diameter, 3 mm thick) of agar containing mycelium were cut from 15-day-old cultures and placed in the center of sterile 9-cm petri dishes containing 20 ml of either PDA or Czapek Dox agar, (Oxoid Ltd products). Inoculated dishes were wrapped in aluminum foil and placed in growth rooms at 15, 19, 25 and 29°C. When mycelia reached the edges of the dishes, cultures were uncovered and exposed to fluorescent light (160 $\mu E.m^{-2}.s^{-1}$) for 16 hours per day in order to induce sporulation. Weekly assessments of sporulation were carried out thereafter by determining the number of spores in a 8 mm-diameter disk of mycelium grown in agar.

Since no sporulation occurred, factors such as a 24 hours of day light exposure, exposure to excised leaves of the host (*D.alata*) and contact with growing plants *in vitro* were tested for induction of sporulation. The fungus was exposed to growing plants *in vitro* by placing mycelium disks (8 mm-diameter) near the stems of four plantlets of *D.alata* clone 7334 (Barbados), grown in Murashige and Skoog medium (MS) in individual tubes for 15 days. Pellets of mycelium were collected from these tubes and transferred to PDA and Czapek Dox agar dishes for incubation at 28°C in the dark. Assessments of sporulation were carried out for three weeks.

After the re-establishment of the spore production capacity of the fungus, an experiment was carried out to test the effects of different media (Czapek Dox and PDA), pretratment with the host, and exposure to light on sporulation. Fourty petri dishes were used for this experiment, 20 containing Czapek Dox and 20 PDA. Half of the dishes containing each medium was inoculated with pellets of mycelium that had been in contact with *in vitro* growing plantlets. The other half was inoculated with pellets of mycelium previously grown in MS medium without plantlets. In order to test the importance of light in enhancing sporulation, half of the dishes of each treatment was exposed to artificial light (160 $\mu E\ m^{-2}\ s^{-1}$,16 hour photoperiod) for one week inside the growth chambers, and the other half was maintained in the dark. Petri dishes were incubated at 25°C. A second experiment tested the effect of temperature on sporulation. Twenty petri dishes containing Czapek Dox agar were inoculated with pellets of mycelium that had been in contact with *in vitro* growing plantlets. Petri dishes were incubated at either 15, 19, 25 or 29°C, in the dark. When the mycelium reached the edges of plates assessments of sporulation were

carried out by counting the number of spores present in 8 mm-diameter disks of mycelium grown in each of the set of conditions described. The experiments were analysed using one-way analysis of variance after arc-sin transformation of data, with 5 replicates.

Inoculum preparation

Inoculum consisted of either spore suspensions or mycelial culture filtrates. Spore suspensions were prepared aseptically using 25-day old fungal cultures grown on Czapek Dox agar plates held at 25°C. Disks of agar/mycelium were cut out of media plates using a 8 mm-diameter cork borer and placed in sterile distilled water contained in 10 ml centrifuge tubes. These were shaken for 5 minutes and the mixture filtered using a 400 µm sieve, to separate the mycelial and agar fractions from the spores. Following washing of the spores two times by centrifugation for 15 minutes at 200 g, the spore concentrations were adjusted to 5.0×10^5 spores ml^{-1} according to the method of Winch *et al.* (1984). Mycelial filtrates were used for inoculating isolated yam protoplasts as carried out by Crucefix *et al.* (1987) for studies on *Bremia* and lettuce interactions. A pellet of mycelium produced from a 8-mm-diameter disk was placed in 25 ml of Czapek Dox liquid medium (Bailey & Deverall, 1971; Van der Bruggen *et al*., 1986) and incubated in 250-ml flasks on a shaker at 25°C in the light. As a control, uninoculated Czapek Dox medium was kept under the same conditions. Cultures were grown for 15 days and then filtered through a 8-µm pore size membrane filter (Millipore), using a Sartorius twin chamber apparatus. The filtrate produced was utilized for the preparation of the protoplast medium.

Plant material

The species and clones of *Dioscorea* sp. held in the Wye yam tissue culture germplasm collection and used in this study were as follows: *Dioscorea alata* - Oriental Lisbon, Barbados clone 7334 (YMA^A), *D.alata* - Crop Lisbon, Barbados clone GHFP (YMA^B); *D.alata* - Crop Lisbon, Barbados clone PW33 (YMA^C); *D.alata* - Oriental Lisbon, Barbados clone 5446 (YMA^D); *D.alata* - cv. Kinabauo, originally from Philippines and cultivated in Solomon Islands (YMA^P); *D.esculenta* - University of West Indies, St.Augustine, Trinidad (YME); *D.cayenensis/rotundata* - INRA yam collection, Guadeloupe, FWI (YMCAY - seedling clone); *D.composita* - Ubatuba, Sao Paulo, Brasil (YMCOM - seedling clone).

Both greenhouse-grown and *in vitro*-cultured plants were used for inoculation experiments. For greenhouse, plants from each of the clones/species (except YMA^P) were transferred from 30 and 60-day-old *in vitro* cultures into a 1:1 vermiculite : potting compost (peat : sand, 9:1) contained in covered propagation trays (22 x 35 x 18 cm). Plants were kept in the glasshouse for 1 month prior to inoculations with *Colletotrichum* spores. *In vitro* plantlets (yam shoot cultures) were grown according to the method of Mantell *et al.* (1978) in MS medium (Flow Laboratories), 2% sucrose, 0.8% agar, pH 5.8, in a 25/29°C (night/day) growth room, in 2.5-cm-diameter x 10-cm-long tubes for 60 days under 16 h photoperiod (160 $\mu E\ m^{-2}\ s^{-1}$).

Protoplasts were isolated from medium-sized leaves (≤ 1.0 cm long) from shoot cultures of eight clones or species of *Dioscorea* cultured under the standard conditions described above. The leaves were finely chopped and placed in a plasmolysing medium for two hours prior to enzymatic digestion of tissues. This medium consisted of Macerozyme (0.1% w/v), Onazuka R10 cellulase (1.5% w/v), $CaCl_2$ (1500 mg l^{-1}), NaH_2PO_4 (150 mg l^{-1}), 2-[N-morpholino] ethane sulphonic acid (1500 mg l^{-1}), soluble polyvinyl pyrolidone (2% w/v) and mannitol (11% w/v), adjusted to pH 5.8. After an 18-hr digestion period the suspension was filtered through a nylon filter, 60-µm mesh size, and centrifuged for 7 min at 100 g to sediment protoplasts. The supernatant was poured off and fresh plasmolysis medium added to wash the protoplasts. This process was repeated three times with centrifugation 100 g for 5 min then protoplasts were mixed with a culture medium composed of full strength MS salts, 20 g l^{-1} glucose, 80 g l^{-1} mannitol, 2 mg l^{-1} 2,4-D, pH 5.8, the osmoticum of which was adjusted prior to autoclaving to 670 mOsm kg^{-1}.

Infection experiments

Whole plants inoculation. Plants from each clone or species were inoculated on the left half of both the upper and lower surfaces of each leaf using a soft-haired paint brush. Controls were distilled water applied on the right half of each leaf on the same plants. A second experiment was carried out but in this case inoculations were followed by wounding the leaf surfaces with a sharp needle as described by

Winch *et al.* (1984). Controls were brushed with distilled water which was applied at random to other leaves on the same plant. Both experiments used ten plants for each treatment, and only one leaf of each plant was inoculated. Observations of disease development were carried out for two weeks following inoculation.

To observe the presence of the pathogen in the lesions, excised leaves, previously infected as described before, were placed in methanol for 24 hr then placed in a 50% aqueous chlorohydrate solution for 5 min and mounted on microscope slides in a drop of 50 % glycerol. Specimens were examined using an Olympus IMT-2 inverted microscope.

***In vitro* plantlets inoculation.** One droplet (0.8 µl) of spore suspension (5 x 10^5 spores ml^{-1}) or distilled water (control) was applied to the upper surfaces of the leaves with a sterile 1.0 ml syringe. Each treatment was applied to an individual plantlet, and only one leaf of each plant was inoculated. Assessments carried out at 7 and 14 days after inoculation, were achieved by counting the number of plantlets that showed lesions and by classifying these as 1 (with lesions) or 0 (without lesions). This experiment was repeated, but inoculating both the upper and the lower surfaces of the leaves, and assessed at 14 and 28 days. Both experiments used four replicate tubes per treatment, and were analysed by one-way analysis of variance after arc-sin transformation of data.

Protoplasts inoculation. Protoplasts were cultured together with the mycelial filtrates at 100, 50 and 25% levels (v/v) in sterile distilled water. Controls consisted of Czapek Dox medium at the same dilutions. To test the possible effects that Czapek Dox might have on protoplasts, sterile double distilled water (SDW) was also utilised as a treatment with mannitol added to 8% (670 mOsm kg^{-1} ± 10% osmotic pressure) after testing different concentrations. The osmoticum of the mycelial filtrate and the Czapek Dox media was adjusted to the same value as the control by adding mannitol, after assessment with an automated freezing-point depression micro osmometer (Camlab MOD 200). The densities of the protoplasts in the solution were adjusted to 1.0 x 10^5 protoplasts ml^{-1}. Droplets (10 µl) of protoplast cultures were pipetted into sterile 5-cm-diameter petri dishes and sealed with nescofilm. Five replicates were used for each treatment. Assessments of protoplast viability were made using 0.01 % fluorescein diacetate (Widholm 1972) from the first day of culture by counting the proportion of viable protoplasts in each culture. The experiment was analysed using one-way analysis of variance after arc-sin transformation of data.

RESULTS AND DISCUSSION

Sporulation of *Colletotrichum gloeosporioides* f.sp. *dioscoreae*.

No sporulation occurred after two years' subculturing of the fungal isolate on either PDA or Czapek Dox media. The exposure of the fungus to natural daylight did not induce sporulation, when this was carried out over a range of temperatures (15, 19, 25, 29°C). There was therefore a possibility that the prolonged period of culture of the isolate away from the host may have resulted in a loss of sporulation capacity. However, when excised leaves of shoot cultures were brought into contact with mycelia, sporulation was still not stimulated. In contrast, the contact of mycelium with intact plantlets of *D.alata* growing *in vitro* appeared to be a significant trigger of sporulation capacity in the pathogen. After only two weeks in contact with *in vitro* plants of *D.alata* yams, the fungus initiated sporulation at all the different temperatures tested. Sporulation reached a peak after two weeks of culture, with no apparent further increase of the numbers of spores per area of mycelium after this time. The optimal temperature for sporulation of the fungal isolate used in this study was 25°C.

After the re-establishment of spore-production capacity of the fungus, experiments were carried out to test the influence of other variables (pretreatment with the host *in vitro*, medium type, exposure to light, temperature) on sporulation. The establishment of host contact appeared to be critical for inducing sporulation as shown by the comparison with the fungus that did not have contact with the plants (Table 1). The type of medium in which the fungus was cultured affected sporulation levels. Czapek Dox was significantly more favorable for sporulation than was PDA ($P = 0.001$), which agrees with the results of previous work by Winch *et al.* (1984). There was no significant effect of artificial light on sporulation. From

the range of temperatures tested the best temperatures for sporulation (in descending order) were 25, 29, 20 and 15°C. All the differences in temperature effect were significantly different at P = 0.001 except between the two lower temperatures.

Inoculation experiments

Whole plant inoculations.

Inoculated plants did not develop symptoms of anthracnose following inoculation of leaves with spore suspensions applied using a paint brush method. However, when spores were applied in the same manner but followed by wounding with a needle, leaves produced a browning reaction at each of the injured areas. Controls with wounding following application of water gave no such reaction. The *D.alata* clones YMA^A, YMA^B, YMA^C and YMA^D showed large (>5 mm diam) necrotic lesions by five days after inoculation, whereas the *D.alata* clone YMA^P showed only a restricted lesion (RL) type of reaction with the formation of small (1 to 2 mm diam) dark-brown or black necrotic lesions after five days. No further development of these initial RL lesions was observed even up to 21 days following inoculations. In the case of the former four *D.alata* clones, lesions continued to extend into healthy tissues which led rapidly to the complete collapse and death of whole leaves and in some cases whole vines. On the other hand, in *D.cayenensis/rotundata, D.composita*, *D. nummularia* and *D. esculenta* RL type of lesions were also observed but usually much later, at 15 days after inoculation. This indicated that there were delayed types of interaction between these four species of *Dioscorea* and the two *Colletotrichum* isolates tested, as compared to the more sensitive types displayed by clones of *D. alata*, *ie* Oriental and Crop Lisbon (YMA^A, YMA^B,YMA^C and YMA^D). Fungal mycelia were found in the borders of the injured tissues while appressoria were found a few millimeters away from each of the wounds.

The results of these two greenhouse tests supported the assumption of Nwankity & Okpala (1984) that the cuticle probably acts as a barrier to infection of leaves by *C.gloeosporioides*. The combined results also suggest that the pathogen probably lacks the ability to produce cuticle-degrading enzymes. This might be due to a change in the physiological state of the fungus during prolonged culture *in vitro*. According to Wijesundera *et al.* (1984), *Colletotrichum* spp. produce different enzymes during the biotrophic and necrotrophic stages of their life cycles. This might be an explanation for the behaviour of this organism in these experiments because a long period of *in vitro* culture may have resulted in the fungal isolate losing its capacity of producing some of the enzymes necessary for the infection process. The fact that appressoria were found inside intact tissues suggests that a certain degree of penetration via cuticles occurred. According to Rossal & Mansfield (1981), their formation depends on the leakage of nutrients (carbohydrates and aminoacids) from the leaves and that glasshouse-raised plants generally release lower amounts of nutrients than field-grown plants. Therefore, the exudation of nutrients from the wounds incited by needles might have increased the ability of *Colletotrichum* spores to germinate, the mycelium so produced causing lesions on leaves.

***In vitro* plantlet inoculations.**

The results of *in vitro* inoculation tests (Figure 1) confirmed that there was a correlation between *in vitro* and *in vivo* reactions of *Dioscorea* tissues to the anthracnose pathogen. *D.esculenta* was the most tolerant of all the species tested followed by *D.composita* and *D.cayenensis* (significantly different at P = 0.05). The *D.alata* group showed a typical sensitivity to the disease compared with the other species. Collections of *Dioscorea* food yams grown for several years at single sites have indicated that generally *D.alata* > *D.cayenensis/rotundata* > *D.composita* > *D.esculenta* in terms of relative sensitivity to outbreaks of anthracnose disease (based on observations made at the University of West Indies Field Station, Champs Fleurs, Trinidad, between 1973 and 1985 by R.H.Phelps and F.Elango (pers. comm.). Amongst the *D.alata* group, the clones YMA^B and YMA^C showed significantly higher tolerance compared with YMA^A and YMA^D (P = 0.05). The cv. Oriental is often found to be more tolerant of anthracnose disease in the field in Barbados compared to cv. Crop Lisbon. There were no significant differences between the disease reaction of YMA^A and YMA^D at this level of significance. The different responses of YMA^B and YMA^D compared with the other species was of interest. In the cases of these two clones, the infection developed faster, since all the total-infected plants showed symptoms by one week. It suggests that in these clones, any possible infection-delaying mechanisms present in the other clones/species may

be lacking. The statistical analysis of variance of these experiments did not consider the values of the controls (inoculation with distilled water), since no effect was found with this treatment (Fig.1). There was no evident pattern in the results of spore inoculations on different leaf surfaces of shoot cultures (Fig.2). In most cases the upper surface inoculations gave better infection rates than lower surface inoculations (eg. YMA^A, YMA^D) whilst in others they did not (eg. YME and YMCOM). There were experimental problems with delivering spores onto the undersides of leaves of cultured shoots. In some cases the inoculation method caused the dropping of the inocula from the lower surface of the leaves, thus possibly reducing overall levels of spore inoculum. The penetration of fungal germ tubes via stomata or directly by appressoria may depend on the number, shape and length of pores (Nwankity & Okpala, 1984). Further studies of these characteristics, as well as cuticle thickness, must be carried out for each clone/species to give a better understanding of the morphological influence of this factor on the tolerance of tissues to the disease.

Protoplast inoculations.

The results of the inoculation of protoplasts with mycelial filtrates of *C.gloeosporioides* were incomplete. This may have been due to the fact that the protoplast isolation procedure yielded *D.alata* protoplasts with low viability levels (45.3 and 36.6 % at 6 and 24 hours after isolation, respectively). Significant differences in the responses of YMA^P protoplast populations to mycelial filtrates were observed (Table 2). Protoplasts lost viability to a greater degree in 100 % mycelial filtrates compared to the controls. Also there was a gradient of response with 50 % and 25 % filtrates in Czapek Dox medium. These results suggested that the mycelial filtrates contained a factor(s) which cause viability loss. Further investigations are required to establish whether such factor(s) represent functional pathotoxin(s) implicit in the disease syndrome. Czapek Dox medium also appeared to have a negative effect on protoplast viability. However, the effects were not strong enough to mask the effects of the mycelial filtrates, since there were significant differences in protoplast viability between treatments containing equal dilutions of either Czapek Dox or mycelial filtrates (Table 2).

Based on the preliminary results presented above, the use of *in vitro* inoculation methods for anthracnose resistant or tolerant plants appears to be a promising screening approach. This method is easier to carry out compared to field trials, and has the advantage of reduced risks of causing inadvertant build up of anthracnose diseases. According to these preliminary results, the same pattern of resistance and/or susceptibility appears to occur in plant tissue and cell cultures as it does *in vivo*. Micropropagation methods for yams are already available and the system of screening *in vitro* plantlets can therefore be readily applied and currently methods are being developed for cultured cell and protoplast culture of the major food yams.

According to Debergh (1983), *in vitro* plantlets lack the waxy cuticle present in normal plants. Since the main pathway to infection by the fungus is usually via the cuticle which according to Nwakity & Okpala (1984) acts as a barrier to infection in resistant yam cultivars, the results obtained from the *in vitro* and glass house experiments appeared to confirm these assumptions. *In vitro* plantlets showed anthracnose symptoms in the cases of all clones in all of the experiments reported here while plants in glasshouse experiments only showed symptoms after cuticles had been damaged deliberately with a needle. Even among the *in vitro* plants used in tissue culture-based screening tests (in which no wounding was carried out during inoculation), marked differences between clones/species were found in their responses to both spores and mycelial filtrates. These results suggest that not only morphological aspects are involved in determining differential disease reactions but also biochemical ones. The biochemistry of the fungus as well as that of yam plants needs to be studied in greater detail so that possible toxic compounds (pathotoxins) produced by the pathogen might be identified and the true nature of resistance of yam cultivars to anthracnose discovered. Any identified compouds can also be used as potential pathotoxins for screening cell suspensions from which anthracnose-tolerant cell lines could be obtained (presuming that hypersensitivity responses to such agents does not complicate or invalidate these selection strategies).

Results of preliminary attempts at screening yam clones to anthracnose disease at the protoplast level were promising but not conclusive. The isolation and purification of yam protoplasts needs to be modified to produce large numbers of viable protoplasts. Recent work in our laboratory suggests that yam protoplast release can be improved to levels which would be acceptable for *in vitro* cell screening (ie. 2-6

x 10^6 protoplasts g^{-1} fresh weight leaves). The screening procedure must be developed also to the extent that any resistant cells can be regenerated into plants. Further studies should be carried out also to see if there is any correlation of tolerance in the field with any that is shown at the cell level. The preliminary results reported here however do seem encouraging in this respect.

ACKNOWLEDGEMENTS

To the financial support of the Brazilian Government (CNPq) for a postgr aduate studentship held by P.M.Costa, and the technical advise in pathology aspects kindly provided by Dr. J.W.Mansfield of the Dept. of Biological Sciences, Wye College. The collaboration of C.Twyford and other colleagues in UAPS is gratefully appreciated.

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Figure 1. Comparison of sensitivity of seven clones of yams to *in vitro* inoculations with a Caribean pathovar of *C.gloeosporioides*. Figures are the mean percentage of inoculated plants showing anthracnose symptoms, after arc-sin transformation, 4 replicates. LSD* = 7.705 (1 week) and 6.142 (2 weeks).

Fig. 2. Comparison of sensitivity of seven clones of yams to *in vitro* inoculations with a Caribean pathovar of *C.gloeosporioides* on both sides of the leaves. 1 = upper leaf surface; 2 = lower leaf surface. Figures are the mean proportion of plants showing anthracnose symptoms, 10 replicates. LSD* = 0.220 (2 weeks) and 0.208 (4 weeks). A, B, C and D are YMA^A, YMA^B, YMA^C and YMA^D, respectively.

Table 1. Effects of light, media and pre-treatment with the host on sporulation of a Caribean pathovar of Colletotrichum gloeosporioides f.sp. *dioscoreae*.

Treatments	Number of spores per cm mycelial mat*
CZ.PL.L	22.48 a
CZ.PL.D	22.13 a
CZ.NP.D	18.67 b
PDA.PL.D	17.73 b
CZ.NP.L	17.38 b
PDA.PL.L	17.26 b
PDA.NP.L	2.52 c
PDA.NP.D	1.79 c

Legend: CZ: Czapek dox agar; PDA: Potato dextrose agar; PL: pre-treatment with the host; NP: without pre-treatment; D: dark; L: light. *Values represent the number of spores/cm. mycelial mat, after log (1+x) transformation. LSD***= 2.362. Figures are the mean of 5 replicate plates.

Table 2. Effects of mycelial filtrates and Czapek Dox on the viability of protoplasts of D.alata YMA^P.

	Mycelial filtrate in Czapek Dox			Czapek Dox			Control	LSD*
Concentrations	100%	50%	25%	100%	50%	25%	-	-
6 hours	14.2	27.0	31.7	32.7	36.3	38.4	45.3	2.8
rank	e	d	c	c	b	b	a	
24 hours	.57	22.5	27.3	26.4	27.4	36.2	36.6	1.9
rank	d	c	b	b	b	a	a	

Figures are the means of 5 replicates, standing for the percentage of viable protoplasts from the total number in culture, after arc-sin transformation. Assessments were carried out 6 and 24 hours after inoculation with different culture media.