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Evaluating use of minitubers in screening yam genotypes for tolerance to fungal rot pathogens

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Abstract Fungal rots are an economically important constraint in yam production, especially during storage. Resistant varieties could contribute to management, but they can only be successfully deployed if a reliable and rapid method for screening large numbers of improved genotypes exists. A method to screen genotypes at an early stage of growth based on yam mini-tubers was investigated. Vines from five yam clones were rooted in sterile rice husks and inoculated with *Botryodiplodia theobromae* or *Fusarium* spp. conidia suspensions. Plants were harvested after three months and assessed for rotting of mini-tubers. The effect of pathogen on shoot biomass, number and weight of tubers formed was also assessed. On all five yam clones, rotting was observed only on the fibrous roots but not on the mini-tubers. The effect of pathogens on plant growth varied. It was concluded that a mini-tuber based screening method is not better than the tuber slice method that is currently used to assess yam genotypes.

Key words: *Botryodiplodia theobromae, Dioscorea* spp., *Fusarium* spp.

Résumé La pourriture de champignon (moisissure) est une contrainte importante dans la production des ignames, spécialement pendant la conservation. Des variétés résistantes devraient contribuer à la gestion, mais elles ne peuvent être employées avec succès que si une méthode sûre et rapide de dépistage d'un grand nombre de génotypes améliorés existe. Une méthode de dépistage de génotypes à un stade matinal de la croissance basée sur des mini-tubercules d'ignames était expérimentée. Les vignes qui viennent de cinq ignames clonées étaient enracinées dans des balles de riz stériles et inoculées avec *Bootrydiplodia theobromae* or *Fasarium* spp. de suspenssion conidia. Les plantes étaient récoltées après trois mois et évaluées pour la pourriture des mini-tubercules. L'effet de pathogène sur la pousse de biomasse, le nombre et le poids des tubercules formés étaient évalués. Sur les cinq ignames clonées, la pourriture était observée seulement sur les fibres des racines, mais pas sur les mini-tubercules, pendant que l'effet des pathogènes sur la croissance des plantes était varié. Il a été conclu qu'un tubercule issu de la méthode de dépistage n'est pas meilleur qu'un tubercule issu de la méthode de tranche, qui est actuellement utilisée pour évaluer les génotypes d'igname.

Mots clés: *Botryodiplodia theobromea, Dioscorea* spp., *Fusarium* spp.

Introduction

Yam (*Dioscorea* species) plays an important role in the daily diet and the agricultural system of over 100 million people in West and Central Africa. The yam producing belt stretches from Cameroon, in the east, to the Bandama river in the Ivory Coast (FAO, 2002) with preferences changing from *Dioscorea rotundata* to *D. alata* westwards. In spite of decreasing yields and competition from cheaper staples, a year round supply is highly desirable and profitable for producers and traders, because yam is deeply rooted in the consumer's culture (Tschannen, 2003). This implies long term storage periods, which are associated with substantial losses due to physiological processes, rots, nematodes and insect pests.

Other constraints are high cost of planting material and difficult marketing conditions (Aighewi *et al.*, 2003). Soon after harvest tubers are in a dormant state during which rotting is minimal but this changes at break of dormancy when rotting increases to severe levels (Degras, 1993). It has been suggested that there are possibly preformed defense mechanisms that protect the tubers in dormancy from infection. The fungi implicated in yam storage rots include *Fusarium* species, *Botryodiplodia theobromae, Nattrassia mangiferae* and *Aspergillus niger* but also bacteria such as *Erwinia* and *Corynebacterium* spp. (Orkwor *et al.*, 1998).

Recommendations for management of storage rots include proper curing of tubers after harvest, construction of suitable storage sheds and application of chemicals. These methods are, however, not always practiced or are costly to implement, hence it is considered that use of rot tolerant varieties would be the most appropriate approach. The potential of tolerant germplasm has not been vigorously investigated, probably due to lack of efficient methods for screening large numbers of germplasm that are available. Field based screening of germplasm may not be appropriate due to spatial and temporal variations in pathogen populations and densities. Also, field based screening methods require maintenance of trials over a long period of time until tubers have formed and are harvested for evaluation, which usually lasts 12 months. Therefore there is a need to develop screening methods that would reduce the time taken to reach a decision and reduce the amount of resources required, especially when large numbers of germplasm are involved. This study was undertaken to investigate the possibility of using minitubers to screen yam for tolerance to fungal rot pathogens.

Materials and methods

Four *D. rotundata* clones (TDr 4490, TDr 1010, TDr 131 c2 and TDr 01/02148) and one *D. cayenensis* clone (TDc 98/136) were obtained from the International Institute of Tropical Agriculture Yam Breeding Unit and used for this study. Vine cuttings extending two leaves long (with one internodal space between leaves) were harvested from mature plants of the five clones. The vines were treated with auxin to promote rooting and planted in carbonated sterile rice husks contained in 15 cm diameter polythene bags. After planting the vines the rice husk was watered well and then placed under a polythene enclosure to maintain high humidity.

The vines remained covered for the first month during which they were watered twice a week. After one month the polythene covering was removed and each plantlet was inoculated by drenching with 100 ml suspension containing 10^5 conidia ml^{-1} *B. theobromae* or *Fusarium solani*. The treated plants remained in the screen house where they were watered once a week until 12 weeks after planting when they were uprooted and evaluated. The experiment was conducted thrice in the same way, except for the third repetition when the period from planting to evaluation was extended from three to four months.

Data recording. Rotting was evaluated using 0 - 4 scale described as follows. 0 = Healthy, less than 10% feeder roots rotted, stem clean; 1 = 25 – 50% feeder roots rotted, tubers and stems healthy. 2 = 25 – 50% feeder roots rotted with slight tuber rot and stem wilt; 3 = 50 – 75% feeder roots rotted, tubers and stems moderately rotted; 4 = All feeder roots rotted, no tubers formed or all tubers and stem rotted with plant dead.

After scoring, mini-tubers and fibrous roots were separated from the shoots and the mass of different plant parts determined separately. The healthy mini-tubers were sliced and inoculated with mycelial plugs taken from freshly growing cultures of the fungal pathogens. For comparison, tubers of the same yam clones varieties that had been harvested after one year of growth and stored for two months were similarly sliced and inoculated with mycelial plugs of the same pathogens. Each mycelial plug measured 6mm diameter and was placed at the centre of the slice. The inoculated tuber slices were placed inside sterile petridishes and incubated at 23 ± 2 °C. The area colonised by pathogen on each slice was calculated after incubating for 7 days

Results

Three months after planting, each of the vines had formed at least one mini-tuber, with the highest average number of 12 tubers produced by clone TDc 98-136, followed by clone TDr 131 c2 (Table 1). After inoculation with either *Fusarium* or *Botryodiplodia*, rotting on all treated clones was only observed on the fibrous roots and not on the minitubers. The highest rotting incidence was on clone TDr 1010 inoculated with *B. theobromae*. On these plants over 50% of the feeder roots were rotted, discoloured and appeared without vitality. Infection of fibrous roots was moderately severe on clones TDc 98-136 and TDr 01/02148 inoculated with *Fusarium* (Table 2).

Table 1. Effect of *B. theobromae* on rotting of fibrous roots and growth of yam vines.

Clone	Rot score	Root weight	Tuber no[1].	Tuber weight	Shoot weight
TDr 4490 - control	-	0.20 (0.02)	2 (0.47)[2]	7.03 (0.12)	1.32 (0.14)
TDr 4490-Bt	2.33 (0.47)	0.30 (0.04)	2 (0.47)	7.61 (0.24)	1.79 (0.51)
TDr 1010-control	-	0.16 (0.02)	1 (0)	4.90 (0.16)	1.12 (0.07)
TDr 1010-Bt	3.00 (0.82)	0.08 (0.01)	1 (0)	2.78 (1.2)	0.82 (0.16)
TDc 98-136 Contr	-	9.43 (5.6)	12 (1.12)	28.9 (9)	11.75 (7.05)
TDc 98-136 Bt	2.00 (1.22)	11.3 (3.54)	5 (2.5)	18.8 (9.06)	11.68 (8.09)
TDr – 131 c2 - cont	-	4.30 (2.29)	8 (3.86)	21.4 (8.01)	15.77 (11.33)
TDr – 131 c2 -Bt	2.33 (0.47)	6.04 (0.17)	10 (0.94)	29.2 (4.26)	30.70 (1.59)
TDr – 02148 - cont	-	0.12 (0.12)	2 (0.63)	1.47 (0.93)	3.54 (2.96)
TDr – 02148- Bt	2.00 (0.6)	0.16 (0.1)	2 (0.63)	1.55 (0.63)	2.96 (1.88)

[1]Average tuber number is rounded to the nearest whole number. [2]Figure in bracket is standard deviation.

Table 2. Effect of *Fusarium* spp on rotting of fibrous roots and growth of yam vines.

Clone	Rot score	Root weight	Tuber no.	Tuber weight	Shoot weight
TDc 98-136 Control	-	9.43 (5.6)	12 (1.12)	28.9 (9)	11.75 (7.05)
TDc 98-136 -Fus	2.75 (0.43)	5.78 (3.1)	9 (3.57)	21.4 (3.68)	12.4 (3.92)
TDr – 131 C2 - Cost	-	4.30 (2.29)	8 (3.86)	21.4 (8.01)	15.77 (11.33)
TDr – 131 C2 - Fus	2.33 (0.47)	5.97 (0.95)	11 (4.32)	33.6 (13.65)	25.5 (9.29)
TDr – 02148 - Cont	-	0.12 (0.12)	2 (0.63)	1.47 (0.93)	3.54 (2.96)
TDr – 02148 - Fus	2.75 (0.43)	0.20 (0.14)	2 (0.58)	1.87 (0.92)	4.22 (2.92)

Figure in bracket is standard deviation.

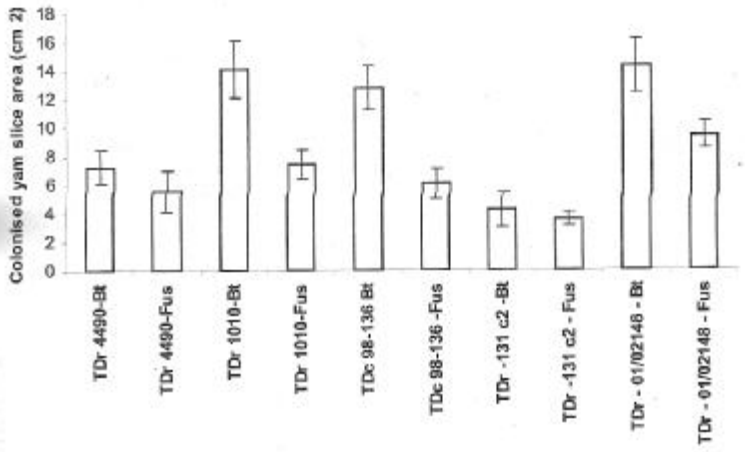

Figure 1. Rotting incidence on slices from 12-month-old yam tubers after inoculation with the rot pathogens *B. theobromae* or *Fusarium* spp. Bars indicate standards deviation. Clone name ending with Bt means inoculated with *B. theobromae*; ending with Fus means inoculated with *Fusarium* spp.

The number of tubers formed was reduced by fungal treatments only on clone TDc 98-136, while the average weight of tubers formed was reduced on clone TDr 1010, when inoculated with *B. theobromae* and on clone TDC 98-136 inoculated with *Fusarium*. On all clones, the mini-tubers were without rot symptoms at time of harvesting, and they also did not develop rot after slicing and inoculating with fungal mycelial plugs.

When slices of 12 month-old tubers of the same clones were inoculated with fungi rot symptoms developed rapidly within 7 days (Fig. 1) with symptoms being most severe on plants inoculated with *B. theobromae*. On all clones the shoot biomass of plants growing from vines was reduced by inoculating with *B. theobromae* on cultivars TDr 1010 and TDr 01/02148 while *Fusarium* infection did not affect shoot biomass on any clone.

Discussion

A method based on three-month old yam mini-tubers was evaluated as an alternative for screening improved yam varieties for tolerance to fungal rot pathogens. The yam mini-tuber technique has been recently developed as a rapid multiplication method for planting material (Shiwachi *et al.*, 2004). Mini-tubers are easy to transport over long distances; they do not require hardening compared to tissue cultured plants and the tubers are planted without splitting into setts, which eliminates injuries that facilitate rotting.

The observation that fibrous roots were infected while mini -tubers remained healthy was surprising and possibly indicates that fungi can penetrate more easily into the fibrous roots as they are formed. Since the five clones were not resistant to infection, as indicated by the rotting when slices of mature tubers were inoculated, it is probable that the mini-tubers at an early stage of growth have a mechanism that helps to resist infection. Such a mechanism could be based on antifungal compounds (Orkwor *et al.*, 1998) or on the skin providing a mechanical barrier (Jatal and Bridge, 1990). It is, however, unlikely that the skin prevented the mini -tubers from rotting, because no rotting occurred after eliminating the skin by slicing and placing the pathogen in contact with internal tissues.

On mature tubers, it has been reported that the incidence of rots increases with time after harvest, which is thought to be linked to physiological changes taking place as dormancy breaks (Orkwor *et al.*, 1998). We hypothesise that yam tubers at an early stage of growth have a different biochemical composition and are at a physiological state that makes them inappropriate for use in screening for tolerance to pathogens. Further work could be done to determine if there are antifungal compounds that accumulate in tubers at an early stage of development. Although some growth parameters, e.g. shoot weight, were adversely affected on some cultivars after inoculation with fungi, this effect may not be reliable as a basis for screening since the target pathogens attack the tuber predominantly. Although the mini-tuber method could considerably reduce the time, space and resources required to screen large numbers of genotypes, the study concluded that the tuber slice method should be preferred for identifying yam genotypes tolerant to fungal pathogens.

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