WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.5 (3&4) : 327-329. 2006

www.world-food.net

Evaluation of the susceptibility of local cocoa germplasms in Nigeria to *Phytophthora* pod rot disease using the leaf discs technique

A. H. Otuonye [1*], A. Y. A. Adeoti [2], S.O. Agbeniyi [1] and P. O. Aikpokpodion [1], O. Enikomehin [2] and T. O. Popoola [2]

*[1]Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Oyo State, Nigeria.. [2]University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Nigeria. *e-mail:alytonye@yahoo.com.*

Received 11 September 2006, accepted 2 August 2007.

Abstract

The *Phytophthora* pod rot disease constitutes a major threat to cocoa cultivation in all cocoa-growing regions worldwide. It is one of the most important diseases limiting the attainment of genetic yield potential of this tree crop. Though available, chemical control methods are often expensive and beyond the reach of peasant farmers that produce more than 95% of Nigeria crop. Also, the limited knowledge of rational chemical application among rural farmers and the adverse effect on the environment makes it an unfriendly approach. Breeding commercially viable resistant cacao genotypes have been advocated to be the most durable control approach against the *Phytophthora* pod rot. Towards achieving this objective, promising cacao materials were selected from the gene pool in Cocoa Research Institute of Nigeria (CRIN) Headquarters, Ibadan. Screening of these eight selected cocoa clones with four isolates of *Phytophthora megakarya* of known aggressiveness in combination-NGRI$_4$, using the leaf discs inoculation method at standard inoculum concentration of zoospores of 3×10^5 ml^{-1} was carried out. Results showed that T12/5 was resistant, while clones T17/11 and T86/2 are moderately resistant. The other clones, T85/5, T85/45 and T20/11, were moderately susceptible. It is therefore suggested that clone T12/5 be integrated into the resistance breeding effort against *Phytophthora* pod rot.

Key words: Cocoa, clones, screening, resistant, isolate, aggressiveness, *Phytophthora megakarya*.

Introduction

The *Phytophthora* pod rot (popularly known as 'black pod' disease) on cocoa (*Theobroma cacao* L.) remains the most important disease having debilitating effect on the health of cacao trees and consequently on their productive potential, worldwide[7]. Data of economic losses incurred from the devastation of cacao by the species of this pathogen from different cacao producing regions, worldwide, is quite enormous. Crop losses estimated at 30% of the world production have been reported [21] but a recent report [20] estimated 44% global annual crop loss attributable to the 'black pod' disease. In Nigeria, losses could be as high as 80% when no treatment is applied [1]. Cocoa farmers in Nigeria lose ₦3,965.16 million Naira of their total net revenue to the incidence of 'black pod' disease [22].

Although chemical therapy of disease control have been developed to reduce the impact of diseases caused by *Phytophthora* species and hence yield losses in cacao, these chemicals are often expensive and beyond the reach of the peasant cocoa farmers who produce over 90% of the world production in developing countries [3, 18]. However, apart from the chemical control method, several control strategies have been reported over the years, but the use of genetic resistance has been advocated as the most effective and economic control method, since the identification of useful levels of resistance within cacao germplasm at different cacao producing countries and the establishment of its heritable nature [2, 3, 7, 14].

In breeding cacao materials (germplasm) resistant to *Phytophthora* pod rot, cocoa germplasm selection through application of different screening techniques against black pod has been practiced in cocoa growing countries for many years [11, 20]. The lineage of many important resistant cultivars could be traced back to germplasm collections [13], but most countries collections remain largely unexplored for their potential as reservoirs of resistance genes [6]. The objective of this study, therefore, was to evaluate and select cacao germplasm resistant to *Phytophthora megakarya,* from the germplasm collection in Cocoa Research Institute of Nigeria (CRIN), that could be incorporated into future national breeding programme.

Materials and Methods

Selection and establishment of Phytophthora isolate for screening: Isolates of *Phytophthora megakarya* for the leaf discs screening test were obtained from naturally infected cacao pods from four different locations, namely, Plots N6/1, N4/4A and N7/1-3 in the Northern part of CRIN headquarters, Plots S2/3A and S2/2 in the southern part, E2/2 and E4/4 in the Eastern part and Plot W23/1-3 in the Western part of Cocoa Research Institute of Nigeria, headquarters. The high aggressiveness of the *Phytophthora* isolates from these plots is well known. Further isolation of the isolates to obtain pure culture of the fungus was done by plating out pieces of the infected cacao pods tissue on potato dextrose agar (PDA) medium and subsequently sub-cultured to obtain axenic cultures of the fungus. Based on preliminary characterization following the pattern of growth in culture, shape and size of sporangia, size and length of pedicel [5], the isolates were identified as *Phytophthora megakarya.* Each isolate of the fungus was then maintained in the laboratory on carrot agar (CA) medium and on fresh mature green pods.

***Cacao genotypes*:** Twenty-one trees amounting to eight cacao clones including two controls (Table 1) were selected for evaluation of their resistance to *P. megakarya.* These clonal accessions represent Trinitario, Forastero and local Amelonado groups maintained at the CRIN germplasm plots.

Leaf discs inoculation test: Fully matured leaves at the interflush-2 stage, corresponding to about two-month-old tissue, were harvested from the region of twigs that were changing from green to brown color. The leaf samples (5-8 leaves/tree) [12] were excised with a semi-automated leaf discs cutter to discs of diameter 15-20 mm, to obtain 15-60 discs or more per tree clone. The leaf discs were then laid out in rows of columns in a plastic inoculation trays lined with moist paper towels, with abaxial surfaces up. There were six replicate trays with each tray containing ten discs of each of the genotypes.

Matured cultures from 10 day old actively growing cultures of the different isolates maintained on carrot agar (CA) medium were each flooded with 10 ml of sterile distilled water (SDW) pre cooled to 10°C, then chilled at –5°C for 30 minutes and transferred into an incubator at 25°C for 30 minutes as described [11] as method of inoculum preparation. This alternating chilling and thawing of the cultures resulted in rupturing of matured sporangia to release motile zoospores into the solution. The resultant zoospore suspension of the isolates was then harvested and mixed together based on the previous screening report with isolates from these plots that showed consistency in their rankings to be of the same aggressiveness (Otuonye, unpublished).

The ensuing zoospore concentration was then adjusted using Malassez haemocytometer and calibrated to standard required inoculum strength of $3x10^5$ zoospores/ml, and labeled $NGRI_4$. Then, with a micro-syringe attached to sterile automated repeatable dispenser a drop of the 10 µl aliquot corresponding to 1 µl, was deposited centrally onto the abaxial surface of each leaf disc from each clone, placed in rows. Inoculation was done across in rows in the plastic inoculating trays. The essence of this was to inoculate a disc from each clone in succession. The inoculation trays were then covered with a lid and enclosed in sealed cellophane or plastic bags and incubated under controlled temperature and humidity conditions of 25°C and 65-85% relative humidity (RH) for 72 hours after inoculation.

Evaluation of infected leaf discs tissues of the cacao clones for resistance to P. megakarya: The leaf discs reactions to the treatments showing disease severity were scored on the 3rd, 4th and 7th day after inoculation on 0-5 point scale [4, 12]. Based on this scale disease assessment of the tissues are indicated as follows: 0 no symptoms, 1 small localized brown or dark-brown penetration points, 2 small penetration points with some connection between them and small expanding lesions, 3 coalescence of brown spots forming intermediate size lesions or coalescence of brown spots, 4 expanding lesions, 5 large dark-brown lesions or uniformly dark-brown large expanding lesions.

Data analysis: The data collected on number of lesions and lesion size from the leaf discs test score were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects and interactions between treatments using the Statistical Package for Social Sciences (SPSS). The means were separated by Duncan new multiple range test (DNMRT).

Results

The analysis of variance (Table 1) done for the disease index rating data, obtained from the leaf discs inoculation, indicated that the reactions of the clones evaluated for resistance to isolate of *Phytophthora megakarya* ($NGRI_4$) were highly significant ($P<0.05$). None of the 8 clones evaluated was totally immune to the isolate of *Phytophthora megakarya* ($NGRI_4$), used in the screening trial. Mean separation of individual clones reaction based on Duncan multiple range test, shows that the six test clones mean lesion score was significantly lower than that of the susceptible N38 control genotype, but varied individually in their reaction to the isolate $NGRI_4$, when compared with the resistant C77 control genotype. T12/5 and control variety C77 were not significantly different in their reaction, but it was observed in the mean lesion score of the leaf discs that T12/5 was 5.99 and 82.16% better than C77 and N38 respectively in its reaction to the isolate $NGRI_4$. However, the control resistant clone C77 showed lower infection scores than clones T86/2, T17/11, T20/21, T85/5 and T86/45 in reaction to $NGRI_4$ (Table 1). Also in the mean lesion score, C77 was better with 22.62, 23.04, 36.52, 40.44 and 50.87% respectively over the five clones in its overall reaction to the isolate $NGRI_4$ of the *Phytophthora megakarya* fungus. These differences observed in clonal responses of the test clones to the isolate of *Phytophthora megakarya* ($NGRI_4$) resulted in the classification of the clones as resistant/or tolerant, moderately resistant, moderately susceptible and highly susceptible. However, analysis of variance for the block treatment effect was not significant ($P<0.05$).

Table 1. Clonal susceptibility classification in response to isolate of *Phytophthora megakarya* from leaf disc inoculation.

No.	Clone	No. of plants screened	Mean value of disease index rating DIR*	Class (Group)
1	T86/2	5	2.82 a	Moderately resistant
2	T12/5	2	2.16 b	Resistant
3	T85/5	5	3.22 c	Moderately susceptible
4	T17/11	4	2.82 a	Moderately resistant
5	T86/45	2	3.47 d	Moderately susceptible
6	T20/21	1	3.14 c	Moderately susceptible
7	N38	1	3.92 e	Highly susceptible
8	C77	1	2.31 b	Resistant

Means of six replications. Means with the same letter are not significantly different ($p < 0.05$).
*DIR: Disease index rating of 0.00 indicates no lesion, 1.00 small penetration point, 2.00 penetration points with connection, 3.00 intermediate size lesion, 4.00 expanding lesion and 5.00 true necrosis.

Discussion

The results showed that there was a significant variation in the resistance of the clones to *P. megakarya* isolates. It has been previously reported [9, 10, 15-17] that varying levels of resistance to *Phytophthora* species were observed among cacao clones. The distribution of the clones showed occurrence of useful levels of resistance in response to the *Phytophthora megakarya* isolate NGRI$_4$. The relative low lesion number observed in clone T12/5, produced from cross between Scavina 12 and Upper Amazon cocoa, indicated useful genotypic resistance level. It has been reported [8, 10, 17] that Scavina 6 and Scavina 12, upper Amazonia Forastero cacao materials from which clone T12/5 was developed, often express intermediate to high level of resistance on progeny cross derived from them. This observation, therefore, suggests the resistance level shown in clone T12/5 response to the influence of the isolate of *Phytophthora megakarya* fungus,in developing fairly low lesion numbers. Sequent to this result the susceptibility-resistant ranking of this cocoa germplasm is resistant. However, the immense value of leaf assay in screening of perennial crops such as cocoa cannot be overemphasized. Provided the conditions for standardizing the method are strictly adhered to, leaf test should enable rapid and early selection of cocoa cultivars least susceptible to *Phytophthora*, correlated to their susceptibility at fruit level [12]. It is hereby suggested, that in future breeding work, the resistant factor in clone T12/5, should be incorporated into national or regional breeding programmes for the development of high yielding resistant cacao cultivars.

Acknowledgements

We wish to thank the Common Fund for Commodities (CFC) for their financial support in carrying out of this research work and in publishing it. We also thank the Executive Director of Cocoa Research Institute of Nigeria, for providing the enabling environment in which this research was conducted. We are also grateful to Mr. Raji for his invaluable assistance.

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