Protocol to Screen Cocoyam Accessions for Resistance or Tolerance to Cocoyam Root Rot Disease in Cameroon

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ABSTRACT

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For the first time in Cameroon, cocoyam (*Xanthosoma sagittifolium*) accessions of cv. Macabo (white) have been screened for resistance or tolerance to cocoyam root rot disease caused by *Pythium myriotylum*. Of 626 cocoyams planted in the greenhouse and screened for 21 days, 72 resistant and 210 tolerant suckers were transplanted in a field in Mamu, Cameroon, known to be infested with *P. myriotylum*. After 3 mo of intensive screening in the field, 42 resistant and 35 tolerant accessions of the Roots and Tuber Research Project (ROTREP) were identified.

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is a land race cultivar and is propagated vegetatively by suckers. One cocoyam plant can produce three to five suckers at a time. Of the three cocoyam cultivars, the pink or red and the yellow are not considered a food source for millions of people in Africa. The white cultivar (Macabo or Tannia), however, is the most important food staple in Nigeria (6), Ghana (3), and Gabon (4) and the second most important staple crop in Cameroon (5). The corms are an excellent source of starch and contain essential proteins and vitamins (1).

Cocoyam root rot is a destructive disease of the cultivar Macabo and has reduced yields up to 90% in some cocoyam plantations in Cameroon (7). Resistance to cocoyam root rot disease (CRRD) has been observed in the pink or red and the yellow cultivars. *Pythium myriotylum* Drechs. was reported as the causal organism of CRRD (7,8,12), but *Fusarium solani* (Mart.) Sacc. and *Rhizoctonia solani* Kühn, which were always associated with *P. myriotylum* in the partially rotted roots of diseased cocoyams, were excluded from the pathogenicity tests (7,8). Also, the disease was known as cocoyam root rot blight complex (apollo disease) caused by *P. myriotylum, F. solani*, and *R. solani* (13). Recently, pathogenicity tests of the three organisms were developed on cocoyam plantlets (plants grown from tissue culture of cocoyam explants and cormels),

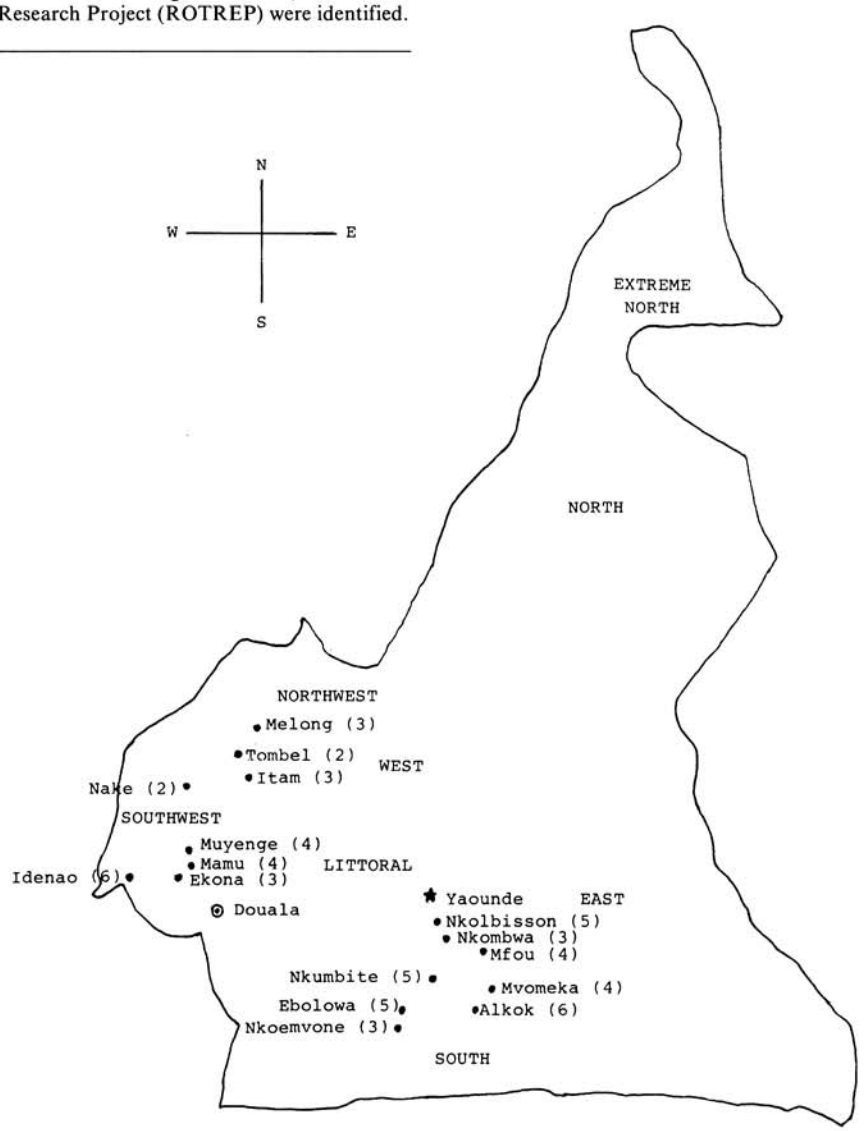

Fig. 1. Locations in Cameroon where the isolates of *Pythium myriotylum* were obtained, with the number of isolates in parentheses.

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and *P. myriotylum* was determined to be the causal organism of CRRD (9,10); the other two fungi were concluded to be opportunistic contaminants (9,10).

High-yielding cocoyam accessions (cv. Macabo) of the Roots and Tuber Research Project (ROTREP) were collected from various areas of Cameroon and bordering countries. All cocoyam accessions from Ghana and Togo seemed resistant to CRRD. Screening of cocoyam accessions for resistance or tolerance to CRRD has never been attempted previously in Cameroon or in any part of the world where cocoyams are grown. The objectives of the study were to screen Macabo plants for resistance and tolerance to CRRD in the greenhouse and to determine whether resistance and tolerance identified in the greenhouse would be expressed under field conditions in soil infested with *P. myriotylum*.

MATERIALS AND METHODS

The study was conducted in the laboratory, greenhouse, and *P. myriotylum*-infested field of the ROTREP, United States Agency for International Development (USAID)/Government of Cameroon (GOC), Ekona Research Centre, Buea, Cameroon. Four isolates of *P. myriotylum* had been obtained from the roots of cocoyam showing symptoms of CRRD from Mamu, Cameroon, and one of these isolates was used in this study. One isolate of *P. myriotylum* was reisolated by the single hyphal tip method (9,10), then by the zoospore method (11) before being used in the screening procedure. Other *P. myriotylum* isolates from various locations in Cameroon (Fig. 1), Ghana, and Togo were obtained from the soil by cocoyam leaf disk baits (9,10) and maintained in the ROTREP laboratory. *P. myriotylum* inoculum was first grown on lima bean sucrose agar (LBSA) for 5 days at 31 C in the dark. LBSA was prepared by adding 10 g of sucrose to 23 g of dehydrated lima bean agar and suspending the mixture in 1,000 ml of sterile distilled water (9,10). Mycelial strands used for inoculating autoclaved soil were obtained by placing *P. myriotylum* grown on LBSA (20 petri dishes) in a Waring blender with 3,000 ml of sterile distilled water and blending the mixture for 5 min. The number of mycelial fragments in the 3,000-ml inoculum solution was counted with a hemacytometer. By means of a galvanized sprinkler system, each screening box (100 × 72 × 14 cm) filled with autoclaved soil was infested with 3,000 ml of the inoculum solution containing 2.4–5 × 10^4 mycelial strands per square centimeter. The soil in each screening box was mixed after inoculum was applied and before cocoyam suckers were planted, and each screening box contained several accessions. The inoculum level in the screening box soil was not compared with that in the field soil. No experimental design or replication system was followed for screening cocoyams in the greenhouse and the field because each cocoyam sucker screened for resistance or tolerance represented one cocoyam accession of the ROTREP or other collections.

The initial screening period in boxes in the greenhouse was 21 days. Cocoyam suckers identified initially as resistant or tolerant to CRRD in the greenhouse were transplanted, 1 × 1 m apart, in a *P. myriotylum*-infested field and screened for resistance or tolerance for 3 mo. This procedure was based on results obtained from pathogenicity tests with *P. myriotylum* and 3- to 7-mo-old cocoyam plantlets that succumbed to CRRD in 4–7 days (9,10).

Cocoyam accessions in the greenhouse and the field were rated *susceptible* if roots were rotted and the leaves chlorotic after 5 days in the screening box (Fig. 2A) or within 30 days in the field, followed by wilting and plant death; *tolerant* if roots were slightly rotted and the oldest leaves yellowing after 5 days in the screening box (Fig. 2B) or 30 days in the field, with succeeding young leaves remaining green; and *resistant* if plants remained green for at least 21 days in the screening box (Fig. 2B) or 3 mo in the field.

RESULTS AND DISCUSSION

Of 626 accessions of the cocoyam cultivar Macabo screened (571 from the ROTREP Ekona Research Centre in Buea, Cameroon, and 55 from Ghana and Togo), 72 were initially identified as resistant and 210 as tolerant. After the

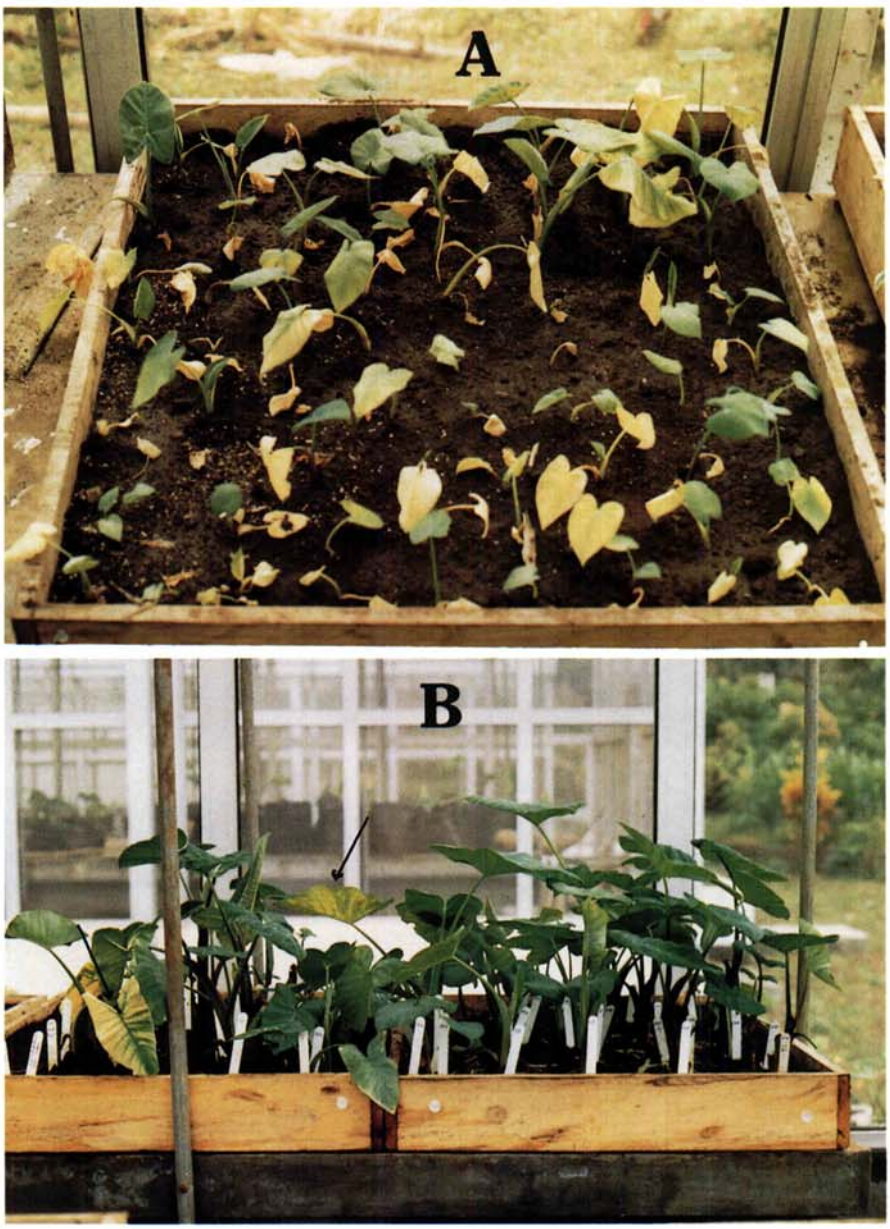

Fig. 2. (A) Inoculated susceptible cocoyams turned yellow and wilted after 5 days in a screening box and died within 21 days. **(B)** Leaves of resistant cocoyams were still green after 21 days; a few accessions with yellowing of oldest leaves (arrows) were identified as tolerant.

final screening in a *P. myriotylum*-infested field in Mamu, Cameroon, one of the ROTREP accessions was rated as resistant (RO1075) and 21 were rated as tolerant (RO1002-1 through RO1002-5, RO1006-1 through RO1006-6, RO1043, RO1054, RO1116, RO1132, RO1199, and RO1144-1 through RO1144-5). Of the Ghana and Togo accessions, 41 were rated as resistant (020-1, 020-2, 021-1, 021-2, 022-1, 022-2, 037, 039-1 through 039-10, 048-1, 048-2, 049-1 through 049-3, 051-1, 051-2, 5-2, 18-9, 21-4, 29-23-1 through 29-23-5, 36-4-1 through 36-4-3, 38-20-1 through 38-20-4, 42-19-1, and 42-19-2) and 14 were rated as tolerant (005-1, 023-1 through 023-4, 041-1, XX-1 through XX-4, and White Osiem-1 through White Osiem-4).

The ratings of a ROTREP accession were expected to be the same in the greenhouse and in the field because the isolate of *P. myriotylum* used for screening cocoyam accessions was obtained from the same field in Mamu, Cameroon. However, only one ROTREP accession initially identified as resistant and only 21 initially identified as tolerant survived final screening in the field. Possibly, the inoculum level of *P. myriotylum* was lower in the screening boxes in the greenhouse than in the field. However, the minimum concentration of *P. myriotylum* propagules causing CRRD symptoms in 3–6 days on 4-mo-old cocoyam plantlets was earlier determined to be 200 zoospores or 180 mycelial strands per square centimeter (11). The inoculum level of *P. myriotylum* in the screening boxes was 7.2×10^7 to 1.5×10^8 mycelial strands. Another possibility is that the isolate of *P. myriotylum* used in the greenhouse study had lost its virulence when maintained and transferred at regular intervals for extended periods of time (2 yr) on artificial culture media (vermiculite-cornmeal-dextrose), compared with a fresh isolate from infected roots or from the soil. Dhingra and Sinclair (2) reported that some fungi maintained and transferred at regular intervals to fresh media became adapted to saprophytic growth and gradually lost pathogenicity. Other possibilities are that the isolate of *P. myriotylum* from Mamu consisted of one of several races of the pathogen in the area and that other fungi and environmental factors influence CRRD resistance in the field.

Because the cocoyam accessions from Ghana and Togo were combined with the ROTREP accessions, they were screened with the isolate of *P. myriotylum* from Mamu. Ratings of these accessions were similar in the greenhouse and the field. However, all the accessions brought from Ghana and Togo to Cameroon seemed resistant to CRRD. If the isolate of *P. myriotylum* from Mamu consisted of several races of the pathogen, the isolates of *P. myriotylum* from Ghana and Togo may also have consisted of several races that differed from the isolates of Mamu. Consequently, the ratings may have been different if the collections from ROTREP, Ghana, and Togo had been screened with the isolates from Ghana and Togo. We therefore recommend studies to determine: 1) what races of *P. myriotylum* are present in the various pools of isolates now maintained in the ROTREP laboratory once the resistance and tolerance of cocoyam cv. Macabo accessions are resolved, 2) if other fungi and environmental factors have an influence on CRRD resistance in the field, and 3) whether isolates of *P. myriotylum* maintained in artificial media for extended periods of time (6 mo to 2 yr) lose pathogenicity to cocoyam.

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