First International Workshop on Cocoyam (Xanthosoma sagittifolium) Cultivation, Genetic Improvement and Disease Management

Programme and Abstract Book

Organized by
IRAD, Cameroon, Ghent University and IITA, Nigeria

October 29 – 31, 2008
IRAD Ekona, Buea-Cameroon
First International Workshop on Cocoyam
(*Xanthosoma sagittifolium*)
Cultivation, Genetic Improvement and Disease Management

IRAD Ekona, Buea, Cameroon
October 29-31, 2008

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Funding
VLIR-UOS, Belgium
IRAD, Cameroon
IITA, Nigeria
Introduction

Organization

The workshop is co-organized by the Institute of Agricultural Research for Development (IRAD) Cameroon, the Ghent University, Belgium, and the International Institute of Tropical Agriculture (IITA), Nigeria.

Duration and venue

The workshop takes place from 29-31 October 2008 at the conference hall of the Regional Research Centre of IRAD, Ekona, some 10 km south of Buea. The workshop is followed by a one-day meeting on 1 November 2008 to give participants an opportunity to brainstorm and set the research agenda for the crop, determine the orientation to take in combating the root rot disease in a sustainable manner, and develop funding proposals to be presented to donors.

Workshop background and rationale

Cocoyam is an important food crop in Cameroon and many other countries of the tropics and subtropics. Over the past three decades, attempts have been made to increase cocoyam production as a means of ensuring food security, alleviating poverty and protecting the environment, major challenges facing developing countries. In many cocoyam growing countries, the most important field constraint to cocoyam production is the cocoyam root rot disease (CRRD), caused by the fungus, *Pythium myriotylum*. Research efforts over the years have therefore focused on controlling the disease under field conditions.

Initial work carried out by IRAD in the 1980s led to identification of the etiology of the disease. Later, epidemiological studies and molecular characterization studies confirmed that *Pythium myriotylum* was in fact the causal agent of the disease. Fairly recently (2001-2006), molecular characterization work conducted on the pathogen by the Ghent University in collaboration with the cocoyam research team of IRAD, focused on geographic and intraspecific variability of the pathogen, and interactions between the pathogen and native soil antagonists.

To obtain durable resistance to the disease, attempts were made by IRAD scientists to develop varieties by making crosses between Cameroon cocoyam varieties and accessions obtained from Latin America and other parts of Africa. Although, cocoyam resistant varieties were not obtained, constraints related to cocoyam breeding were established, as well as possibilities for overcoming these constraints. Members of the Cameroon research team will be present in the workshop to share their experiences with other participants.

The major handicap in cocoyam breeding which is the narrow gene pool is being circumvented by a mutation-breeding approach, recently initiated in Cameroon with funding from the International Atomic Energy Agency (IAEA) in Vienna, Austria. In this project, cocoyam plant material from Cameroon was gamma-
irradiated, to increase variability in the species. The results of this novel research work will also be presented during the workshop.

Another approach, which has been used to control the cocoyam root rot disease has been the use of disease-free planting material obtained through micro and macro-propagation of healthy cocoyam corms. The results obtained so far will also form important issues for discussion during the workshop.

A decade ago, Ghent University and IRAD jointly initiated research on biological control of the cocoyam root rot disease within the framework of a project entitled, “Ecologically sustainable management of the cocoyam root rot disease caused by Pythium myriotylum.” Potential biological control agents were screened from the rhizosphere of healthy cocoyam plants growing in diseased fields, and their disease suppression mechanisms determined. Some of these biological control agents are being studied at the genomic level in the Plant Pathology laboratory in Ghent University.

When various soil types were evaluated for their conduciveness to the cocoyam root rot disease, an andosol from Mount Cameroon was found to be naturally disease-suppressive. In further investigations the main factors responsible for soil suppressiveness were identified. Later studies were conducted to control the CRRD in field systems in Cameroon by using suppressive composts. Currently, IRAD together with Ghent University are conducting fundamental research on the interaction of soil type with indigenous Pythium myriotylum antagonists. This later initiative is also trying to evaluate disease suppressive effects of some cultural practices in order to develop an integrated disease control strategy that can be proposed to cocoyam growers.

In the past few years, plant host-\textit{Pythium} interaction studies have been conducted in a laboratory at the University of Yaounde I, Cameroon. Some of the results obtained by this research team on mechanisms of resistance and resistant markers will be presented during the workshop. At the same time a lot of research is being conducted in other countries on management of various cocoyam diseases as well as on genetic improvement of the crop, as the crop continues to gain importance in the growing regions of the world. Despite the volume of information now available on the crop, there have been very few opportunities for scientists to exchange their experiences on this important crop, as well as limited opportunities to disseminate the information now available to end-users (extensionists and growers).
Programme

Wednesday, October 29, 2008

8.00 h – 9.00 h
Arrival of Participants and Registration

9.00 h – 11.00 h
Opening Ceremony

- Welcome address by the General Manager, IRAD Cameroon
- Address by the Representative of Ghent University, Belgium
- Address by the Representative of the IITA
- Keynote address by Dr. Samuel Nzietchueng
- Opening speech by H.E., The Governor of the South West Province, Cameroon
- Family photograph
- Exhibition
- Cocktail

Lunch

13.30 h – 18.00 h
Scientific Session 1: Cocoyam cultivation, production constraints, genetic improvement
Chair: Dr. Jacob M. Ngeve, Geneticist/Plant Pathologist, National Scientific coordinator of Root and Tuber research (IRAD)

O1: Socio-economic analysis of cocoyam production constraints in agro-ecological zone IV in Cameroon
J.C. Mboua (IRAD, Ekona)

O2: Status of Cocoyam research, production and utilization in Nigeria
G.O. Chukwu (IITA, Nigeria)

O3: Collection, morphological characterization and conservation of phytogenic resources of Xanthosoma species in Costa Rica
F. Saborio (Universidad de Costa Rica)
O4: Commercial adoption by farmers of single bud multiplication technique, production and distribution of disease-free plants, germplasm collection and characterization, flowering induction and sequence characterization of DsMV isolates from cocoyam (Xanthosoma spp.) in Nicaragua
G. Reyes Castro (Universidad Nacional Agraria, Nicaragua)

Coffee/tea break

O5: Recent advances in cocoyam tissue and biotechnology in the “Plant Physiology and Biochemistry Laboratory” (LAF314), ENS, University of Yaoundé 1.
N. Omokolo (University of Yaoundé)

O6: Application of tissue culture techniques to cocoyam (Xanthosoma sagittifolium Schott) production
A. Sama (IRAD, Ekona)

O7: Preliminary study on radiation sensitivity of in vitro cultures of Xanthosoma (Macabo) in Cameroon
X. Ndzama (IRAD, Ekona)

O8: In-vivo mass production of Pythium-free cocoyam planting material
A.M. Ngone (IRAD, Ekona)

O9: Rapid multiplication using PIF Techniques to overcome scarcity of Cocoyam planting material in Cameroon
G.A. Manga (IRAD, Njombe)

Thursday October 30, 2008

9.00 h – 12.00 h
Scientific Session 2: Cocoyam diseases and their management (I)
Chair: Prof. Denis Omokolo, Biologist and biotechnologist, University of Yaoundé I

O10: Molecular applications for the advancement of cocoyam research: pathogen identification and breeding for disease resistance
J. T. Tambong (Agriculture and Agri-Food Canada)

O11 (a,b,c,d): Pythium myriotylum: molecular detection, phylogeny, host range
F. Saborio (Universidad de Costa Rica)

O12: Response of cocoyam (Xanthosoma sagittifolium) accessions to Pythium myriotylum inoculation
D.A. Fontem (University of Dschang)
Coffee/tea break

O13: Hybridization in *Xanthosoma* for genetic improvement of major agronomic traits and root rot disease resistance  
*X. Ndzana (IRAD, Ekona)*

O14: Variation of *Pythium*-induced cocoyam root rot severity in response to soil type  
*A. Amayana (IRAD, Ekona)*

Lunch

14.00 h – 16.00 h

**Scientific Session 3: Cocoyam diseases and their management (II)**  
*Chair: Prof. Dominic Fontem, Plant pathologist, University of Dschang*

O15. Factors associated with organic matter-mediated cocoyam root rot suppression in field systems  
*A. Amayana (IRAD, Ekona)*

O16. Chitosan and benzo (1,2,3) thiazole-7-carbothioic acid S-methyl ester (BTH) induced oxidative stress in *Xanthosoma sagittifolium* (cocoyam)/*Pythium myriotylum* interaction  
*H.D. Mbouobda (University of Yaoundé)*

O17. Pseudomonas bacteria antagonistic to *Pythium myriotylum* associated with cocoyam (*Xanthosoma sagittifolium*) in Cameroon  
*M. Höfte (Ghent University, Belgium)*

O18: Incidence and distribution of viruses infecting Cocoyam (*Xanthosoma sagittifolium*) in two Southwestern States of Nigeria  
*T. Oben (IITA, Nigeria)*

O19. Cocoyam diseases in the forest zones of Ghana  
*E.L. Omenyo (IITA, Ghana)*

Coffee/tea break

16.30 – 17.30

**Scientific Session 4: Cocoyam research: future prospective**  
*Chair: Dr. J. Tambong, (Agriculture and Agri-Food Canada)*

O20: Cocoyam rebirth in Nigeria  
*G.O. Chukwu (IITA, Nigeria)*
O21: Possibilities of IRAD cocoyam germplasm regeneration and duplication from Global Crop Diversity Trust
J. Kengue (IRAD, Yaoundé)

Friday October 31, 2008

9.00 h – 12.00 h
Field Visits

Lunch

14.00 – 16.00 h
General discussion and concluding remarks
Abstracts – Oral presentations

O1. Socio-economic analysis of cocoyam production constraints in agro-ecological zone IV in Cameroon

Institute of Agricultural Research for Development (IRAD), Ekona, Cameroon

Cocoyam (*Xanthosoma sagittifolium*) is one of the main food crops which are grown in Cameroon. Over the past years its production has been declining due to several factors which have not yet been fully elucidated. In this study, attempts were made to analyze cocoyam production constraints in socio-economical point of view in agro-ecological zone IV which is among the leading cocoyam producing areas in Cameroon. In 2005, a survey was conducted within this agro-ecological zone. Data were collected on farmer’s management practices, cocoyam yield losses due to major diseases and pests, and inputs use. Survey results show that cocoyam production is constrained by a number of factors including the root rot disease, tuber scale insect (*Stictoccocus sp.*), lack of suitable planting material, labour and land scarcity, and cost of chemicals. Contributions of the above mentioned factors to cocoyam yield reduction are discussed in this paper.

**Key words:** socio-economic analysis, cocoyam production constraints, agro-ecological zone IV, Cameroon.
O2: Status of Cocoyam research, production and utilization in Nigeria

Chukwu G.O., Nwosu K.I, Okoye B.C, and Onyeke J

International Institute of Tropical Agriculture, Nigeria
O3. Collection, morphological characterization and conservation of phytogenic resources of *Xanthosoma* species in Costa Rica

G. Chacón (1), F. Saborío (1)

(1) Laboratorio de Biotecnología de Plantas, Centro de Investigaciones Agronómicas, Universidad de Costa Rica, San José, Costa Rica

Cocoyam (*Xanthosoma* sp.) is an important crop in the region of Central America and the Caribbean where, in addition of being a source of carbohydrates, it is a cash crop for small farmers that export this crop to United States and European countries. The center of origin of this crop is believed to be the Orinoco Valley in Venezuela, however in Costa Rica and Nicaragua many wild relatives of this crop are commonly observed. In order to evaluate these species as possible sources of variation for breeding programs, we collected, introduced and multiplied in vitro these relatives, 37 from Costa Rica and 15 collected from other countries. After *in vitro* multiplication we planted at least 10 plants of each accession and they were morphologically characterized during 12 months according to IPGRI’s descriptors and the taxonomic information available. We confirmed the presence of *X. mexicanum*, *X. robustum*, *X. undipes* and *X. wendlandii* but 4 new species not reported previously in Costa Rica were found: *X. mafaffa*, *X. violaceum*, *X. atrovirens* and a species that differs from the ones described in the literature. The characterization of the cultivated showed that it is possible to differentiate these based on the anthocyanin pattern on the petiols, shape of the leave and cormel’s flesh color.
O4. Commercial adoption by farmers of single bud multiplication technique, production and distribution of disease-free plants, germplasm collection and characterization, flowering induction and sequence characterization of DsMV isolates from cocoyam (Xanthosoma spp.) in Nicaragua

Guillermo Reyes Castro PhD Agr.

Universidad Nacional Agraria
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In the period 1990-2004 cocoyam (Xanthosoma spp.) was the most important export root and tuber crop in Nicaragua. The national and international demand of cocoyam has increased, but total area and yield has declined due to root rot disease (RRD) and DsMV. There is a big national demand of good quality planting material. In 2005 a guide for rapid and easy multiplication using individual buds from corms and cormels was published. Farmers had been trained and some of them have created plant nursery small enterprises. Disease-free plants from meristem of two cultivars and from indirect organogenesis in five cultivars have been distributed to farmers. Field performance of the plants in non-traditional areas and their multiplication using single bud multiplication technique has been evaluated. A national collection of Xanthosoma germplasm (wild and cultivated species) was carried out; morphological characterization of 61 accessions is ongoing. 18 accessions of X. violaceum, 8 of X. sagittifolium, 1 of X. robustum, 6 of X. mexicanum, 6 of X. wendlandii and 22 non identify wild Xanthosoma were collected. Agromorphological characterization (phenology, yield, DsMV and RRD incidence and severity) in a non-traditional and in a RRD infected area of 23 cultivated accessions is in progress. 14 RAPD primers have been selected for molecular characterization of the collection. The AG3-induced flowering process in nine cultivated accessions was studied. Differences in number of flower and flowering moment between cultivated accessions were found. Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the coat protein (CP) and 3’-untranslated regions for ten Nicaraguan DsMV isolates. The isolates showed high nucleotide identity to DsMV isolates from USA, Eastern Asia and Australasia. All Nicaraguan isolates, except one, shared a tandem repeat in the N-terminus of the CP. The Nicaraguan isolates formed two distinct subgroups correlated with cultivars.
O5. Recent advances in cocoyam tissue and biotechnology in the “Plant Physiology and Biochemistry Laboratory” (LAF314), ENS, University of Yaoundé 1.

Omokolo Ndoumou Denis, Boudjeko Thaddée, Mbouobda Hermann Désiré, Tsafack Takadong Julie Judith

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Cocoyam (Xanthosoma sagittifolium) is an important source of energy for populations of the humid and semi humid tropics. It consists of three cvs different from each other by their caryotype, the color of their flesh; their productivity and their root rot disease scores viz: the white cv (2n=26) sensitive and productive, the red cv (2n=26) intermediate and productive and the yellow cv (2n=56) resistant and unproductive. In our laboratory, investigations have been conducted since early 90s on 1): the biodiversity of Cameroon germplasm, 2): in vitro micropropagation and tuberization and 3): the biochemical characterization of Xanthosoma sagittifolium/Pythium myriotylum interactions.

Based on IPGRI’s morphological and agronomic descriptors for tuber crops, the biodiversity studies revealed an important morphological polymorphism in the Cameroon cocoyam germplasm. Positive and significant correlations such as pigmentation were established between some morphological and agronomic parameters, suggesting the existence of a common gene for these characters. Enzyme polymorphism showed variability among the accessions studied. The analyses of enzymatic polymorphism through peroxidases (Pox), polyphenoloxidase (PPO) and amylase profiles revealed eleven loci; four of which were monomorphs and seven polymorphs. This study revealed two polymorphic isoforms (Pox d and Amy c) specific to the yellow cv and two others (Pox c and Amy b) specific to the white one.

Plant regeneration was achieved in apex and callus culture. After 70 days of culture and from one single explant we could obtain from our own method (25)10 to (310)30 shoots through three subcultures. Four different methods were developed for microtuberisation 1): by an appropriate growth regulator balance in the culture medium, 2): by using relatively high concentration (80 g/l) of sucrose in the culture medium, 3) by an appropriate variation of the photoperiod and the thermoperiod and 4): by appropriate nitrogen nutrition.

The evaluation of total Pox activity in X. Sagittifolium and P myriotylum interaction showed the existence of a correlation between the genotype sensitivity and Pox activity. PAGE electrophoresis of the soluble fractions of the resistant cv. revealed the appearance of three new bands (Rf 0.25; 0.38 and 0.85) specifically in inoculated root. The electro focusing (IEF) profile revealed two cationic (pI 8.7 and 9) and two neutral (pI 6.67 and 7) isoperoxidases.

Following plant infection, total amino acid contents increased after infection and glycine could be detected. Total phenol contents increased also but, this increase was more important in the yellow cv. HPLC analysis of phenols revealed that plant infection was followed by an increase in paracoumaric acid and the appearance of two new compounds in the white cv while in yellow one, just one new unidentified compound was detected.

Ultrastructure analysis showed that during colonization of roots by the fungus, cell wall compounds, notably pectin were destroyed at the early stage of the infection.
The plant reacts by an accumulation of cellulose and callose in the contact of the pathogen. Biological control revealed that when cocoyam was stimulated with BTH or chitosan, biochemical indicators such as total peroxide (H$_2$O$_2$), ascorbate peroxidase (A-pox) and guaialcol peroxidase (G-pox) increased. This phenomenon was more pronounced in the tolerant yellow cv.

In conclusion, although we are facing many problems that are common to most developing countries, e.g. funding problems, shortage of equipment and facilities and the time it takes to establish efficient collaborative links between research and business, as well as between molecular biologists and agronomists/plant breeders, cocoyam biotechnology in our laboratory is progressing and is contributing to fruitful achievement.

Keys words: Biochemical markers, micropropagation, P. myriotylum, tuberization, X. sagittifolium,
O6. Application of tissue culture techniques to cocoyam (*Xanthosoma sagittifolium* Schott) production

E. Sama and S. Zok

*Jay PJ Biotechnology Biotechnology Laboratory, Institute of Agricultural Research for Development (IRAD), Ekona, Cameroon*

Cocoyam has been successfully micro-propagated from shoot tips initiated on liquid B5 basal medium either stationery or agitated. Soil media were beneficial at the multiplication, elongation and rooting stages. Shoot proliferation was significantly enhanced by 2µM TDZ, 20 µMBA and a combination of both levels, producing an average of 30 micro-shoots/culture. Shoots produced with BA were larger, more normal in appearance and rooted easily than those produced with TDZ, which were small, compressed and stunted. Upon transfer from culture into non-sterile soil for establishment in *vivo*, plantlets with a well developed shoot and root system survived greenhouse conditions, irrespective of the acclimatization method employed. This high level of survival was attributed in part to the few and sunken stomates found on both adaxial leaf surfaces of in vitro cultured plantlets, the epicuticular wax content and high rate of rooting.
O7. Preliminary study on radiation sensitivity of in vitro cultures of *Xanthosoma* (Macabo) in Cameroon

*X. Ndzana*, *S. Zok.* and *A.E Sama.*

*Jay P. Johnson Biotechnology Laboratory*  
*IRAD Ekona Regional Centre*  
*PMB 25 Buea, Cameroon*

In vitro grown cocoyam genotypes were exposed to $^{60}$Co $\gamma$-irradiation at varying doses (4 to 20 Gy) in order to determine a suitable lethal dose (LD) for eventual use as orientation for selection of effective mutagenic treatments that can induce useful genetic changes. The LD$_{50}$ was more appropriate than the LD$_{30}$ to be used as orientation for dose selection. The three cocoyam cultivars differed in their reaction to the irradiation, with the red being more sensitive than the white. Some phenotypic changes following irradiation included growth reduction and transformation of plantlet leaf shape, indicating thereby the possibility of distinct changes in the plant’s genetic makeup. This study indicates that variability within cocoyam species could be increased through induced mutations at a dose rate of $\sim$9 Gy.
O8. In-vivo mass production of Pythium-free cocoyam planting material

A. Amayana, A. M Ngone, D. Oumar and A. C. Njandjoa

Jay PJ Biotechnology Laboratory, Institute of Agricultural Research for Development (IRAD), Ekona, Cameroon

One of the major constraints to control Pythium-induce cocoyam root rot is the lack of diseased-free planting material. A study was conducted at Ekona Research Centre in 2007 to develop an affordable method of mass production of Pythium-free cocoyam planting material. White cocoyam corms were cut into pieces (50, 100 and 150g), and then treated with metalaxyl before being grown in four media including top soil (control), two Pythium-suppressive compost types (C1 and C2) and soil amended with various composts. The set up was incubated in the greenhouse at 25-26°C for two weeks, and then data were collected on the number of sprouting buds and rotting corm pieces to determine the suitable substrate for corm germination. The results show that compost substrate, used either alone or mixed with soil, enhanced corm bud germination. Fifty gram-corm pieces scored the best bud germination rate as determined by the germination index (total number of sprouting buds/total weight of corm pieces in counting) x 100, irrespective of the substrate type. However, 50g-corm fragments were much more prone to rotting than 100g- and 150g-corm pieces, especially with compost C2. Suckers from 50g-corm pieces scored a low survival rate (20-50%) as compared to those from corm pieces of 100g (30-100%) and 150g (50-100%). Growing suckers from corm pieces were individualized and grown in the various media for 8 weeks. Data were collected on time-to-next division and survival rate to determine the plant multiplication rate. Suckers survived better in compost C1 either when used alone (50-100%) or mixed with soil (50-100%). Suckers grew faster on compost C1 or compost C1 mixed with soil (6 weeks), than on other test media (8 weeks). The results show that about 40 000 cocoyam suckers can be obtained from a corm of 1kg within six months, using compost C1 as the plant growing substrate.
O9. Rapid multiplication using PIF Techniques to overcome scarcity of Cocoyam planting material in Cameroon

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Traditional Araceae propagating methods by corms and cormels portray a low multiplication rate and have not been able to satisfy national demand in planting material. In view of decrease of Cameroon production of Cocoyam attributable to the scarcity of good quality material associated with root rot disease, trials were carried out at IRAD Njome research station to develop rapid multiplication and propagation techniques of Cocoyam (Xanthosoma sagittifolium L.) through the use of corms and cormels following the PIF Technique as developed in Plantains.

Corms from two cultivars (Red and White) obtained from farmer field were treated following the PIF Technique and introduced in nursery bed in a screen house. The substrate of nursery beds was a sterilized mixture of fine soil and pouzzolane. Germination was assessed two months by counting the number of sprouted shoots with mean values ranging from 10 to 20 sprouts per corm. Sprouted shoots were removed from corms and transferred in ridges. Establishment rates evaluated one month after was 81% and 100% for red and white cultivars respectively. Corms from previous trial were treated according to the PIF and minisets techniques with sawdust as nursery substrate in open air. Sprouted shoots number per corm varied according to weight of initial corm and sett sizes with values ranging from 10 to 40. Sprouting rate evaluated two months later was 98% for the minisett and 95% for the PIF technique with reactivation possibilities after removal of sprouts greater for the PIF technique than the minisett technique. Transferred in field, 100% establishment was observed for the two methods and cultivars with no signs of root rot.

These results suggest that the PIF technique could be adapted and efficiently used for large scale production of Cocoyam planting material to overcome present shortages of good quality planting material and in dissemination of newly developed research accessions.

Keywords: Cocoyam, rapid multiplication, PIF, sett; planting material
Cocoyam is an important food staple in developing countries where it is grown. Its tubers have a higher protein content and high market value compared to cassava with the potential to improve food security and family income of the population. Cocoyam is largely produced by small holders who rely on traditional and labor-intensive practices that do not allow for optimal exploitation of the crop’s productive potential. Although production constraints differ with agroecology, cocoyam suffers severe yield losses due to the root rot disease caused by *Pythium myriotylum*. In addition, ongoing national cocoyam research programs have shown limited progress in the development of high productivity and disease resistant varieties partly due to employed classical methods. This paper will review molecular tools instrumental to the advancement of cocoyam research and highlight recent molecular data that have effectively enhanced our understanding of the cocoyam root rot disease pathogen. In additional, molecular aspects of breeding for cocoyam resistance will be discussed. Our understanding of *P. myriotylum* isolates that attack cocoyam has greatly been enhanced by molecular data but the road to the development of resistant/tolerant varieties is still uncertain. Employing molecular techniques is crucial in future cocoyam breeding research to generate new knowledge with a priority on target traits. These creative approaches could be used to exploit genetic diversity and genomic data in germplasm enhancement and improvement of the cocoyam. For example, it would be helpful in integrating conventional cocoyam breeding with Marker Assisted Breeding in a multidisciplinary collaborative effort involving molecular biologists and biotechnologists. This could be achieved by establishing and fostering relationships and linkages with national partners and Advanced Research Institutes. Increasing cocoyam production will have measurable positive benefit on the economy and on the lives of over 200 million people for whom cocoyam is the major staple food and source of family income, especially in West and Central Africa.
O11(a). Molecular identification of the causal organism of cocoyam root rot disease in Cocoyam (Xanthosoma spp.) using PCR-RFLP technique

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Cocoyam (Xanthosoma sagittifolium) is an herbaceous plant that produces edible cormels, which are part of the daily diet of millions of people around the world. Root rot disease caused by *Pythium myriotylum* seems to be the most important production constraint with reductions of up 90% reported in Costa Rica, Cameroon and Puerto Rico.

Pathogenic and non-pathogenic isolates of *P. myriotylum* from cocoyam, *P. myriotylum* from other host crops and other species of *Pythium* were compared using PCR-RFLP technique. Amplicons from the internal transcribed spacer (ITS) regions of rDNA and the cytochrome oxidase subunit II (COXII) gene were obtained. Digestion of the former with 2 restriction enzymes and the latter with five, showed that the pathogenic isolates to cocoyam could be clearly differentiated from the other isolates.

These results create an easy, fast and precise identification method for cocoyam pathogenic isolates. In addition, it adds evidence to previous biochemical and molecular studies that have revealed proof of speciation for the *P. myriotylum* associated to cocoyam.

O11(b). Phylogenetic relationships between *Pythium myriotylum* isolates from cocoyam (Xanthosoma sagittifolium) and *P. myriotylum* from other host crops and other species of *Pythium* based on cytochrome oxidase I, cytochrome oxidase II and β-Tubulin gene sequences.

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*Pythium myriotylum*, the causal agent of Cocoyam Root Rot Disease (CRRD) is considered the most destructive disease of cocoyam (*Xanthosoma sagittifolium*). Reductions of up 90% have been reported in Costa Rica, Cameroon and Puerto Rico. Biochemical and molecular studies have revealed evidence of speciation for the *P. myriotylum* associated to cocoyam. Sixty isolates representing 38 species of *Pythium* were chosen to investigate intra- and intergeneric relationships with sequence analysis of three genomic areas: one from the mitochondrial DNA, the cytochrome Oxidase I (cox I) gene; and two from the nuclear DNA, a 563 bp of the cytochrome oxidase II (cox II) gene and a 658 bp fragment from the β-tubulin gene. Neighbor-joining analysis of the three DNA regions revealed that, *P. myriotylum* from cocoyam form a different cluster from *P. myriotylum* from other host crops and from other *Pythium* species.
O11(c). Presence of *Pythium myriotylum* in soils with different crop coverage (cobertura vegetal) on the Northern Zone of Costa Rica

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Root rot disease (RRD) caused by *Pythium myriotylum* is the most important constraint on cocoyam (*Xanthosoma sagittifolium*) production. Cultivated areas where this disease has been observed cannot be planted again with cocoyam because there is a significant chance of observing higher incidence and severity of RRD. Soil analysis to determine if the pathogen is present, and therefore if the disease could develop, is a high priority for farmers. In order to approach this need soil samples from 6 different soil conditions (cassava, banana, citrus, pineapple, primary forest and cocoyam) were collected. The presence of the pathogen was tested using three methods: (1) growing *in vitro* derived plants on each soil and checking for symptom development, (2) by macroarray analysis of DNA isolated from each soil using oomycete specific primers and (3) macroarray analysis of DNA isolated from roots of *in vitro* derived plants growing on each soil. RRD symptoms were observed only on plants grown on RRD infected soil. Macroarray analysis of the soil DNA did not detect the presence of *P. myriotylum*, but macroarray analysis of DNA isolated from roots shown that the pathogen was present in artificially and naturally RRD infected soil as well as in plantain cultivated soil.

O11(d). Evaluation of the host range of *Pythium myriotylum* isolated from cocoyam (*Xanthosoma sagittifolium*) infected plants.

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*Pythium myriotylum* is considered the major drawback in cocoyam production. It can cause the complete destruction of the root system. Studies of isolates from cocoyam infected plants have shown signs of host specificity. In order to evaluate the range of this specificity, plants closely related to cocoyam (*Colocasia* sp., *Phylodendron* sp., *Spathiphyllum* sp., *Aglaonema* sp.) or plants commonly affected by *Pythium myriotylum* (tobacco, sorghum and pineapple, were inoculated with various isolates of this pathogen. Three types of isolates were used: (a) isolated from cocoyam but not pathogenic, (b) isolated from cocoyam and pathogenic from Cameroon and (c) from Costa Rica. It was found that the only the pathogenic isolates, either from Costa Rica or Cameroon, were pathogenic to cocoyam, and only cocoyam plants showed infection symptoms.
O12. Response of cocoyam (Xanthosoma sagittifolium) accessions to Pythium myriotylum inoculation

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Continuous cocoyam production in Cameroon is usually handicapped by the susceptibility of the plant to the root rot disease incited by Pythium myriotylum. To investigate potential sources of disease resistance, ten (8 Cameroonian, 1 Costa Rican, 1 American) cocoyam accessions were artificially inoculated with a highland isolate of P. myriotylum during 2002 in Dschang, Cameroon. Shoots of three-month old cocoyam plants were soaked in sporangial suspensions (2.5 x 10^4 sporangia/ml) of the fungus for 5 min, planted in plastic pots containing sterilized soil and placed in a screenhouse. Control plants were inoculated with sterile distilled water. Disease and plant growth characteristics were rated weekly for two months. No significant differences (P > 0.05) were observed among the accessions for disease incidence, disease severity and growth characteristics. Leaf and root symptoms did not develop on non inoculated plantlets while inoculated plantlets showed symptoms of root rot infection (decay roots, root pruning, yellowing of shoots, reduced leaf size and leaf number, and stunted growth). Inoculated plantlets had disease incidence of 35% on leaves and 46 % on roots. The lack of differential responses in the accessions suggests that alternative sources of resistance may be required in the sustainable management of cocoyam root rot disease in Cameroon.
O13. Hybridization in *Xanthosoma* for genetic improvement of major agronomic traits and root rot disease resistance

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Improvement of cocoyam (*Xanthosoma sagittifolium*) mainly involves breeding for high yield and resistance to the cocoyam root rot disease (CRRD). Randomly selected hybrids derived from crosses between white and red accessions of *Xanthosoma* were evaluated along with their parents at Ekona Research Station to investigate the effect of genetic recombination in *Xanthosoma* improvement. Results show that the overall performance of the red cultivar was significantly low as compared to the white cultivar and the hybrids for all the traits studied, except for resistance to CRRD. The hybrid clones had low performance with respect to single plant cormel yield and cormel weight compared to the white accessions. The root rot disease scores were significantly lower in the hybrid population compared to the white and red accessions. Correlations between major agronomic characters were significant except between cormel size and cormel number for the red and hybrid clones. Highest and positive correlations were recorded between corm weight and cormel number \((r = 0.89**)*\) among the white accessions; corm weight and single plant cormel number \((r = 0.81**)*\) among the red; and between single plant cormel yield and cormel weight \((r = 0.77**)*\) among the hybrid derived clones. Significant negative correlations were also recorded between CRRD scores and single plant cormel yield, cormel number and single corm weight. This study indicates that intervarietal hybridization is a potential means to genetically improve *Xanthosoma* especially with respect to root rot disease resistance.
O14. Variation of *Pythium*-induced cocoyam root rot severity in response to soil type

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In Cameroon, andosols are suspected to be suppressive to cocoyam (*Xanthosoma sagittifolium*) root rot disease (CRDD) caused by the Oomycete pathogen *Pythium myriotylum*. To determine factors involved in disease suppressiveness, andosols were studied in comparison to ferralsols known to be disease-conducive. Soil samples were collected from six sites of which three were in andosols around Mount Cameroon (Boteva, Njonji, and Ekona) and the three others in ferralsols (Bakoa, Lapkwang, and Nko’o canane). Greenhouse plant experiments were used to assess soil suppressiveness. Soils were artificially infested with two levels of *P. myriotylum* inoculum (100 and 300 mycelia strands.g⁻¹ soil) prior to planting cocoyam. Disease severity was significantly higher in ferralsols than in andosols. Andosols partly lost their suppressiveness as a result of autoclaving and could recover suppressiveness following recolonisation by their original microflora. Soil microbial groups implicated in the disease suppression were investigated by assessing the effect of fungicide, bactericide and pasteurisation on andosol suppressiveness. Andosols suppressiveness was significantly reduced following pasteurisation and treatments with fungicide and bactericide. The possible influence of microbial biomass on andosols suppressiveness was investigated by comparing microbial populations of suppressive andosols to those in andosols that had lost suppressiveness.

A comparative analysis of suppressive and conducive soil properties was performed to identify soil variables, which may contribute to soil suppressiveness. Soil chemical analysis results show that organic matter content was higher in andosols than in ferralsols. In addition, the content of mineral nutrients such as Ca, K, Mg and N, were higher in andosols than in ferralsols. These soil variables negatively correlated with disease severity. By contrast, sand and clay, which were higher in ferralsols than in andosols, were positively related to disease severity. This study has confirmed the suppressive nature of andosols from Mount Cameroon to CRRD. The results suggest that high organic matter content is likely mediating the cocoyam root rot suppression in andosols by improving soil structure, increasing soil mineral nutrient and microbial biomass, and sustaining microbial activities.
O15. Factors associated with organic matter-mediated cocoyam root rot suppression in field systems

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Major factors that influence suppression of cocoyam root rot disease by organic matter amendment were investigated from 2005 to 2007 in Cameroon. Cocoyam plant experiments were conducted over three sites of which one was from andosol soil type at Ekona and two others from ferralsol soil type at Matomb and Akonolinga. Plots were amended with three types of compost A, B and C, known to suppress the cocoyam root rot disease (CRRD) in the greenhouse, at the rate of 20 t/ha. Plots without compost, and plots treated with fungicide (metalaxyl) were used as control in the various experiments. The results show that compost amendment raised soil pH and soil nutrients content including total nitrogen, organic carbon and cations, and it significantly reduced the CRRD incidence. However the level of disease suppression varied according to compost type and location, compost C and Ekona, being the best disease suppressing compost and site of the highest disease suppression (68-70%), respectively. \textit{Pythium myriotylum} density was lower at Ekona (3.73 x 10\textsuperscript{2} cfu/g soil) as compared to Matomb (5.43 x 10\textsuperscript{2} cfu/g soil) or Akonolinga (5.27 x 10\textsuperscript{2} cfu/g soil), suggesting that poor disease suppression in Matomb (20-30%) and Akonolinga (10-24%) might be due to high inoculum level. In a different experiment, compost C was applied at three different times March, April and May, and controls were made of no compost- and mineral fertilizer-treated plots, to see whether time of compost application could influence the level of disease suppression. Disease suppression level varied from 11 to 68% according to time of compost application, March (onset of rainy season), being the best time to apply compost. The study suggests that the level of inoculum that exists in soil before organic matter application is critical for effective disease suppression. Timely application of good quality organic matter is likely essential to maximized suppression of CRRD in the field.
Chitosan and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), well known chemical inducer of systemic acquired resistance (SAR) were tested for their ability to protect cocoyam (*Xanthosoma sagittifolium*) from root rot disease following inoculation with *Pythium myriotylum*. Three months old cocoyam plants sprayed with chitosan (0.2 mg.ml$^{-1}$) or BTH (0.2 mg.ml$^{-1}$) to induce stimulation 7 days before inoculation with fungal. The potential stimulation was evaluated in terms of hydrogen peroxide (H$_2$O$_2$), malondialdehyde (MDA) and phenol contents, guaiacol peroxidase (G-Pox) and polyphenoloxidase (PPO) activities. Results were found that the activities of Pox and PPO enhanced totally in roots and leaves organs in response to the stress. Furthermore, antioxidant Pox and PPO activities increased in the initial stimulation and decreased afterwards. These activities ascended to a max at day 5 and 10 in both leaves and roots of yellow and white cvs respectively. H$_2$O$_2$ and total phenol contents showed the same tendency during treatment. As a lipid peroxidation parameter, MDA content in leaves and roots increased in the beginning, dropped afterward and increased again after 20 days. The MDA content was conversely correlated to Pox and PPO activities. To characterize the importance of this stimulation, qualitative analysis of phenol compounds using HPLC reveals the appearance of cafeoshikimic acids derivatives after spreading of white and yellow cvs with BTH and chitosan respectively. Polyacrylamide gel electrophoresis showed the presence of two and three new Pox bands for white and yellow cvs. These results indicated the potential effect of both chitosan and BTH to induce cocoyam defense system which could improve the defense against *P. myriotylum*.

**Keys words:** cocoyam; BTH; chitosan; elicitation, lipid peroxidation.
**O17. Pseudomonas bacteria antagonistic to *Pythium myriotylum* associated with cocoyam (*Xanthosoma sagittifolium*) in Cameroon**

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Forty pseudomonads were isolated from the rhizosphere of healthy white and red cocoyam plants appearing in natural fields in Cameroon, heavily infested with *Pythium myriotylum*. White cocoyam is highly susceptible to cocoyam root rot. In contrast, the red cocoyam has a certain degree of field tolerance against the cocoyam root rot disease. Intriguingly, *P. myriotylum* antagonists could be exclusively retrieved in this study from the rhizosphere of red cocoyam. Except for one isolate, all antagonistic isolates produced phenazine antibiotics. Results from whole-cell protein profiling showed that the antagonistic isolates are different from other isolated pseudomonads, while BOX-PCR revealed high genomic similarity among them. Our results seem to suggest that different pseudomonad groups inhabit the rhizosphere of white and red cocoyam.16S rDNA sequencing of two representative strains within the group of antagonists confirmed their relatively low similarity with validly described Pseudomonas species. These antagonists are thus provisionally labelled as unidentified Pseudomonas strains. Among the antagonists, Pseudomonas CMR5c and CMR12a were selected because of their combined production of phenazines and biosurfactants. Both CMR5c and CMR12a showed excellent in vivo biocontrol activity against *P. myriotylum*. In addition, phenazines and biosurfactants act synergistically in the control of *P. myriotylum*.

We hypothesize that the red cocoyam may have evolved a strategy of stimulating and supporting specific groups of autochthonous antagonistic microorganisms as a first line of defense against *P. myriotylum*. This hypothesis is supported by the observation that red cocoyam is as susceptible to *P. myriotylum* as white cocoyam in sterile soil.
O18. Incidence and distribution of viruses infecting Cocoyam (Xanthosoma sagittifolium) in two Southwestern States of Nigeria

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Cocoyam (Xanthosoma sagittifolium) is a tropical tuber and staple in Nigeria that is fast assuming great importance in other West and Central African countries because of its nutritional value and income generating potentials particularly for the rural farm families. Diseases caused by viruses are important production constraints. Farms in 44 villages of 22 Local Government Areas (LGAs) of 2 highly populated Southwestern States (Oyo and Osun) of Nigeria, the highest world producer of this crop were surveyed in 2007 for viruses infecting cocoyam. Five virus-like symptomatic and asymptomatic samples were randomly collected from 2 farms in each LGA. They were indexed serologically by Protein A antibody sandwich (PAS) and Antigen coated plate (ACP) - Enzyme-linked immunosorbent assay (ELISA) for Dasheen mosaic virus (DsMV) genus Potyvirus, Cocoyam badnavirus virus (CBV) known to infect this crop and Cucumber mosaic virus (CMV) genus Cucumovirus which is cosmopolitan in Nigeria. Nucleic acid extracts of samples that were positive for these viruses by ELISA, were further identified using Polymerase chain reaction (PCR) and Immunocapture-Reverse transcription (IC-RT)-PCR. Out of 220 samples collected from the 2 states, 33.6% (74 out of 220) were positive for both Dasheen mosaic virus and Cocoyam badnavirus and none were positive for Cucumber mosaic virus.

Incidence of DsMV was higher than that of CBV as 28.18% (62 out of 220) of samples were positive against 5.45% (12 out of 220) for CBV. Osun State harboured more CBV (9 positive samples against 3 for Oyo) while the reverse was the situation for DsMV with 40.9% (45 out of 110 for Oyo state against 15.5% (17 out of 110). Of all the LGAs surveyed in both states only cocoyam samples from Aiyedade tested negative for all the viruses. 15 of the 22 LGAs (7 in Oyo and 8 in Osun) were free of CBV while DsMV was present in 21. Latent infection was noted in 2.3% of asymptomatic samples (5 out of 220) and also mixed infection of these viruses was noted in 4 samples (2 from each State). Bands of expected sizes at about 900bp and 600bp respectively were obtained on viewing 70% agarose gel under UV-light on running IC-RT-PCR and PCR for DsMV and CBV for ELISA positive samples using degenerate Potyvirus and Badnavirus primers. This is the first time DsMV and CBV are reported on cocoyam in Nigeria and also this is the first report of their occurrence.

Keywords: Cocoyam, Dasheen mosaic virus, Cocoyam badnavirus, enzyme-linked immunosorbent assay, polymerase chain reaction.
Cocoyam diseases in the forest zones of Ghana

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Cocoyam is an important staple food crop in the forest zones of Ghana. In the forest belts of Ghana, cocoyam is a common volunteer crop that sprouts out of the soil after land preparation (particularly after burning). Cocoyam cormels store better than most root and tuber crops. This attribute makes it an important food security crop in several rural communities in forest zones of Ghana. Cocoyam production is now receiving the needed attention that it deserves as a food security crop after many years of neglect. In Ghana, both cormels and the leaves of cocoyam are eaten. As part of the initiative to improve production, the National Root and Tuber Improvement and Marketing Programme (RTIMP) ordered a diagnostic survey in 2008 to define the level of diseases affecting cocoyam production in two major cocoyam producing districts, Asante Akim South (in the Ashanti Region) and Fanteakwa (in the Eastern Region). Incidence of brown leaf spot disease in the Fanteakwa District was 80%. On the farms, 20% of cocoyam plants were infected with brown spots. Severity of leaf spot disease in the Fanteakwa District was 2.6 on a 1-5 scale. In the Asante-Akim South District, incidence of brown leaf spot was 80% and disease severity was 2.6. Leaf blight was recorded in the Asante-Akim South District at an incidence of 20%. Incidence of Dasheen mosaic virus was 100% in both districts. Three Farmer Field Fora (FFF) have been established in three cocoyam producing communities in the Asunafo North District (a major cocoyam producing area in the Brong- Ahafo Region) to facilitate the sharing of knowledge in improved cocoyam production practices including disease control. Ninety farmers from the three communities, agricultural extension agents, a breeder and a plant pathologist are the major interactive partners in the established fora. Cocoyam trials have been set up in each of the fora communities as the study fields.
O20. Cocoyam rebirth in Nigeria

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O21. Possibilities of IRAD cocoyam germplasm regeneration and duplication from Global Crop Diversity Trust

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The Global crops diversity trust is an international organisation temporary hosted by FAO and Bioversity International. Its objective is to promote a sustainable long term conservation and availability of plant genetic resources for food and agriculture (PGRFA) in the aim of achieving food security in line with the provisions of the International treaty for food and Agriculture and the Gobal plan of action for the conservation, utilisation of PGRFA.

As a first step of its action, a series of consultation held around the world identified PGRFA regeneration and safety duplication as a pressing need shared by many collections. At this aim some priority collections were targeted among which IRAD cocoyam (Xanthosoma sagittifolium) collection.

This paper aims at introducing the activities which are yet still to start and collaborators

Key-words: Cocoyam, germplasm, regeneration, safety duplication
Abstracts – Posters

P1. Pathogenicity of *Pythium myriotylum*, *Fusarium* sp. and *Rhizoctonia* sp. on cocoyam (*Xanthosoma sagittifolium*) plants under different soil water conditions

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Cocoyam Root Rot Disease (RRD) has been associated to several pathogens. In some countries *Pythium myriotylum* is considered the causal agent while in others *Fusarium solani*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Sclerotium rolfsii* (*Corticium rolfsii*) and some bacteria as *Erwinia* sp and *Pseudomonas* sp. have been associated to this disease. In order to identify the causal agent under Costa Rican conditions, the organisms present on symptomatic plants were . Three organisms, *Pythium aff. myriotylum*, *Fusarium solani* and *Rhizoctonia solani* were isolated and inoculated on *in vitro* derived plants growing on sterile soil. In addition, since RRD have been correlated with high water content in the soil, the inoculated plants were grown under four different soil water content (1.0, 0.33, 2 y 10 bars). Three days after planting on *P. aff. myriotylum* infected soil, wilting symptoms were evident and all plants died after 10 days. Plants growing on *F. solani* and *R. solani* infected soil at any water content were symptomless. Re-isolation of all three pathogens was possible from roots.
P2. Evaluation of different biological agents on the control of *Pythium myriotylum* attack on cocoyam (*Xanthosoma sagittifolium*) plants.

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Cocoyam (*Xanthosoma* sp.) is an important crop in the region of Central America and the Caribbean where it is a cash crop for small farmers that export this crop to United States and European countries. Cocoyam Root Rot Disease (RRD), caused by *Pythium myriotylum* is considered the major drawback in cocoyam production. It can cause the complete destruction of the root system. Due to the high cost of chemical control of this disease and the negative effects of its use on the environment, organic control strategies have been proposed. In this study the effect of several organic compounds were tested on *in vitro* derived plants that were inoculated on sterile soil inoculated with cocoyam-pathogenic isolates of *Pythium myriotylum*. It was found that only the vermicompost derived from cow manure significantly reduced the symptoms of RRD.
P3. Some pests of cocoyam, Xanthosoma sagittifolium (L.) Schott in Cameroon

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Cocoyam is an important food crop in Cameroon and several other countries in tropics and sub-tropics. Decline in cocoyam production observed since some decades is largely attributed to cocoyam root rot disease (CRRD), principally caused by Pythium myriotylum. Therefore, effort in increasing production has been devoted to crop improvement against CRRD and pest aspect has been overlooked. Here we reported on some insect pests that may contribute to cocoyam yield reduction and suggest studies in their bio-ecology been undertaken to develop management strategies.
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