COLLABORATORS MEETING

IITA-JIC-NRI

GATSBY-FUNDED BIOTECHNOLOGY PROJECTS

EXTENDED ABSTRACTS

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Report
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This progress report covers the period from April to October 1999

Contents

PROJECT 1

A. Application of molecular genetics to assist breeding of yams (*Dioscorea rotundata* and *D. alata*)

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1 Study of genetic diversity of cultivated yams, *D. rotundata* and *D. cayenensis*

Comprehensive characterisation of genetic resources is very useful for their efficient utilisation. During the two cropping seasons of 1996 and 1997 over 340 accessions of yam varieties collected from West and Central Africa were characterised on the basis of morphological and molecular markers and then classified into cultivar groups. The results obtained were confirmed in 1998. In the 1999 cropping season, additional yam cultivars from Nigeria, which were not studied previously, are being characterised by morphological, biochemical, (isozyme) and molecular markers. The underground organs (tubers) will be characterised at harvest in December- January 2000.

Recently, the Ghana Plant Genetic Resources Centre (PGRC), under a special project on root and tuber improvement project funded by IFAD, requested IITA's expertise in the characterisation of yams and other tuber crops. A workshop on conservation and characterisation of root and tuber genetic resources of Ghana (yams, cassava, sweetpotatoes, frafra-potatoes) was organised by the PGRC, Bunso, Ghana (22-26 June, 1999). IITA was invited to present the current approach of yam germplasm characterisation in West and Central Africa. That approach was adopted and research is ongoing to implement it in Ghana.

2 Development of molecular markers linked with specific agronomic traits e.g. resistance to anthracnose, virus and nematodes.

2.1 Development of a genetic linkage map in *D. rotundata*

A genetic map of yam, based on molecular markers, is being developed for identification of markers associated with resistance to yam mosaic virus (YMV) and nematodes. Four mapping populations were developed for this project. Of these, one population derived from a cross between a popular landrace cultivar (TDr 93-2) as female parent and a breeding line (TDr 87/00211) as male parent, was initially used as the mapping population. This population consisted of 169 F_1 individuals. Although a genetic linkage map could be generated using this population size, it was found that a mapping population with over 180 F_1 individuals could lead to a better genetic linkage map. Therefore, a second population derived from a cross between TDr 93-1, another popular landrace cultivar (female parent) and TDr 87/00211, a breeding line (male parent) was selected to generate a linkage map.

Based on results from pre-screening of the parental lines using 64 EcoRI/MseI AFLP primer combinations (PCs), 10 PCs were selected to fingerprint 180 randomly selected individuals. Polymorphic bands were co-dominantly scored and genotypes allocated based on the assumption that the markers are segregating as in a cross-pollinated population. The data set that consisted of 360 markers was subjected to

mapping analysis using the Joinmap software program. The data were split into separate sets, one for each parent. For both parents a genetic linkage map was generated.

IITA, in collaboration with the University of Saskachtewan, Canada (USASK), is currently developing micro-satellite markers for yam. Thirty micro-satellite markers or simple sequence repeats (SSRs) were isolated from the cultivar TDr 93-2 and tested for polymorphism on restricted yam germplasm at USASK. These SSR are being tested for the level of polymorphism in the parental lines of the mapping populations. Of the thirty SSR primers tested so far, twenty SSR primers amplified yam DNA and yielded a variable number of polymorphic bands between the parental lines. Effort is being made to integrate these SSRs markers on the yam AFLP linkage map.

2.2 The genetics of resistance to Yam Mosaic Virus (YMV) in *D. rotundata*

Crosses are being made among progenies segregating for reaction to YMV. These involve the following combinations of parents: Resistant x Susceptible; Susceptible x Resistant; Susceptible x Susceptible; and Resistant x Resistant. ELISA tests are being carried out on the mapping populations currently under evaluation in the field (for more details see report 1B below).

2.3 Nematode resistance screening of *D. rotundata* mapping populations

Screening of the segregating populations for resistance to yam nematode (*Scutellonema bradys*) and root-knot nematodes (*Meloidogyne* spp.) is in progress. This involves plantlets multiplied in vitro and minitubers obtained from tissue culture derived plantlets. Results are yet to be fully analysed. In addition, tuber setts of the parental lines used to develop the mapping populations were screened both in pots in a screenhouse and in the field. The pot experiment is completed, but the field component is yet to be completed . The most highly susceptible clone was TDr 93-2 while the less susceptible ones were TDr 93-1 and TDr 87/00211. These results will be confirmed and the current study is expected to provide us with the genetic information on resistance of yams to the nematodes.

2.4 Development of a genetic linkage map in *D. alata*

Based on a pre-screening of the parental lines (TDa 95/00328 and TDa 87/011091) using 64 EcoRI/MseI AFLP PCs, 10 PCs were chosen to fingerprint 180 randomly selected individuals. The choice of PCs was based on the density, the complexity and the number of polymorphisms in the fingerprints. Polymorphic bands were co-dominantly scored and genotypes allocated based on the assumption that the markers are segregating as in a cross- pollinated population. The data set that consisted of 364 markers was subjected to mapping analysis using the Joinmap software program. Following this initial mapping analysis, the total data set of 264 markers was mapped. Some linkage groups contained three or less markers. These markers could not be used in the analysis. An increase in the number of markers may help to assign linkage groups to the same chromosomes. However it is also possible that regions not covered by the markers are not polymorphic in this cross. Finally 338 markers were mapped in 20 linkage groups with a total length of 1055 cM . Two linkage groups consisted of

parent 1 markers only and 4 linkage groups have only parent 2 markers. These six linkage groups probably represent specific parental regions.

2.5 The genetics of resistance to anthracnose

The genetics of resistance to anthracnose disease (caused by the fungus *Colletotrichum gloeosporioides*) was investigated in 1998 in a cross between *D. alata* genotype TDa 95/00328, which has consistently exhibited field resistance across locations, and a susceptible landrace TDa 95-310. Isolates representing two distinct populations of the pathogen were used in the screening experiments: a fast growing salmon (FGS) strain and a slow growing grey (SGG) strain. Screening with the FGS strain resulted in a 5:1 resistant:susceptible segregation ratio, which is consistent with the presence of a single dominant resistance locus in the duplex configuration in the hybrid TDa 95/00328 (RRrr). In contrast, inoculation with the SGG strain revealed an excess of susceptible progenies, indicating that the resistance gene in TDa 95/00328 reacts differentially to strains of *C. gloeosporioides*. In order to confirm these segregation ratios, a second mapping population derived from the cross between TDa 92-2 and TDa 85/00257 was subjected to a similar screenhouse screening procedure as described above. The experiment is completed and data are being analysed. In addition to this screenhouse screening, the two populations are currently being evaluated for their reaction to anthracnose disease under field conditions.

2.6 Development of anchor RFLP markers for yams, *D. rotundata* and *D. alata*

Yams are known to be polyploid and the lowest level is tetraploid. The ploidy varies in the cultivated species *D. rotundata*, *D. cayenensis* and *D. alata*. Most cultivars are tetraploid 2n=4x =40, but some are hexaploid 2n=6x =60 or octoploid 2n=8x=80. The basic chromosome number is 10. Mapping polyploid genomes with unknown genetic constitution (allo- versus autopolyploid) is particularly demanding in terms of finding useful mapping markers. The strategy so far adopted was to identify single dose (SD) and double dose (DD) markers in the F_1. These SD markers are used to generate the linkage map and the DD markers used to bridge linkage groups between female and male maps. At the end of the exercise, the maximum number of linkage groups expected is 40 with no assumption of the genome constitution (allo- or autopolyploid). To identify the homologous linkage groups belonging to the same chromosome, one approach is to use locus specific markers (RFLPs, SSRs). The current yam map being developed is based on AFLPs, which most of the time are scored as dominant markers (except Keygene, with their proprietary software that can allow a co- dominant genotyping of individuals).

In order to generate anchor RFLP markers, Jacob Mignouna has been visiting JIC (July-November, 1999) to carry out RFLP mapping of the yam genome. In the first instance, cereal conserved sequences (Monocot.) from the EGRAM (The European Union project on Gramineae genomics) Project were generously provided by Dave Laurie, Cambridge Laboratory. Noel Ellis generously provided pea probes (Dicot.) from another EU legume mapping project. These two sets of probes were used to hybridise yam Southern blots. Cereal conserved sequences hybridised with yam DNA while pea probes failed to show any signal thus confirming the classification of yam as a monocot. Although the cereal conserved sequences did show some hybridisation signals, these signals were rather weak owing to the fact that the hybridisations were

carried out at low stringency conditions. From a taxonomic point of view the lineage leading to yam is a very deep division within the monocots. This means that yam sequences are very different from any others studied to date. In consequence we are somewhat limited in the probes that can be used for RFLP analysis. In a preliminary screening of polymorphism between the parental lines using 24 cereal probes, only two probes were found to be useful. As reported above, there is a problem with the cereal conserved sequence probes which is that they have to be used at low stringency hybridisation. This tends to generate high background on the autoradiographs and often the bands are 'fuzzy', so some potentially useful data may be lost for technical reasons. Consequently, it was thought that it would be appropriate to generate some yam probes. Some yam genomic clones that had been generated in 1995 were considered but these would not be ideal; cDNAs would be better, but so far we are not aware of a good source of these probes. Therefore, plans are being made to generate a cDNA library soon at IITA. Another source of RFLP probes has been drawn from gene sequence database available in data banks. So far 12 genomic sequences have been selected. PCR primers were designed and the corresponding sequences in *D. rotundata* and *D. alata* amplified. So far 9 primers amplified yam genome, 6 of which were purified and are being used for hybridisations. The remaining 3 genes are yet to be tested. This is still a rather low number of probes and effort will be made to generate more yam cDNA clones.

3. Phenotypic assessment and clonal evaluation of the mapping populations (*D. rotundata*)

Clonal evaluation in order to generate data for genetic analysis and mapping purposes is a very labour intensive effort for a vegetatively propagated and bulky crop where mislabeling of genotypes at harvest and/or planting could be high if great care is not taken at all steps. The four mapping populations were planted during late May to June, 1999 and the plants were fully established by early August. This late establishment was due to the late commencement of the rainy season this year. The mapping populations are:

BC1 : (TDr 87/00571 x TDr 87/00211): 109 genotypes
BC2 : (TDr 93-2 x TDr 87/00211): 169 genotypes
BC3 : (TDr 93-1 x TDr 87/00211): 304 genotypes
BC4 : (TDr 87/00571 x TDr 89/01444): 180 genotypes

The published list of yam descriptors is being used for population evaluation both at juvenile and adult stages. These descriptors were selected to be the most stable across environments. In addition, some specific traits useful in current yam breeding were given priority in the evaluation. These characters were at juvenile stage: early emergence, juvenile habit, leaf spot if any, nature of spines, young spine colour, viral symptoms at seedling stage. At adult stage I, the increase or decrease of spine density, leaf blight, anthracnose damage. At adult stage II, spine colour, viral symptoms, inflorescence habit, early or late maturing type, nematode damage for early maturing type. Currently, the populations are at the second adult stage and will be later evaluated at harvest stage (December- January 2000). The traits to be considered will be: multiple tubers per plant, tuber-rooting system, dormancy period after harvest, colour of the tuber flesh. The 762 genotypes were replicated six times. Tuber set size and other conditions were kept uniform. The evaluation is still in progress.

4 Phenotypic assessment and clonal evaluation of the mapping populations (*D. alata*)

The *D. alata* mapping population consisting of 72 individuals (TDa 95/00328 x TDa 95- 310) was planted in the field for anthracnose resistance screening. Currently some genotypes are flowering profusely and are being used for crossing to develop backcross populations. The type of inflorescence (male or female) of each genotype is also being scored. These data will be compared to the juvenile stage data collected in the screenhouse at previous years. A second mapping population (TDa 95/00328 x TDa 95/01091) with over 300 F_1 progeny was established in the screenhouse. Morphological characterisation is in progress. The population will be screened for anthracnose resistance for the first time in 2000.This population is being duplicated in tissue culture using nodal cutting.

5. Future plans

Develop a cDNA library for mapping and sequencing to generate ESTs.
Develop more microsatellite markers for mapping and genetic studies.
Integrate RFLP and SSRs markers to the current yam maps.
Confirm the screening of yams for resistance to nematodes using tissue culture plantlets, minitubers and regular planting setts.
Screen mapping populations for nematode reaction under field conditions.
Screen *D. alata* mapping population [TDa 95/00328 x TDa 87/01091] (tissue culture plantlets) for anthracnose reaction in controlled environment and in the field .
Identify molecular makers linked to YMV and nematode resistance in *D. rotundata*.
Identify molecular markers linked to anthracnose resistance in *D. alata*.
Test any useful markers for marker-assisted breeding.

6. Conclusion

Considerable progress has been made in the last six months particularly in developing a genetic linkage map for both species *D. rotundata* and *D. alata* based on AFLP markers. For the first time, a genetic analysis of molecular markers revealed that yams are rather allo-polyploid, which is extremely important for genetic analysis and breeding strategies. Clonal evaluation of the mapping populations and their reactions to disease (virus, anthracnose) and pest (nematodes) are also progressing to our satisfaction. When completed, the genetic control of these traits will be confirmed and markers linked to these traits identified as more markers are to be added to the present linkage map. Building on the present framework linkage map, anchor markers based on RFLP, SSRs and ESTs will be extremely useful in order to use the map for germplasm management and allele mining as well as marker assisted yam genetic improvement.

7. Publications, Training and Impact

Two papers have been published and two others accepted for publication in peer review journals.
A workshop organised by PGRC, Ghana gave us the real opportunity to transfer the knowledge of yams and tuber germplasm characterisation to Ghana. The approach developed under this project was adopted as a model and is being implemented by

PGRC, Bunso, Ghana and also all the Ghanaian Research Institutes involved in the root and tuber improvement project funded by IFAD.
A visiting scientist from Ghana (M.Sc. level) is presently being trained at IITA on molecular markers for roots and tuber germplasm characterisation for efficient use in breeding. Another breeder from the Savannah Agriculture Research Institute (SARI) Nyankpala, Ghana is expected at IITA next for similar training in molecular marker application for root and tuber germplasm enhancement. He will be funded by GTZ, Ghana.

Titles of the published papers:

1. Morphological diversity, cultivar groups and possible descent in the cultivated yams(*Dioscorea cayenensis/D. rotundata)* complex in Benin Republic. Genetic Resources and Crop Evolution 46: 371-388, 1999.
2. RAPD analysis of *Colletotrichum gleosporides* isolates from yams. African Crop Science Journal vol.xx, 1999
3. Morphological and isozyme diversity of the cultivated yams belonging to *Dioscorea cayenensis* and *D. rotundata* of Cameroon. Genetic Resources and Crop Evolution: 1999 in press
4. Using isozyme polymorphism for identification and assessing genetic variation in cultivated yam (*Dioscorea cayenensis/ Dioscorea rotundata* complex) of Benin Republic. Genetic Resources and Crop Evolution. 1999 in press

B. Identification of resistance to yam viruses in *Dioscorea* species and genetic analysis of resistance to yam mosaic potyvirus in *Dioscorea rotundata* Poir.

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Research Objectives

- Identify resistance to yam mosaic potyvirus in West African landraces of white yam (*D. rotundata*)
- Identify resistance to yam mosaic potyvirus in *D. alata*, semi-domesticated and wild *Dioscorea* species in West Africa
- Determine reaction to other major yam viruses (e.g. yam potyvirus I, *D. alata* badnavirus and *D. dumetorum* potyvirus) in cultivated and wild *Dioscorea* species
- Establish the inheritance of resistance to yam mosaic potyvirus in landraces of *Dioscorea rotundata*

Expected Outputs

- *D. rotundata* and *D. alata* landraces confirmed as resistant to yam mosaic potyvirus
- Semi-domesticated and wild *Dioscorea* species confirmed as sources of resistance to yam mosaic potyvirus
- Reaction to other major yam viruses established for selected *Dioscorea* species
- Inheritance of resistance to yam mosaic potyvirus in *D. rotundata* established

Vector Studies

The ability of four new vectors to transmit yam mosaic potyvirus (YMV) has been confirmed. These are *Aphis spiraecola, Pentalonia nigronervosa, Toxoptera aurantii* and *Rhopalosiphum nymphaea*. Virus-tested plantlets of TDr 87/00211 (a susceptible breeder's line of *D. rotundata*) were used in this study and the mosaic symptoms characteristic of YMV-infected TDr 87/00211 were induced. Previously reported vectors of YMV are *Aphis gossypii, A. craccivora, Rhopalosiphum maidis* and *Toxoptera citricidus*.

Objective: Identify resistance to yam mosaic potyvirus in West African landraces of white yam (D. rotundata)

1.1 Screenhouse evaluation

All the 24 *D. rotundata* accessions planted in 1998 were re-planted in a screenhouse for re-evaluation. After mechanical inoculation of these accessions with YMV, TDr 95-115, TDr 95-128, TDr 93-48, TDr 93-46 exhibited no symptoms and tested negative for the virus when subjected to ELISA. Furthermore, these four accessions, together with TDr 93-49, TDr 35, and TDr 2224, remained uninfected when inoculated with YMV using the vectors *T. citricidus, A. craccivora* and *R. maidis*.

Eighteen newly collected accessions of *D. rotundata* landraces were planted in a screenhouse and subsequently indexed serologically for infection by YMV, DAV and CMV. Seven accessions that tested negative for virus infection were mechanically inoculated with YMV. All the seven clones proved uninfected when indexed serologically. These will be re-planted in a screenhouse for further evaluation.

1.2 Multi-site field evaluation

Twenty of the *D. rotundata* accessions evaluated in 1998 and two breeder's lines (susceptible checks) were planted at four sites in Nigeria: Ubiaja, Ibadan, Abuja, and Jos. These sites represent the Forest, Forest/Savanna transition, Southern Guinea savanna, and Mid-altitude savanna ecologies, respectively. Scoring for severity of virus symptoms from July through September has been completed, while scoring for the month of October is in progress.

Objective: Identify resistance to yam mosaic potyvirus in *D. alata*, semi-domesticated and wild *Dioscorea* species in West Africa

2.1 Screenhouse evaluation of *D. alata*

Thirty-two accessions of *D. alata* were subjected to mechanical and vector inoculation with YMV in a screenhouse. Two accessions, TDr 291 and DAN 087, did not show symptoms of virus infection, and tested negative serologically.

2.2 Field evaluation of *D. alata*

The number of *D. alata* accessions evaluated at Ibadan during 1998 planting season was increased to 41 and these were planted for evaluation in March at Ibadan. These

genotypes (39 landraces and 2 breeder's lines) were scored for virus incidence and symptom severity at about one month after emergence. The field was later infected by anthracnose disease, which prevented subsequent scoring.

2.3 Screenhouse evaluation of related wild *Dioscorea* species

In vitro plantlets of wild and semi-domesticated *Dioscorea* species were transplanted into a room at a temperature of about 20°C and with supplementary lighting in order to 'harden' them. These were indexed serologically and one accession of each of the following species tested negative for the presence of YMV: *D. praehensilis, D. togoensis, D. bulbifera, D. hirtiflora* and *D. abyssinica*. The accessions of *D. bulbifera* and *D. togoensis* have been mechanically inoculated with YMV to test them for resistance to the virus, and will be indexed serologically to detect infection from inoculation.

Objective: Establish the inheritance of resistance to yam mosaic potyvirus in landraces of *Dioscorea rotundata:*

3.1 Controlled hybridisation

Controlled crosses were made between male and female parents (total of 11) selected from the *D. rotundata* landraces under evaluation in the multi-site trial. Selection of parents was based on results obtained from last year's evaluation for resistance. F_1 progenies from the various cross combinations (resistant x susceptible, resistant x resistant and susceptible x susceptible) will be assessed in a screenhouse next year for seed-borne viruses and for reaction to YMV.

PROJECT 2

Genomic mapping of cowpea

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Placement of additional DNA markers on the existing genome map of cowpea.
A genetic linkage map of cowpea is being developed using DNA markers. The primary aim of developing the linkage map is to provide additional selection tools for cowpea breeders so that selection for desirable traits can be done more effectively. The mapping population comprises a set of 94 recombinant inbred lines (RILs) that resulted from a cross between an improved cowpea line (IT84S-2246-4) and one of its cross compatible wild relatives, *V. unguiculata* var. *pubescens*, (TVNu 110-3A). The markers being generated for placement on the map are RAPD, AFLP and SSRs (microsatellites). DNA has been extracted from all of the 94 RILs and these are being used to study the segregation of the markers that were polymorphic between the two parents. Fourteen microsatellite and seventy-seven RAPD markers have been assigned to loci on the linkage map using the Mapmaker programme. Three morphological traits V-mark on leaves, pod dehiscence and pod colour have also mapped on different linkage groups. The markers (microsatellites, RAPD and morphological) are distributed on 12 linkage groups spanning about 800 cM. Thirteen marker loci span the longest of the linkage groups which is about 129 cM.

Use the map to identify DNA markers linked with loci with effects on resistance to insect pests.
The cowpea lines that were crossed to generate the mapping population differ in their response to damage by the cowpea seed storage bruchid (*Callosobruchus maculatus*). The wild cowpea is susceptible to the insect while the improved line (IT84S-2246-4) delays the emergence of the adults. The build up of bruchid population in stored seeds of the latter is delayed thereby causing less damage to the seeds. Farmers would be able to keep seeds obtained from such varieties that delay insect emergence over a longer period of time. Seeds obtained from each of the 94 RILs were inoculated with male and female insects. Female insects were allowed to lay eggs on seeds which, were then kept in separate containers placed on a bench in the laboratory. The number of days from inoculation to adult emergence was recorded on each seed lot and values compared with DNA marker segregation patterns. Two regions of the cowpea genome were found to have quantitative trait loci (QTL) for delayed emergence of adult insects. These QTL contribute to the variation in number of days to insect emergence observed among the RILs. DNA markers spanning the two regions have been identified.

Two RILs have been generated from crosses between cowpea lines that are susceptible and those that show low levels of resistance to flower bud thrips. If not controlled with insecticide flower bud thrips can cause significant seed yield losses in susceptible lines. A susceptible cowpea line VITA 7 was crossed to two individuals (one from Ghana and the second from Nigeria) that show low levels of resistance to

this insect pest. We would like to ascertain if same or different DNA markers span the resistance loci in the two RILs. The aim is to accumulate resistance genes in improved breeding lines thereby increasing the level of resistance beyond what is presently available in any of the two parents.

PROJECT 3

Transformation of cowpea to obtain virus resistant plants

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Background

The legumes and woody plants are often referred to as recalcitrant to transformation because many representatives of these groups have proven difficult to transform. The main obstacle in transforming legumes (grain legumes, including cowpea, being more difficult to transform than forage legumes), is the lack of a *de novo* regeneration system that allows recovery of intact plants from plant cells. Success in "coupling" regeneration and transformation is a pre-requisite for introduction of traits that cannot be introgressed by conventional breeding means. Regeneration-independent transformation procedures are also possible, and have the advantage that transgenic plants are obtained with no or minimal tissue culture. Each of these two approaches is being pursued at IITA.

Progress

During the Gatsby-funded Biotechnology Projects collaborators meeting in July 1998, we reported that transgenic cowpea plants with Bt and virus resistance genes were obtained. However, after rigorous molecular analysis and bioassay, they are confirmed to be not transgenic. Intensified efforts to develop a reliable transformation system are now in progress at IITA. The areas being examined include choice and level of tissue culture (antibiotic) selection systems for putative transformants and use of alternative, improved regeneration systems that can be coupled to transformation. The purpose of the antibiotic study was also to provide baseline information on *Agrobacterium* growth control and suitable selective agent(s) for use in *in vitro* cowpea genetic transformation studies. Ampicillin was identified as an effective alternative to cefotaxime in suppressing *Agrobacterium tumefaciens*. It shows no toxicity to cowpea tissues at a concentration of up to 500 mgl^{-1}. Cefotaxime did not inhibit shoot regeneration or growth but ampicillin is more economical than cefotaxime. This study also examined the effect of four different aminoglycoside antibiotics (geneticin, paromomycin, kanamycin and neomycin) on the regeneration of cowpea decapitated embryos, in an attempt to develop a selection system for *in vitro* cowpea transformation and regeneration. Plant regeneration was completely inhibited by geneticin (50-500 mgl^{-1}) and kanamycin (200-500 mgl^{-1}). Paromomycin and neomycin, at 300-500 mgl^{-1} and 400-500 mgl^{-1} respectively, completely inhibited plant regeneration. Kanamycin (200 mgl^{-1}) and geneticin (10 mgl^{-1}) are suggested as potential agents for selection of transformed cowpea tissues.

An *in planta* regeneration system has now been developed and is in use for transformation using electroporation and a technique similar to the floral dip technique recently developed for *Arabidopsis*. We will report on new transformation efforts using cotyledonary nodes as explants using the GUS reporter gene as well as virus and insect resistance genes. We are using G418 and hygromycin for selection of

transformed tissues, having established thresholds for use in transformation. We have also established new rooting media (containing 0.05 mgl^{-1} NAA) and found this essential for hardening tissue culture derived plantlets in peat pellets. Initially, about half of the plantlets generated from tissue culture died prior to establishment in soil.

We anticipate that a discussion based on progress achieved to date will enable members of the virus transformation team to work out a feasible future plan for this collaborative project.

In addition to transformation experiments, Gatsby funds were used to set up a new, one-room laboratory that is now functioning for media preparation and explant initiation.

Final report to project end (May 1999)

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Summary
To assess the feasibility of a 'dual-binary' co-transformation strategy for the segregation of selectable marker genes away from required transgenes, a model system was tested. Using *Nicòtiana benthamiana*, a host for our target potyviruses, transgenic plants were assessed for virus resistance and kanamycin resistance in the T_0 and T_1 generations after co-transformation with novel vectors developed at JIC. Initially, plants were identified which appeared to fulfill the desired criteria. However, molecular analysis suggested that co-transfer of the two binary vectors had been inefficient. Further use of these vectors in cowpea transformation was not recommended.

Introduction
The aim of this last phase (Jan – June 1999) of Project #3 was to complete the assessment of the dual binary approach to the generation of marker-free transgenic cowpea plants. The rationale to this work was to separate the gene of interest (virus coat protein gene) from the selectable marker gene on two binary constructs, which could co-exist within agrobacterium. Agrobacterium-mediated transformation could then deliver both genes on separate T-DNAs to the same cell but provide the option to segregate away the independent insertion events in subsequent generations. The success of this approach depended upon a range of factors, including the efficiency of co-transfer of T-DNAs and the relative positioning of the sites of insertion of the separate T-DNAs. This strategy was similar to one published in the early phase of this work (Daley et al., 1998), which showed that 50% of transformed tobacco plants had been co-transformed with both plasmids and 50% of those plants had segregatable inserted genes. With regard to the transformation of cowpea plants to give virus resistance it was important to establish that the system adopted would give a similar proportion of plants with segregatable genes. Since cowpea transformation had not been optimized and the numbers of transformed plants was likely to be limited, the strategy was tested on *Nicotiana benthamiana*, a readily transformable host for *Cowpea aphid-borne mosaic virus* (CAbMV), and *Blackeye cowpea mosaic virus* (BCIMV), the two target potyviruses. The dual binary system adopted was one

established at the John Innes Centre (P. Mullineaux, personal commun) that utilised two compatible binary vetors with distinct origins of replication. The two plasmids, pGreen and pSa-Rep, co-existed at different copy number within a single agrobacterium cell. pGreen had fewer copies and carried the selectable marker gene ; pSa-Rep with more copies carried the virus coat protein gene.

Project details

Transformation and binary vectors

The transformation of *N. benthamiana* was performed by a modified version of the leaf disc method of Horsch et al. (1985). We used *Agrobacterium* strain LBA4404 carrying two binary vectors. One vector, pGreen029, contained the *nptII* gene conferring resistance to kanamycin in plants. The other binary vector, pSaRepB, was constructed by cloning the *BglII* fragment from pGreen000 into the pSaRep polylinker. This fragment contained a polylinker site flanked by left and right borders. The two coat protein genes were cloned independently into this polylinker site. The cDNA of the coat protein gene + untranslated region + polyA tail from CAbMV strain 70.12 gave rise to pSaRepB-CP70, and a similar cassette from CAbMV strain Ibadan produced pSaRep-CP81.

Three different transformation experiments were conducted. A total of 150 T_0 kan^r plantlets were grown in the greenhouse, 68 from transformation with the CP70 construct and 82 with the CP81 construct.

Bioassay

Transgenic T_0 plantlets were challenged twice by virus inoculation with the strains CAbMV-70.12 or -Ibadan, homologous to the transgene potentially present in the plants, and were scored two and four to six weeks later for visible symptoms, or by immunoblotting of extracted plant sap using monoclonal antibodies 7D9 (for detection of CAbMV-70.12) or 5H5 (for CAbMV-Ibadan). Six CP70 plants and 15 CP81 plants (see Table 1) appeared to be resistant to virus challenge and were included in a further analysis for the segregation potential of transgenes in the T_1 generation. All non-transgenic control plants were completely susceptible.

T_1 in vitro- and bioassay

From the four possible gene combinations in T_1 plants, *npt^+/CP^+*, *npt^+/CP^-*, *npt^-/CP^- and npt^-/CP^+*, the last is the most interesting for indicating the possibility of obtaining virus-resistant plants devoid of a selection gene. To identify candidate lines with possible single insertion loci for *nptII*, T_1 seed (160) were germinated on kanamycin-containing medium. The lines demonstrating a kan^r:kan^s proportion of 9:1 or lower (Table 1) were maintained for the subsequent bio-essay.

Selected lines were re-sown on compost and after three weeks were subjected to kan^r-assay by leaf painting and virus bioassay by inoculation. The assays also included non-transgenic *N. benthamiana*, transgenic-kan^R *N. benthamiana* and cowpea variety CB5. Per line, 40 T_1 seedlings were screened. The results are summarized in Table 1. From these assays, it appeared that a total of nine lines had at least one T_1 plant with

the kan^s/vir^r phenotype. Two other lines, 28124B and 38116A, also appeared to have this phenotype but had shown delayed infection in the initial T_0 screen.

PCR and Southern blot analysis on T_0 plants

A molecular analysis of the DNA from T_0 plants was also undertaken. Surprisingly, flanking *CP* primers used for PCR identified only two lines (27049A and 38116A) as containing the *CP* gene. Southern blots were also carried out and again only these two lines showed the presence of *CP*. Unfortunately, one of the plants, 27049A, had a poor seed set and could therefore not be screened in the T_1 bioassay, while the other, 38116A, did not display virus resistance with the re-screening and 25% of its T_1 seedlings appeared to be albinos. The Southern analysis is being repeated.

Conclusion

This analysis of the 'dual binary' approach has been puzzling. The strategy aimed to identify plants for which the selectable marker gene may be segregated away from the gene of interest. Because of the numbers of plants and the scale of the analysis necessary, it was appropriate to do a feasibility study in a more amenable model species, *N. benthamiana*. Based upon the prediction that transgene-mediated virus resistance might be seen in 10-20% of *CP* gene positive plants, and on published data (Daley et al., 1998) that showed that 25% of plants could have a segregatable selectable marker gene, we expected that one in 20-40 transgenic T_0 plants could give rise to progeny with a kan^s/vir^r phenotype and a null/*CP* genotype. In fact, we identified nine from 150 T_0 plants with this phenotype. However, the molecular analysis failed to identify the *CP* gene in any of these nine plants. Several questions arise : 1. If these plants had no *CP* gene, why were they resistant?; 2. If only two plants contained the *CP* gene does this imply that T-DNA transfer from the two binary vectors was unequal? 3. Is it worth continuing with these dual-binary vectors in the cowpea transformation efforts?

To answer the last first, we have recommended to IITA that these constructs are not used in the cowpea transformation programme. Considering the previous difficulties with cowpea transformation it seemed unwise to use valuable material and effort when the prospects were not good. We remain confident that the original series of vectors carrying both the *nptII* and *CP* gene will generate effective resistance. The two former questions could relate to the efficiency of the molecular analysis. Although very unlikely, the data could be explained by the techniques only being able to detect multicopy insertions. Some of the plants are being analyzed independently to check this. Otherwise, the data are difficult to explain. Such a systematic analysis of pGreen and pSA-Rep in co-transformation experiments has not been carried out before and our data might indicate a problem with the basic strategy. It seems very unlikely that the data represent low efficiency in virus inoculation and virus detection since control non-transformed plants were included in all experiments.

These experiments do not support the idea that co-transfer of T-DNA from dual binary vectors provides an effective way to remove the selectable marker gene from transgenic plants. Hence this data contradicts material published by other groups (Daley et al., 1998). However, uncertainty over the binary vectors chosen and some

aspects of the analysis means that further work would be required to reach a confident conclusion.

Literature

Daley M, V. Knauf, K. Summerfelt, J. Turner (1998). Co-transformation with one *Agrobacterium tumefaciens* strain containing two binary plasmids as a method for producing marker-free transgenic plants. Plant Cell Rep 17, 489-496.

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Table 1. Analyses of T_0 plants and their progeny

plant number	virus infected[a]	CP gene[b]	kan segr in vitro[c]	in vivo segr of T1 seedlings[d]
27002B	- -	-	6 :1	9:**2**:27:2
27002C	- -	-	8 :1	4:**2**:31:2
27008A	- -	-	5 :1	10:**0**:26:3
27024A	- -	-	9 :1	not enough seed
27049A	- -	+	14 :1	not enough seed
27052A	- -	-	42 :1	-
18102A	- -	-	5 :1	7:**1**:28 :4
18111A	- -	-	sterile	-
28103A	- -	-	4 :1	5:**5**:8:22
28109C	-	-	6 :1	10:**3**:22:4
28114B	- -	-	sterile	-
28115A	- -	-	sterile	-
28124B	- - +	-	8 :1	10:**3**:20:7
28129B	- - -	-	not tested	7:**3**:24:6
28135A	- -	-	38 :1	-
28137A	- -	-	8 :1	9:**2**:22:7
28139A	- -	-	7 :1	10:**7**:18:5
28143A	-	-	14 :1	-
28156A	- -	-	10 :1	-
28159A	- -	-	11 :1	-
38108A	- -	-	7 :1	4:**7**:16:12
38116A	-	-	sterile	-
	- - +	+	5 :1	4:**4**:15:10

[a] detected by immunoblotting with monoclonal antibodies, 1 to 3 screenings on T_0 plants

[b] detected by PCR and Southern blot analysis of DNA extract of T_0 plants

[c] proportion of kan^r : kan^s T_1 seedlings.

[d] proportion of kan^s/vir^s : **kan^s/vir^r** : kan^r/vir^s : kan^r/vir^r T_1 plantlets.

Publications

Phylogenetic analysis of two potyvirus pathogens of commercial cowpea lines: implications for obtaining pathogen-derived resistance

*Jos van Boxtel[1], Carole Lesley Thomas & Andrew John Maule**

Virus Genes – In press

Abstract. As a prelude to developing engineered resistance to two important potyvirus pathogens of cowpea, a phylogenetic analysis of strains of *Cowpea aphid-borne mosaic virus* (CAbMV) and *Bean common mosaic virus* - blackeye cowpea strain (BCMV-BlC) was undertaken. Nucleotide sequences for the coat protein genes and 3'-untranslated regions of four CAbMV and one BCMV-BlC strains were determined and included in an analysis with published sequences. While all the newly sequenced viruses showed strong homology with the existing respective sequences in the database, the CAbMV group showed a divergence into two subgroups. These groups differed from each other by more than some CAbMV strains differed from the *South African Passiflora virus* (CAbMV-SAP), which has distinct biological characteristics. The implications of the sequence analyses are discussed with respect to a strategy for the generation of engineered resistance to both groups of viruses.

Resistance of cowpea (*Vigna unguiculata* (L.) Walp.) breeding lines to *Blackeye cowpea mosaic* and *Cowpea aphid-borne mosaic potyvirus* isolates under experimental conditions

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Journal of Plant Disease & Protection – In Press

Summary

Virus diseases cause substantial yield reduction in cowpea (*Vigna unguiculata* (L.) Walp.) production in West and Central Africa. Improved cowpea varieties have been developed and distributed to various national programs but these varieties were tested only against local virus isolates. To identify resistance applicable to a wider cowpea distribution, fourteen cowpea lines were analyzed under glasshouse conditions for susceptibility to infection by three isolates of *Blackeye cowpea mosaic virus* (BCMV-BlC) and ten isolates of *Cowpea aphid-borne mosaic virus* (CAbMV). The potyvirus isolates were representative of diverse geographical origins. The cowpea lines, derived from a Nigerian breeding program, comprised ten elite lines, three progenitor lines and one improved local variety. The elite lines differed widely in their susceptibility and did not always show a correlation between field performance and resistance to virus infection under experimental conditions. The two cowpea lines displaying highest resistance were still susceptible to several CAbMV isolates. In most cases it should be possible to complement these resistance deficiencies from genes in other lines. For the CAbMV-Morocco isolate, for which no resistance was

identified, the development of engineered virus resistance may be appropriate. In general, the work argues for a combined approach in assessing new cowpea lines for disease resistance, where plants are challenged under both field and experimental conditions.

PROJECT 5

Development of robust molecular diagnostic tests for yam potyviruses for use by West African national programs

E. Canning and S. Seal, NRI

Testing of dried down reagents on field samples from Ghana (July 1999)

Dried reagents were taken to Ghana via Nigeria as hand luggage to compare against Pharmacia dried reagents and the existing TAS and PAS ELISA tests for YMV and DaV respectively. Samples were collected from around the Kumasi region and stored in a cool box and in RNA later (Ambion) a preservative medium. Work was carried out at the University of Ghana, Crop Sciences Department, Legon, Accra in the laboratory of Dr S. Offei. Forty two identical samples were subjected to tissue printing, ELISA and PCR using the Pharmacia reagents and NRI dried reagents. ELISA results indicated that 24 samples were infected with YMV and that no samples were indicated to be DaV infected. Tissue printing confirmed ELISA results for YMV, samples were not tested for DaV through tissue printing. PCR results were identical irrespective of the dried reagents used. All the YMV positives found by ELISA were confirmed, but one additional YMV PCR-positive D. alata plant was also identified that had been missed by the YMV TAS ELISA, and two DaV PCR-positive D. alata plants that had been missed by the PAS ELISA. Duplicate samples stored in RNAlater were tested on return to NRI, UK and were found to give identical results to the fresh samples tested whilst in Ghana. The use of this non-toxic storage buffer will greatly ease the sampling problems encountered in field surveys around Africa.

PROJECT 6, extension.

A. Improvement of Indexing Techniques for Banana Streak Badnavirus as an adjunct to Plant Quarantine and Approaches to Controlling the Virus.

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1B. Which integrant is responsible for producing activated episomal infection in Obino l'Ewai.?

A problem has been encountered with obtaining the sizes of chromosomal DNA from *Musa* required for investigating this question as the current techniques result in broken DNA. However Dr. Jaroslav Dolezel, Institute of Experimental Botany, Sokolovska, Olomouc Czech Republic has very recently improved the quality of DNA in sorted *Musa* nucleii. Once enough nucleii are available the structure of the large (150 kb) and small (50 kb) BSV integrants in Obino l'Ewai will be investigated to try to determine which is responsible for the observed episomal infection. There is now a flow cytometer at the Norwich Research Park site on which Pat Heslop-Harrison has been carrying out some preliminary Musa work.

1C. When does activation occur?

Knowing the mechanism of activation of BSV may lead to approaches to prevent it. We can now distinguish, using PCR, between integrated BSV sequences in Obino l'Ewai and Onne strain BSV by exploiting differences in sequence between the two forms. In particular there is a 9 bp deletion in the episomal sequence at +90 compared to the integrated sequence. This could allow detection of the early activation steps of viral sequences in tissue culture, for example using particular primers for RT-PCR to investigate whether the integrated sequences are transcribed. Phillipe Lepoivre, Faculte Universitaire des Sciences Agronomiques, Gembloux is working with different *Musa* cultivars that we are now investigating for suitable primer combinations. Our collaborator Michel Charles has left the Faculte and is currently being replaced. Phillipe Vain has initiated a *Musa* (plantain) transformation project at JIC. This can also provide a source of tissue culture material for investigating this question. Gene silencing may be involved in the suppression of activation or otherwise involved in the appearance and disappearance of symptoms. A recently discovered characteristic of silencing is the finding of small (25 nt) RNA molecules with sequence homology to the silenced gene. The possibility of using this characteristic as a diagnostic technique will be investigated for *Musa*/BSV under different growth conditions.

1D. Can activatable and non-activatable integrants be distinguished?

By PCR, most if not all *Musa* appear to possess integrated BSV sequences. FISH of metaphase spreads of various *Musa* with BSV Onne sequences show signals indicating their presence. However genomic Southern hybridisation of some A genome *Musa* (e.g. Cavendish AAA) give no signal. This apparent contradiction is being investigated. A further puzzle is the appearance of Onne strain BSV in some

Cavendish Grand Nain plantations in a manner strongly suggesting an internal source for the virus. However, the episomal virus from Grand Nain contains the 9 bp sequence deleted in the Onne strain activated in tetraploids. This indicates that the infections in Grand Nain did not arise by horizontal transmission of episomal virus activated in tetraploids but raises some further questions on this virus. It also gives a good diagnostic for the episomal virus activated from tetraploids.

2. Determine whether there are other activatable BSV sequences.
It is possible that there are different strains of BSV integrated in cultivars which are not being widely propagated by tissue culture and can be activated by other stresses. Other BSV strain DNA has been prepared for analysis.

4A. Transgenic approaches to BSV resistance and control.
Two research approaches are being used to investigate the potential of transgenic control of BSV. A simple method of inoculating *Musa* with BSV is required and agro-inoculation is being developed. The one and a bitmer (infection competent form) Onne BSV sequence has so far resisted various cloning attempts including direct introduction into *Agrobacterium*. Other BSV strains will be investigated for their ability to be cloned.

The second approach is to investigate gene silencing for BSV control. As noted above gene silencing may be involved in the aetiology of the disease and the results from 1C will indicate whether this approach is feasible for the control of either episomal infection and/or activation of integrants.

PAPERS, CONFERENCES AND REPORTS

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B. Establishment of a delivery system for healthy improved *Musa* germplasm with field tolerance to banana streak virus (BSV).

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Project Objectives

- To combat two disease problems (banana streak virus and black sigatoka disease) which contrain *Musa* production by the dissemination of improved hybrid *Musa* germplasm

- To apply recently developed diagnostics for detection of viral BSV to provide planting materials of the best assured health status with currently available technology

Project Description

1. National programmes in Ghana and Uganda will obtain improved virus-indexed germplasm from the INIBAP Transit Centre. Candidate genotypes with black sigatoka resistance and BSV field tolerance are:

Origin IITA: TMPx 4479-1
TMPx 7152-2
TMBx 1378
TMBx 5295-1

Origin FHIA: FHIA 1
FHIA 3

2. With technical backstopping from IITA, national programmes would use (TAS) ELISA and ICPCR protocols to screen the health status of *Musa* plantlets in tissue culture. Only those materials which test negative to viral BSV would be advanced for further multiplication in tissue culture and subsequent establishment in nurseries. Materials in nurseries would be monitored for occurrence of BSV symptoms and rogued when necessary.

3. Young plants of good health status will be field established in plantation nurseries, at a density of 2500 plants/ha (2m x 2m spacing) or higher. These plantations would be source plantations for healthy suckers for delivery to farmers and extension services. They would receive optimal crop management for sucker development and crop sanitation. Random checks for BSV could be carried out if thought necessary, even if all plants are asymptomatic.

4. Where feasible these suckers would be used for establishment of secondary nursery plantations at other locations, after local staff have received training in crop management and sanitation practices.

Project Schedule

Year 1 : Activities 1 and 2. Activity 3

Year 2 : Management of Activity 3. Commencement of outputs

Year 3 : Delivery of outputs from Activity 3, Activity 4

If the project commences in 1997, delivery to extension services and farmers would commence in the 1998 planting season. There would be measurable impact in farmers' fields by the year 2000.

Indicators

The indicators of impact would be:

- The number of suckers delivered to the extension agents/farmers
- The number of hectares in Ghana and Uganda planted with healthy improved germplasm

Earlier progress

The project was initiated in August 1996 by introducing five IITA hybrids into the tissue culture laboratory at Kawanda, Uganda. These materials consisted of three plantain hybrids (TMPx 4479-1, 7002-1, and 7152-2) and two cooking banana hybrids (TMBx 1378 and 5295-1). The hybrids were imported as virus-tested tissue cultures (5 cultures each) and immediately subcultured for further micropropagation. All hybrids were selected for their black sigatoka resistance and good yield. Four of the five hybrids are also believed to exhibit some form of tolerance or resistance to BSV (all except TMPx 7002-1). By February 1997, these in vitro culture had been multiplied up to the following numbers of propagules:
- TMPx 4479-1 (PITA-17): 264
- TMPx 7002-1 (PITA-8): 505
- TMPx 7152-2 (PITA-14): 154
- TMBx 1378 (BITA-2): 254
- TMBx 5295-1 (BITA-3): 224

giving a total of 1401 propagules in 6 months, starting from 25.

In addition to these five IITA hybrids, another six genotypes were selected for inclusion into this project: two FHIA hybrids (FHIA-1 and FHIA-3), two East African highland banana landraces (Mbwazirume and Kisansa), and two dessert bananas (Grand Nain and Km-5, both virus-tested). Some 60-140 in vitro propagules of each of these six genotypes had been multiplied up by February 1997.

In August 1997, in vitro propagated plantlets were planted in virus-free temporary sheds in Masaka (1566 plantlets) and Rakai (1552 plantlets), Uganda, for 3 months. BSV-infected plantlets and off-types were counted and rogued out for each cultivar.

Healthy plantlets were planted in the field in November 1997 at a spacing of 2m x 2m, which was later increased to 4m x 2m by uprooting whole plants that were transferred to different districts. Plants that did not show BSV infection were later treated by false decapitation to induce profuse suckering. Plants showing virus symptoms were rogued. Suckers were harvested at 3.5 months after decapitation, leaving only one sucker in the field for future multiplication. Suckers produced following decapitation in the two multiplication plots ranged from 795 to 2034, depending on the hybrid. During 1998, between 440 and 490 suckers of each cultivar were planted on 48 farms in 13 locations (in 12 different districts), covering both traditional and non-traditional banana-growing areas (see 1998 report; Kubiriba et al., 1999), where they were evaluated for disease resistance, agronomic performance, and consumer acceptability. Farms in the first phase of this project will serve as secondary distribution centres following the completion of the evaluation process.

Another 'virus-resistant' IITA hybrid, PITA–16 (TM3x 15108-6), which in Nigeria and Uganda has shown to be high-yielding, black sigatoka-resistant, wind-resistant, and probably nematode- and weevil-resistant, was introduced from IITA and multiplied in vitro in 1998. More than 1000 plants were produced, of which a few hundred will be planted in the Masaka, Rakai and other nurseries during 1999.

1999 Progress: Evaluation of BSV-tolerant hybrids

In 1998, the materials were planted on farmers fields and observed under farmer management conditions in 1999. The main evaluator was the farmer, assisted by extensionists and researchers of the Uganda National Banana Research Programme. The materials were evaluated for disease/pest response, agronomic performance and end-user acceptability. The materials will be functionally classified on-farm, the best ones identified, and a farmer-based recommendation of which cultivars to multiply will follow.

Suckers of five IITA hybrids (PITA-14, PITA-17, PITA-8, BITA-3 and BITA-2) were obtained from mother gardens in Masaka and Rakai districts in Uganda, cleaned by paring and planted on farmers fields at 13 locations in the North-East, East, Central and Western parts of Uganda, along with landrace cooking bananas as local checks. PITA-16 was recently planted in Rukungiri, Hoima, Jinja and Kamuli.

The evaluation process for agronomic performance, disease and pest response, and consumer acceptability are on-going. The following is the progress to date:

The sites planted with the BSV-tolerant materials of IITA and covered during the on-going assessment are indicated in Table 1. The Hoima sites were the first to be planted. Farmers have already harvested some of the cultivars and are showing interest in the hybrid PITA-14 (TMPx 7152-2) as a roasting banana. The demand for the planting material of this cultivar is currently high in Hoima district. Farmers have already removed suckers of this cultivar at this site, but it was difficult to estimate the numbers. BITA-2 (TMBx 1378) is already known to take long to mature and is similar to a cultivar locally known as Serere (Pisang awak). At one site in Hoima, however, this hybrid had severe fusarium wilt symptoms. The farmer was planning to

uproot this cultivar. BITA-3 (TMBx 5295-1) was found to have a long weak stem, which breaks immediately after flowering. PITA-17 (TMPx 4479-1) was found to be a good juice banana and farmers are already distributing it among themselves.

In Pallisa district, at the Butebo site, the farmers had almost similar observations, but had no cases of hybrid TMBx 5295-1 breaking on flowering. The hybrids were all generally doing well (Table 2). The farmer at this site was planning to uproot TMBx 1378 because it behaves like Serere cultivar (Pisang awak) by succumbing to wilt.

At the sites where these hybrids have been harvested, none of the farmers like them as much as cooking bananas (matooke). Rather, they are appreciated when used as plantains. It is, however, too early to make a conclusion on utilisation of these hybrids since only two of the hybrids (PITA-14 and 17) have as yet been harvested at only one site in Hoima (Kinogozi) and Pallisa (Butebo).

Due to vigorous growth of these plants at most sites, the farmers are removing the suckers to plant in their own gardens. It is therefore difficult to estimate suckering capacity of these cultivars.

Visiting of other sites where the materials were planted as per previous report is also on-going. The feedback from the remaining sites will be reported in the next report.

BSV symptoms were observed on some plants of the hybrids at most of the sites (Table 2). Only hybrid BITA-2 has so far not shown BSV symptoms at all the sites. The affected plants were, however, growing vigorously suggesting high tolerance. It requires a specialist to detect presence of the disease, because affected and non-affected plants look equally vigorous.

Table 1. Location of the BSV-tolerant plants (10 plants per cultivar were planted at each of the sites below).

District	County	Sub-county/Site name	Farmer
Lira	Moroto	Omoro	Mary Akoko
	Moroto	Oloi	Charles Ocin
	Dokolo	Dokolo	John Angel
Soroti	Kasiro	Bugondo	Mr. Opolot S.P
Kumi	Kumi	Kumi	Clement Ogwang
Pallisa	Butebo	Butebo	Steven Malinga
	Budaka	Ikiki	Nathan Kasanga
Tororo	Tororo	Usukuru	Paul Eparut
	Tororo	Oliyoi	Michel Ekoleit
	Tororo	Kayolo	Ocen Mbuye
	Tororo	Usukru	Michel Etiang
Iganga	Kigulu	Nakigo	Mr. Tibairira
	Bunya	Imaniro	Mr. Mukakanya
	Kigulu	Nambale	Mr. Zikusoka
	Busika	Bulange	Mr. Tito Tibaire
Kamuli	Nawanyago	Nawanyago	Mr. G. Kinosa
	Buzayo	Bugulubya	Mr. Moses Waidha
	Buzayo	Kisozi	Mr. Jerome bamutira
	Buzaya	Nawanyago	Rose Mutekanga
Luwero	Bamunanika	Bamunanika	Mr. Odoi
	Bamunanika	Kyotamigavu	Mr. Kasule
	Bamunanika	Kibanya	Ms. Nabukalu
Hoima	Bugahya	Kyabigambira	ADC-Hoima
	Bugahya	Kyabigambira	Mr. Charles Kahwa
	Buhaguzi	Buhimba	Mr. Solomon Mabona
	Buhaguzi	Busisi	Mr. Irumba

Table 2. BSV incidence on the BSV-tolerant hybrids at the evaluation sites.

District (Sites)	Genotype	No. of plants sampled	No. of plants infected
Hoima	TMBx 5295-1	20	0
	TMBx 1378	20	0
	TMPx 4479-1	30	0
	TMPx 7152-2	28	12
	TMPx 7002-1	54	18
	Mudwale	31	0
	Musakala	30	0
	Nakitembe	10	0
Iganga	TMPx 4479-1	6	6
	TMPx 7002-1	5	5
	TMPx 7152-2	-	-
	TMBx 5295-1	-	-
	TMBx 1378	-	-
	Mudwale	20	0
	Musakala	20	0
	Nakitembe	20	0
Kumi	TMBx 1378	10	0
	TMPx 4479-1	10	0
	TMPx 7002-1	10	3
	TMPx 7152-2	6	1
	TMBx 5295-1	-	-
	Mudwale	1	1
Lira	TMBx 5295-1	30	3
	TMBx 1378	30	0
	TMPx 4479-1	30	10
	TMPx 7002-1	30	6
	TMPx 7152-2	30	3
	Mudwale	30	0
	Musakala	30	0
Luwero	TMBx 5295-1	10	0
	TMBx 1378	10	0
	TMPx 4479-1	10	0
	TMPx 7002-1	9	2
	TMPx 7152-2	10	0
	Mudwale	30	0
	Kisansa	20	0
Soroti	TMBx 5295-1	10	0
	TMBx 1378	10	0
	TMPx 4479-1	10	0
	TMPx 7002-1	6	5
	TMPx 7152	10	0
	Musakala	10	0
	Mudwale	10	0
Tororo	TMPx 4479-1	24	4
	TMPx 7002-1	13	8
	TMPx 7152-2	5	5
	TMBx 1378	-	-
	TMBx 5295-1	-	-
	Musakala	40	0
	Mudwale	36	23
Pallisa	TMPx 4479-1	20	1
	TMPx 7002-1	20	0

TMPx 7152-2	13	4
TMBx 1378	20	0
TMBx 5295-1	-	-
Musakala	10	0
Kisansa	20	5

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D. Establishment of a delivery system for healthy improved *Musa* germplasm with field tolerance/resistance to BSV and resistance to black sigatoka

F.O. Anno-Nyako, K.R. Green, J.d'A. Hughes & A. Tenkouano

Project Purpose

To disseminate improved *Musa* hybrid germplasm and promising landraces to combat two disease problems (banana streak virus (BSV) and black sigatoka disease) that constrain *Musa* production.

Project Objectives

1. To mass propagate selected hybrids and landraces using in vitro and rapid vegetative multiplication techniques.
2. To apply recently developed diagnostics for the detection of viral BSV so that the health status of the germplasm can be assured.
3. To routinely index and evaluate plants of the selected hybrids and landraces.
4. To distribute proven varieties to farmers through the establishment of community-based nurseries.

Progress

1. To mass propagate selected hybrids and landraces using in vitro and rapid vegetative multiplication techniques.

The following hybrids and landraces have been selected for multiplication to produce 3300 plantlets in total for planting in 2000.

1. PITA-2 [TMPx 548-9] (available in Ghana)
2. PITA-5 [TMPx 2796-5] (available in Ghana)
3. PITA-11 [TMPx 2637-49] (available in Ghana)
4. TMPx 2481 (available in Ghana)
5. FHIA 21(available in Ghana)
6. BITA 2 [TMBx 1378] (recently arrived in Ghana from INIBAP)
7. BITA 3 [TMBx 5295-1] (recently arrived in Ghana from INIBAP)

8. Asamienu (Ghanaian landrace)
9. Apem oniaba (Ghanaian landrace)
10. Apantu brodewiuo (Ghanaian landrace)
11. Apantu osoboaso (Ghanaian landrace)

These hybrids and landraces are all in tissue culture and are being multiplied at Department of Botany, University of Ghana, Biotechnology and Nuclear Agricultural Research Institute, and Crops Research Institute (CRI), Kumasi. Consumables and reagents were provided to the Gatsby *Musa* project scientists in Ghana by IITA to facilitate *in vitro* multiplication.

In addition, all of the varieties apart from BITA 2 and BITA 3 (recently arrived from INIBAP), are being maintained in the field. The nursery sites for rapid vegetative multiplication are being prepared at Agricultural Research Station (University of Ghana) Kade and CRI for planting in early 2000.

Three primary sites of one hectare each at Kade, Fumesua and Assin Fosu will contain all the eleven varieties produced from both tissue culture and vegetative propagation. The primary sites will be planted in April/May 2000.

2. To apply recently developed diagnostics for the detection of viral BSV so that the health status of the germplasm can be assured.

As a result of the training at IITA previously done under this project, F.O. Anno-Nyako was able to confirm the natural occurrence of viral BSV by enzyme-linked immunosorbent assay (ELISA) on field-grown FHIA 21. S.K. Offei also confirmed by ELISA that Ghanaian landraces are also infected with viral BSV. Supplies of polyclonal and monoclonal antibodies raised at IITA were given to the Gatsby *Musa* project scientists in Ghana to facilitate the diagnostics at Department of Crop Science, University of Ghana and CRI.

3. To routinely index and evaluate plants of the selected hybrids and landraces.

Indexing and agronomic evaluation will be undertaken at the primary and secondary multiplication sites from May 2000.

4. To distribute proven varieties to farmers.

One secondary multiplication site (on station) has been identified in each of the six major plantain-growing regions. Extension staff from the Ministry of Food and Agriculture will work with farmers close to the secondary sites who will be encouraged to establish community nurseries (satellite sites) incorporating proven varieties. Secondary and satellite sites are expected to be planted in May 2001.