COLLABORATORS MEETING

IITA-JIC-NRI

Gatsby-funded Biotechnology Projects

Extended Abstracts

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MISSION STATEMENT

'The objective is to deepen the collaborative work of agricultural scientists in Africa and the UK in ways which ensure that current scientific insights are harnessed to the ultimate benefit of smaller scale farmers.'

PROJECT 1

Assist yam breeding through molecular genetics

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

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Background

The current genetic improvement breeding of yams at IITA has been based on a conventional approach. With the advance of molecular techniques, new tools are now available for breeders and germplasm managers to enhance their capacity and improve the efficiency in breeding. This project has given IITA the opportunity to use the tools of molecular genetic to get a better insight into yam gene-pool organisation, gene flow between various cultivated and wild relatives, the inheritance of resistance to important pests, and to initiate the development of the first genetic linkage map in yams.

1. Phylogenetic studies

The definition of gene-pools in any crop, which is made of cultivated, weedy and wild relatives is essential for both germplasm management and genetic improvement. In yams, (*Dioscorea* ssp), the phylogenetic relationships between wild yam species and cultivated yams of West and Central Africa were established based on isozymes, RAPD and AFLP markers. Cultivated yam (*D. rotundata*) formed one group with the wild species *D.praehensilis D. abyssinica* and *D. liebrechtsiana*, while the other yam *D. cayenensis* formed another group with the wild species *D. burkilliana*. The other wild species were genetically distant from these two groups. Some farmers in West and Central Africa select genotypes from the wild for cultivation which appear to be the products of natural interspecific hybridization between the cultivated yam species *Dioscorea rotundata, D. cayenensis* and wild relatives such as *D. praehensilis, D. abyssinica*, *D.burkilliana D. liebrechtsiana* and *D. togoensis*. Controlled crosses were made in the 1997 cropping season, between wild and cultivated yams to further investigate the phenomenon. In May 1998, progenies from these crosses were sown in seedling nurseries to generate minitubers. These minitubers will be planted in the field in the 1999 cropping season for morphological and genetic analysis.

2 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 varietal groups. The results obtained were confirmed in 1998. One of the most important conclusions was that the yam germplasm from Cameroon represents a distinct pool of genetic diversity as compared to the rest of material from West Africa. This constitutes an asset to the yam breeding effort at IITA and consequently within the national programmes.

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

for the development of a genetic map for marker assisted selection in crop breeding. Such populations were not available in yams before the initiation of this project. Through the present project, five mapping populations in *D. rotundata* and one in *D. alata* were obtained in 1995 and 1996. These populations were established in screenhouse in 1997 and micropropagated *in vitro* to produce sufficient plant materials for both screening for particular traits and for DNA analysis.

3.1 Highlight of findings 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. Five mapping populations were developed for this project. Of these, one population derived from a cross between TDr 93-2, a popular landrace (female parent) and TDr 87/00211, an improved breeding line (male parent) was used as the mapping population consisting of 72 heterozygous F1 individuals. Twenty-eight single dose markers (15 RAPDs, 2 isozymes and 11 AFLPs) and 39 markers (20 RAPDs, one isozyme and 18 AFLPs) have been assigned to the female and male parents, respectively. Preliminary maps for the female and male parents have been developed. The map for the female comprises 12 mapped markers assembled in four linkage groups with 16 unassigned markers. The linkage groups rang in size from 27.5 to 120.4 cM and spans 222 cM of the yam genome. The other map comprises 8 mapped markers distributed in 4 linkage groups with 31 unassigned markers and spans 111cM of the yam genome. The maps are still scanty and more markers need to be added to enhance their usefulness in mapping the YMV and yam nematode resistance genes. The genetics of these traits is being investigated. This effort would greatly facilitate the breeding of this important crop species.

3.2 Highlight of findings in *D. alata*

The genetics of resistance to anthracnose

The genetics of resistance to anthracnose (caused by the fungus *C. gloeosporioides*) was investigated in a cross between tetraploid *D. alata* genotypes. The tetraploid nature of the parental lines and F1 progeny was determined by flow cytometry. The hybrid breeding line TDa 95/00328, which has consistently exhibited field resistance across location, was crossed to the susceptible landrace TDa 95-00310. 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 with strains of *C. gloeosporioides*. This is the first resistance gene described in the genus *Dioscorea*.

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

DNA isolated from the above mentioned parental lines used to generated F1 progeny is being screened for polymorphism using RAPD. A total of 348 primers screened between the two parents yielded 1385 fragments of which only 337(24%) were polymorphic. These fragments will be tested for single dose markers in the F1 and used for developing a genetic linkage map of *D. alata*.

PROJECT II

Molecular mapping of cowpea

A Genetic Linkage Map of Cowpea (*Vigna unguiculata*)

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A molecular marker based linkage map of cowpea is being developed for purpose of assisting breeders in making effective selections for desirable traits. 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 a wild relative TVNu110-3A (*V. unguiculata* var. *pubescens*). 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 while 94 RAPD markers have been generated. Seventy seven of these RAPD markers have been assigned to loci on the linkage map using the Mapmaker programme. Three morphologic traits V-mark on leaves, pod dehiscence and pod colour have also mapped on different linkage groups. The markers (RAPD and morphological) are distributed on 12 linkage groups spanning about 670 cM. The longest of the linkage groups at this stage is about 175 cM.

Different primer combinations have been identified for generating AFLP markers for the map. The primer combinations tested have revealed between 10 and 25 polymorphic bands between the two parents that were crossed to generate the mapping population. A non-radioactive detection system is being used for the AFLP analysis.

The purpose of developing the linkage map for cowpea is to be able to identify markers that are linked with loci with effects on desirable traits. This linkage map has been used to identify markers associated with QTL that have effects on some agronomic traits such as days to flower, days to maturity, pod length and some seedling traits. In some cases it was observed that the same markers spanned the loci with effects on more than one trait. Seeds are being produced from F10 plants to be evaluated for resistance/susceptibility to bruchids (*Callosobruchus maculatus*), a trait for which the population is segregating. The cowpea line IT84S-2246-4 is resistant to this insect pest. This will enable us to identify markers that are associated with bruchid resistance in cowpea. At the same time populations are being generated for the mapping of loci with effects on flower bud thrips resistance in cowpea.

Insect Resistance in Cowpea

Establishing the Molecular and Biochemical basis of Insect Resistance - Primary and secondary metabolites of *Vigna* and non-*Vigna* legumes

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What Genes? This is a key question when one talks about insect resistance and genetic engineering of plants for this trait. For cowpea resistance to *Maruca vitrata* Fab. (syn. *M. testulalis* Geyer) this has become a critical issue because of the apparent absence of good levels of cowpea resistance to this insect pest. If it is necessary to search for resistance genes beyond the primary gene pool of cowpea, it is normal to extend the search to the wild progenitors of the species, then to explore the use of alien (or foreign) genes from other *Vigna* germplasm and edible crops, especially food legumes. In this paper we address the scenario of resistance genes from other food legumes, with particular focus on the African yam bean (AYB), *Sphenotylis sternocarpa* (Hochst. ex A. Richard) Harms and *Mucuna* spp..

Preliminary studies have showed that a number of non-*Vigna* food legumes (*S. sternocarpa* and *Lablab purpureus* [L.] Sweet), several wild *Vigna* accessions and species within the genus *Mucuna* such as *M. pruriensis*, are highly resistant to *M. vitrata* and, in certain cases, *Clavigralla tomentosicollis* Stal. and *Callosobruchus maculatus* Fab as well. Since these are different species in different genera, the only avenue open for gene exploitation is alien gene transfer through genetic engineering. As a first step, studies were initiated to investigate the mechanisms and basis of the observed resistance. Two main approaches were followed. The first was to determine the primary proteins that were involved and thus provide a basis for gene identification, characterization and transfer; the second was to identify any secondary metabolites that may be conferring resistance to these genotypes.

Both AYB and lablab bean are used as food in the eastern and northern parts of Nigeria, and also in certain parts of Cameroon and other countries in east and central Africa. AYB is generally grown between September and March in West Africa. Lablab can be grown any time of the year but mainly during the main cropping season (July to December). It is used both as human food and as green manure. Although there are reports about its use as grain, *Mucuna* spp.are used mainly as a cover crop, and for weed smothering in most parts of W. Africa especially S. Guinea, Ghana and and in derived savannas of Africa. Being legumes, these crops also improve soil fertility. The important thing from our current perspective is all these crops suffer little damage from *M. vitrata* and other field and storage pests of cowpea.

I. Primary Metabolites

In legumes, including cowpea, primary metabolites from seeds e.g. lectins, protease,α-amylase inhibitors and possibly thionins are good targets for use in engineering resistance against insects The modes of action of protease inhibitors (PI) is believed to be through inhibition of insect digestive proteases or neurotransmitters, resulting in abnormal development/death due to deficiency of essential amino acids. For example the α-amylase inhibitor from the common bean (*Phaseolus vulgaris*) inhibits α-amylases in bruchids (*Callosobruchus maculatus*), whereas the cowpea trypsin inhibitor (CpTI) inhibits both mammalian and insect serine proteinases (trypsin

and chymotrypsin). Other proteases, and hence other insect systems may not be affected. Lectins possess at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide making them potent antimetabolites. Thionins are also toxic proteins, but unlike lectins and PIs, they have not been studied in detail with respect to resistance against insects. However, they have been reported to be toxic to insects, and to possess α-amylase inhibition activity (Bohlmann, personal communication). What has been unequivocally demonstrated is their role in fungal resistance. In addition to or combination with, Bt-based strategies, the genes encoding lectins, thionins and PI's are thus excellent candidates for transfer to cowpea for protection against pre-and post-flowering pests

Highlights of findings:

- African yam bean seeds have been shown to contain large amounts of lectin

- Albumin fractions from AYB and lablab seeds incorporated in artificial diet and assayed using neonates of *M. vitrata* recorded greatly reduced growth and high larval mortalities. These results suggest high levels of antibiosis of the extracts.

- Lectin specificity assays have been conducted on several AYB, cowpea and wild cowpea accessions through sugar inhibition assays. From these studies, we have been able to determine which sugars bind the different lectins

The galactose-specific lectin from one AYB accession (95-3) has been purified by affinity chromatography and other procedures and is being used in insect bioassays partially purified proteins from *Mucuna* spp. are being separated for use in bioassays.

A genomic clone with good homology to arcelin, a lectin-like insecticidal protein from *Phaseolus* spp. has been isolated from AYB. A thionin-like genomic DNA fragment has been obtained from AYB but not from other *Vigna* spp. A 3'-RACE product has also been isolated, but only from seed mRNA and not from other tissues.

Next Steps:

- Purified AYB and *Mucuna* proteins will be sequenced N-terminally and derived amino acid sequences used for gene isolation

- Further bioassays will be conducted on the purified lectins

- Protein sequencing and gene cloning will follow as appropriate.

II. Secondary Metabolites

Secondary plant products are known to confer resistance to insects attacking many crop plants. These phytochemicals act either as antimetabolic agents (antibiotic resistance), or prevent the use of the plant for food, shelter or oviposition (antixenotic resistance).

Highlights of findings:

Investigations are being conducted to quantify and characterize the secondary metabolites involved in the antibiosis observed in the test plants. Post-ingestive effect of extracts or fractions include toxicity, growth retardant activity, low % pupation and % emergence and low fecundity.

Levels of PAL activity have been determined for various cowpea lines which are also being investigated with respect insect resistance, lignin content (correlated with digestibility) and their utilization as grain and for fodder. Variations between the cowpea lines may indicate a genetic basis for the observed differences in PAL activity.

Putative genomic and cDNA clones have been isolated for genes encoding secondary metabolites, including phenyalanine ammonia lyase (PAL) and chalcone synthase (CHS) from cowpea and AYB using a degenerate PCR primer approach.

III. Next Steps

1. Bioassay directed Isolation of resistant factor(s) using chromatographic techniques
2. Identification of the secondary compounds responsible for resistance
3. Identification of specific biosynthetic pathways
4. Enzyme assay and purification.
5. Isolation and characterizatioon of full length clones of genes e.g. PAL and CHS
6. Determine which points of biochemical pathways will be targets for genetic manipulation

PROJECT III

Transformation of cowpea to obtain virus resistant plants

Topic: Cowpea Transformation for Virus and Insect Resistance

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Background

The legumes and woody plants are often referred to as recalcitrant to transformation because many representatives of these group have proven difficult to transform. The main obstacle in tansforming legumes (grain legumes, including cowpea, being more difficult to tranform that forage legumes), is the lack of a *de novo* regeneration system that allows recovery of intact plants from plant cells. Success in "coupling" regeration and transformation is a pre-requisite for introduction of traits which cannot be introgressed by conventional breeding means. A protocol for cowpea regeneration via direct organogenesis has been established at IITA, paving the way for the development of a pertinent transformation system. Optimisation of the procedures is still underway due the low efficiency of regeneration and transformation. Moreover, not all transformation procedures involve the use of a *de novo* regeneration system. 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 are being pursued at IITA.

Highlights of findings

Some progress has been made using *Agrobacterium*-mediated trasformation methods, notably in transformation using *Agrobacterium* inoculation of flower buds, but success using electroporation of nodal has not been forthcoming Problems with incidence of escapes and chimeras rather than entire transformants

Putative transgenic plants containing Bt and viral genes (supplied by JIC) are being raised and analysed at molecular level especially with respect to gene expression. Preliminary Western analysis suggests that the Bt toxin is expressed in at least some of the lines that have been assayed so far. Bioassays are also in progress with *Maruca vitrata* and pod sucking bugs as targets for control.

Transformation of cowpea with plant-derived insecticidal genes has been initiated

Next steps:

IITA scientists wish to develop a protocol that can be used for transformation of more cowpea genotypes. Development of a somatic embryogenesis system could pave the way for more efficient transformation systems that cut out the incidence of chimeras.

Transformation with other genes (relevant to insect and virus control) or constructs is anticipated, especially "green" constructs (from JIC) that allow elimination of the selectable marker genes

Progress Report on genetically-engineered virus resistance in cowpea (February 1997 - June 1998)

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JIC, Norwich, UK.

1. Screening of elite cowpea lines and progenitors by inoculation with potyviruses

Five elite cowpea lines and 3 progenitor lines were inoculated under green house conditions, each with 2 strains of blackeye cowpea mosaic virus (BlCMV), 9 strains of cowpea aphid-borne mosaic virus (CAbMV) and one unidentified potyvirus strain. The severity of symptoms was scored and infection of plants confirmed by immunoblot assays. We found that all elite lines showed some degree of resistance to 7 of the strains. Five strains consistently infected all elite lines. With regard to the progenitor lines, only four progenitor x strain interactions demonstrated resistance. Given the susceptibility of the progenitors to challenge with 12 strains, resistance of IITA elite lines could not be attributed exclusively to the progenitors considered here. Screening of 5 more IITA elite lines is ongoing.

2. Characterisation of potyvirus strains at CP gene nucleotide sequence level

The coat protein (CP) genes of 4 elite line-infecting strains (potyvirus-81.11, CAbMVs Morocco, SA93/1510 and 70.12) and three non-infecting strains (BlCMVs- Florida and IT16, and CAbMV-Monguno) were isolated and sequenced at the nucleotide level. From the sequencing results it appeared that strain 81.11, which formerly was considered as BlCMV, should rather be classified as CAbMV or SAPV (South African *Passiflora* virus). A second result was the observation of 99% homology between the nucleotide sequences of the CP-genes of CAbMV's Morocco, SA 93/1510 and 70.12, strains which are of very distinct geographical areas. Symptomatically, however, the strains were distinguishable from each other by their virulence to cowpea lines and different severity of stunting and leaf chlorosis. Sequencing of CP genes of strains which are not infective for the elite lines, revealed various amino acid changes in the N-terminal of the peptide sequence, compared to infective strains.

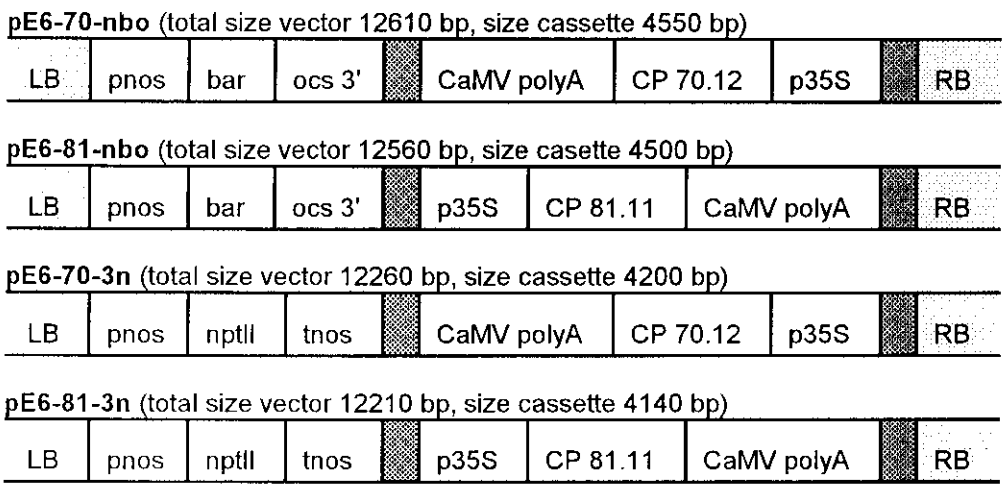

Figure 1. Transformation vectors with viral coat protein sequences (CAbMV 70.12 and potyvirus 81.11). LB = left boarder, RB = right boarder.

3. Transformation vectors for inducing virus resistance

A first series of transformation vectors carrying viral sequences was sent to IITA in September. These vectors carry the coat protein genes of 2 virulent CAbMV isolates and the *bar* gene as

selective marker (Figure 1). A second series of vectors, in which *bar* is replaced by *nptII* (kanamycin resistance), was sent in November. All vectors have now been used at IITA for transformation purposes.

4. "Green constructs"

During the February 1997 meeting at IITA emphasis was laid on strategies related to the biosafety aspects of transgenic release. To avoid recent criticism in the popular and scientific press about the inclusion of unwanted genes in the transgenic lines, a further series of transformation vectors are being made. The main feature of these vectors ("green constructs") is that they provide the opportunity to segregate the selectable marker gene (e.g. herbicide resistance) away from the gene of interest. Such transformation vectors are now nearly ready for being studied, firstly in *Nicotiana benthamiana*. *Agrobacterium* cells for transformation will contain two binary vectors, one carrying the gene of interest, the other a selectable marker gene (Figure 2). If the transgenes appear to be sufficiently unlinked after cointegration in the cowpea genome, the gene of interest can be segregated away from the selectable marker gene in the next generation. A recent report describing the use of a similar method with tobacco and rapeseed showed 50% cointegration of transgenes, from which 50% segregated away in the next generation (1). In their case effective transformation was thus reduced by 1/4.

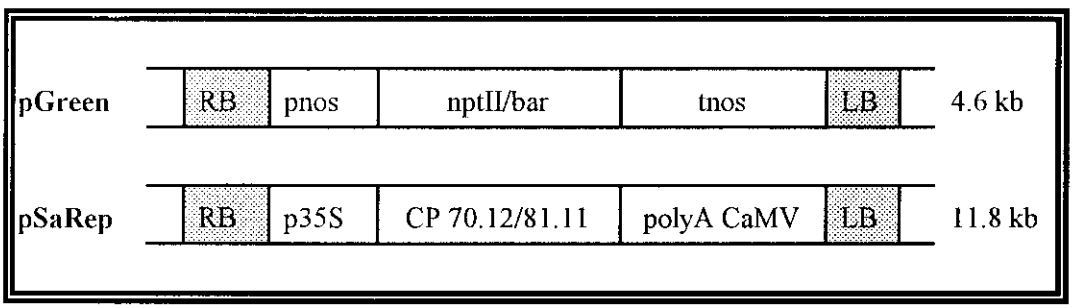

Figure 2. ***Agrobacterium tumefaciens*** **dual binary vector system for cowpea transformation**

5. Perspectives

For the remaining 6 months of the project we intend to conclude all ongoing studies:

1. Screening of five IITA elite lines with 12 strains,
2. Complete the sequencing work on CP-genes,
3. Give help in producing and analysing putative virus-resistant transgenic plants,
4. Study segregation of transgenes by using the dual binary vector system for *N. benthamiana* transformation, and transfer the technology to IITA,
5. Amalgamate results obtained during the project and submit 2 papers for publication.

Reference

1. Daley et al. 1998 Plant Cell Rep 17: 489-496

Biosafety

Biosafety

Situation Report in Sub-Saharan Africa

IITA

Since IITA is located in Nigeria its activities as they relate to the handling of genetically modified organisms are regulated by the laws governing such activities in the country. As a part of the Nigerian biosafety guidelines each organisation that works with genetically modified organisms such as transgenic plants in the country should have in place the Institutional Biosafety Committee (IBC). IITA has already established an IBC with Dr. S. Adetunji as chairman and Drs. R. Asiedu, L. Jackai, N. Sanginga and G. Thottappilly as members. Later Dr. F. Quin became the chairperson. Dr. R. Asiedu was nominated as the institute' s biosafety officer. This committee met a number of times in the past. The terms of reference of the committee are as stipulated in the Biosafety Guidelines for Nigeria (see pages 13 to 19 of the enclosed document). IITA has containment facilities attached to screen houses for testing genetically modified organisms (GMOs).

Nigeria

Biosafety guidelines have been developed by Nigeria with IITA facilitating the process of producing the guidelines. The development of the biosafety guidelines in Nigeria started in 1993 and the final document was produced in 1994. Early in 1995 the Biotechnology Advisory Commission located at the Stockholm Environment Institute, Sweden sponsored a workshop to develop Nigeria's capability for implementation of biosafety guidelines. The four resource persons at the workshop were invited from Australia, India, Sweden and USA. There were 13 Nigerian participants drawn from universities, ministries of Health, Agriculture, Science and Technology, as well as the Plant Quarantine Services, Federal Environment Protection Agency, and Research Institutes.

The biosafety guidelines for the country had been developed before this workshop took place here at IITA and at the closing ceremony of the workshop Dr. Aliyu, the Director of Agricultural Sciences in the Federal Ministry of Agriculture reported that the Honourable Minister for Agriculture had signed the document. It is after this that the Minister will take the document to the Council of ministers' meeting for further consideration. If the Council

approves the guidelines then it will be placed in the government's gazette. The first step in implementing the guidelines will be the inauguration of the National Biosafety Committee. The DG (Dr. Brader) while in Abuja in March was informed by the Minister for Agriculture that the date of April 16, 1998 had been fixed for the inauguration of the NBC. With the NBC in place IITA and any organisation that wish to test or develop genetically modified organisms in the country can now seek the permission of the body.

Other areas outside of Nigeria

Recognising that the development and release of GMOs to the public domain may have implications beyond national boundaries IITA had made contact with the OAU/STRC with office in Lagos with the aim of developing a regional set of biosafety guidelines in Africa. The BAC in Stockholm organised a workshop on biosafety in Harare, Zimbabwe from April 6-10, 1997 in collaboration with the African Regional Biosafety Focal Point (ARBFP). The African countries are at different levels of developing and testing of GMOs. It can be stated however, that most of the countries do not yet have active research in developing transgenics. In West Africa for example, only IITA (in Nigeria) and to some extent WARDA (in Cote d'Ivoire) are engaged in developing or testing GMOs using their containment facilities. It is worthy of note that national scientists in many other countries in the region have shown interest in the establishment in their countries of biosafety guidelines. In East and Southern Africa more countries have active research in biotechnology along with biosafety guidelines. South Africa, Zimbabwe, Kenya and Uganda have developed and are operating their biosafety guidelines. Zambia is also initiating the process.

PROJECT V

Identification of yam potyvirus variability

Detection and Characterisation of Yam Potyviruses

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Potyviruses are the most diverse of the viruses affecting yams. Of these, yam mosaic virus (YMV) and yam mild mosaic virus (YV1) are especially important because of their high incidence and wide distribution amongst the two most widely cultivated yams; *Dioscorea alata* and *D. rotundata-cayenensis*. To allow the international exchange of breeding materials and in an attempt to gain a better knowledge of the variability found amongst yam potyviruses, work on improving an Immunocapture based Reverse Transcription Linked Polymerase Chain Reaction (IC RT-PCR) and the sequence variability found in isolates has been undertaken. Further work has been initiated with the aim of producing antibodies for yam potyvirus detection from coat proteins expressed *in vitro*.

Selected potyvirus isolates have been sequenced to confirm previous findings. A total of 43 complete sequences have been obtained covering the final 3' 700 bases of the potyviral genome. Phylogenetic analyses of these sequences supports previous findings of the division of yam potyviruses into four distinct groups. Though it was previously thought that these were linked to host species, it has now become clear that the two main viruses, YV1 and YMV can be found in both *D.alata* and *D. rotundata-cayenensis*.

Over 200 isolates were collected from Cote d'Ivoire and Cameroon during a visit to these countries in July and August of 1997. Ninety of these have been tested and Yam mild mosaic virus (YV1) and Yam mosaic virus (YMV) was readily detected with the exception of four *Dioscorea alata* and two *Dioscorea rotundata* isolates from Cameroon. ELISA tests indicate that these isolates contain YV1, but to date no specific RT-PCR amplification products have been generated using either specific or degenerate potyvirus primers. Only one example of a double infection was found.

The IC RT-PCR has been improved resulting in increased sensitivity, an internal control and stabilisation of enzymes for room temperature storage.

Improvements to the wash protocols have improved the sensitivity of the RT-PCR step.

1. Sequence information has led to the re-design of one of the YV1 primers with an concurrent improvement in amplification.

A YV1 isolate has been modified to act as a positive internal control for the RT-PCR step. This can be transcribed and the RNA added to the reaction at low levels in order to obtain an amplification product in the absence of target virus.

2. The use of tissue degrading enzymes to improve the immunocapture stage has been investigated.
3. The use of trehalose has been investigated to stabilise enzymes. Though a sensitive and straightforward test, the IC RT-PCR is particularly dependent on the quality of enzymes used. Where storage facilities are unreliable this immediately puts the test in doubt. Results show that it is possible to store Taq polymerase, AMV reverse transcriptase and dNTPs at room temperature for extended periods.

Primers have been designed for the amplification of YMV coat proteins to allow expression for antibody production. Optimisation of the amplification is in progress prior to cloning and

expression, but difficulties are being experienced obtaining suitable primers.

The IC RT-PCR method has been published in the Journal of Virological Methods 1997 volume 69 p73-79. The sequence analysis and PCR optimisation work is being prepared for submission to scientific journals.

PROJECT VI

Development of indexing techniques for banana streak virus

Banana streak virus control project (Uganda)

Prospects for breeding for agronomically superior Musa with reasistance or tolerance to BSV

Gatsby Funded Project 6. Phase 2

Development of indexing techniques for BSV

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Phase 1 was concerned with developing BSV diagnostics for testing the IITA advanced Sigatoka resistant *Musa* hybrids. BSV appeared at high frequency in the progeny from the breeding programme and presented a severe problem in the distribution of the improved germplasm. We obtained infected plants from the IITA field station at Onne, Nigeria and purified viral bacilliform particles. The sequence of the DNA genome revealed it to be an isolate of BSV and from the sequence data we developed a PCR assay for the virus. Results using this test showed widespread if not universal presence of BSV sequences in the IITA and other *Musa* germplasm which led to the suggestion made at the last Gatsby meeting that BSV sequences are integrated into the *Musa* genome.

The second phase was concerned with investigating this phenomenon and developing alternative diagnostic methods to the episomal disease causing form of the virus and to the integrated form.

Detection of BSV

IC-PCR with Ganesh Dahal and George Thottapilly, IITA

A direct PCR protocol for the sensitive detection of BSV from *Musa* plants was developed which indicated the widespread if not universal presence of BSV sequences in *Musa*. In contrast, use of methods such as symptom inspection, and serological methods indicated a much lower incidence of the virus. Genomic Southern analysis revealed the presence of BSV hybridising bands with different sizes to episomal BSV restriction fragments. These findings and other reports indicated the possible integration of BSV sequences into the *Musa* genome. Integrated sequences do not necessarily lead directly to observable disease symptoms as there are plants documented that are and have been apparently disease free. It was important therefore to develop a sensitive method to detect only episomal BSV, and to uncover the relationship between integrated BSV sequences and episomal BSV. IC-PCR, a technique in which an antigen (e.g. virus coat protein) is specifically captured by antisera and the contained DNA is subsequently amplified by PCR can provide a rapid, sensitive assay system. The approach used here has been to use a "composite" antisera generated against numerous different BSV isolates to trap virus particles and a subsequent PCR with primers to the aspartic protease and reverse transcriptase of the Onne isolate BSV sequence. Degenerate badnavirus sequence primers are available (Lockhart, Thottapilly) for the amplification of different strains of the virus.

IC-PCR consistently amplified a BSV-specific product from crude leaf extracts or virus minipreps prepared from BSV-infected *Musa* spp. whereas no such product was amplified from crude leaf extracts of other plant species or from PCR solutions containing no DNA template. IC-PCR was at least as sensitive, and for some samples, more sensitive than standard-PCR probably due to the removal of inhibitory substances by the washing steps during immuno-capture stage. *Musa* nuclear chloroplast mitochondrial sequences could be detected in *Musa* genomic DNA by direct PCR. However these sequences could not be detected in the IC-PCR tubes in which BSV was detected. The results show that episomal virus can be specifically detected by IC-PCR with high sensitivity and specificity under conditions in which integrated BSV sequences

are not detected. *Musa* nuclear, mitochondrial or chloroplast genomes which may contain BSV integrated sequences are not captured by the anti-sera or by the tubes in which the assay is carried out. The ease of sample preparation for this technique is suitable for the handling of large number of samples. Under limited laboratory facility conditions, either antibody-coated or empty Eppendorf tubes could be hand-carried or shipped by collaborating scientists, and the tubes returned back after loading of samples and washing to complete PCR assay. Degenerate primers are available for amplification of isolates with widely varying sequence. The technique is suitable for the large-scale screening of *Musa* necessary for germplasm movement. This method is currently in use at IITA.

Parallels with another plant pararetrovirus may explain variation in BSV symptom expression. Systemic symptom development in CaMV-infected *Brassica oleraceae* initially elicits a host response causing suppression of CaMV replication leading to plant recovery. The suppression involves a gene silencing mechanism causing viral RNA degradation while transcription of the viral minichromosome continues in the nucleus. In this case the supercoiled viral minichromosome forms a major component of the unencapsidated virus-specific nucleic acids and can be identified by subjecting total DNA (of banana leaf tissue showing symptoms and from plants in which there is remission of symptoms) to 2-D gel electrophoresis in which the 1st dimension conditions are native and the 2nd dimension conditions are denaturing. The gel is then blotted and probed with BSV sequences. This technique has been shown to separate and characterise the unencapsidated DNA forms of other pararetroviruses and to identify the supercoiled form. This procedure will provide a diagnostic for detection of potentially symptomatic plants.

Uganda is currently experiencing a severe epidemic of BSV in some of its principal Musa growing regions. We have shown that the isolate found in the Rakai province is different to that found in IITA Onne. Isolates are being sought from accessions at INIBAP and other sources to determine sequence variation and particularly to enable primers to be designed that anneal to the widest range of BSV isolates.

Integration of BSV

with Julian Osuji and Pat Heslop-Harrison, JIC.

The experiences with BSV in the tetraploid breeding programme at Onne strongly suggest that there is an integrated viral sequence which is activated by stress. The aim of this part of the project is to identify the activatable integrant so that a diagnostic to distinguish it from the non-activatable forms presumably present in all *Musa* can be developed. We focussed on the Onne isolate of BSV isolated from the tetraploid progeny TMP 4698 and parental lines Calcutta 4 (male, AA) and Obino l' Ewai (female AAB)

Fluorescent in situ hybridization (FISH)

To examine whether BSV sequences were present in *Musa* nuclear chromosomes, double target *in situ* hybridizations were conducted on chromosomes from Obino l'Ewai root tips. Three probes were used, 2 to BSV sequences, a short BSV probe covered the region of the viral genome containing amino acid sequence homologies to aspartate protease and reverse transcriptase (same sequence amplified during the PCR assay) and a longer BSV probe (BSV6294-1679) covered a region with little or no homology to other badnaviruses or retroelements. The other probe (MusaOL) was to a sequence adjacent to the BSV insert (see below). All probes gave hybridization signals on chromosomes of Obino l'Ewai. A major hybridization site to BSV6294-1679 was detected on both chromatids of one chromosome in each

metaphase and at least one weaker hybridization site was regularly seen on another chromosome. Hybridization of the shorter probe, was weak and less clearly discriminated from diffuse background although a single site was normally detected, co-localizing with BSV6294-1679 in double-target experiments. The MusaOL probe showed hybridization to multiple sites throughout the genome, including near the major BSV site, but was not uniformly dispersed. Neither of the two BSV signals was on the same chromosome which hybridized with an rDNA probe.

S-SAP

Sequence-specific amplification polymorphism (S-SAP) using a specific primer downstream of the 5' end of the BSV 35S RNA and random *Taq*I primers was used to detect BSV/*Musa* interfaces in nuclear DNA. This approach gave a number of products which hybridized with BSV and which were cloned. Twenty of the clones were sequenced and these fell into three families. Clones in one family contained both BSV and non-BSV, presumed *Musa* sequence and called MusaOL. The interface was at around the 5' nucleotide of the BSV 35S RNA. The MusaOL sequence has no significant homology to any EMBL/GENBANK database sequences, either as nucleic acid or protein.

Another family of S-SAP products contained a mosaic of segments of BSV sequence in various orientations. This indicates a rearrangement of this BSV sequence identical with the sequenced λ library insert of Ndowora, Lockhart and Olszewski and shows that the 35S RNA transcription start site of BSV occurs in more than one arrangement.

PCR analysis of integrated BSV sequences

Using a primer based on the MusaOL sequence and reverse primers based on BSV sequence, the linear extent of the BSV insert was explored by PCR. The expected size of fragments was found with primers up to position 5.8 kbp on the BSV genome but with primers to positions 6.1 kbp and beyond the fragments decreased in size, indicating that this integrant is contiguous BSV sequence from the position of the 5' end of the 35S transcript to about 5.8 kbp and is then disrupted. This sequence disruption is detectable by other BSV specific primer pair combinations and sequencing and is also in accord with the sequence of the λ library insert.

Fibre stretch

To examine the structure of the *in situ* hybridization sites in the *Musa* genome, stretched DNA fibres were prepared on slides from Obino l'Ewai nuclei. Following hybridization with BSV6294-1679, conspicuous rows of punctate hybridization sites ('dots') were observed. Dual hybridization showed that the MusaOL sequence was present both at sites associated with the BSV6294-1679 hybridization sites and independently. Two different structures, represented by rows of dots 51± 14 μm (~ 150 kb) long and 17±6 μm (~ 50 kb) long, were seen in approximately equal numbers. It is considered likely that the longer structure corresponds to the major hybridizing site seen on metaphase chromosomes and the shorter structure to the minor hybridizing site. The longer dot pattern can be interpreted as comprising five elements each with a MusaOL hybridizing signal at one end; the overall structure had the MusaOL signal at one end and not at the other. The elements differed in detail of distribution of BSV hybridizing signals. The shorter dot pattern contained three sub-repeats.

A crucial stage in the replication of retro- and para-retroviruses is the formation of the 35S RNA template. In the simplest model a complete functional BSV sequence is integrated with a promoter upstream and a terminator downstream. More complex models involve the necessity for recombination at the DNA level either between both parents in the cross or within one parent or at the transcriptional level with the 35S RNA being produced by template switching of the polymerase. As yet we have no evidence for an integrant that could simply be activated. The

integrant that we have detailed knowledge of would require a recombination event to restore a compete contiguous sequence.

Conclusions

All *Musa* have some integrated BSV sequences: PCR using a primer based on the MusaOL sequence and reverse primers based on BSV sequence on DNA isolated from different *Musa* accessions shows that similar structures are found in other *Musa* including both A and B genomes.

The presence of integrated sequences that can give rise to episomal BSV infections in *Musa* has profound consequences. If, as suspected the activation occurs during stress then processes such as breeding and tissue culture will be prone to this event. It is possible that local stress e.g infection with pathogens or (even other BSV isolates), drought etc. could provoke strong reactions and lead to BSV epidemics.

Knowledge of the particular arrangement of BSV sequences in potential *Musa* parental lines could minimise or even eliminate problems of activation. Alternatively a transgenic approach, to BSV resistance or suppression of activation can provide a suitable *Musa* genomic background for the IITA advanced Sigatoka resistant *Musa* programme.

Next steps

- Integration; the major question is which integrant is activatable? This will allow a diagnostic integrated sequences to be used in the selection of suitable parents in breeding programmes.
- Are there integrants of other strains of BSV activatable by other stresses.
- Diagnostics for unencapsidated BSV.
- Transformation; for BSV resistance or the suppression of integrant activation in suitable IITA *Musa* germplasm.

Topic: **Banana Streak Virus Control Project (Uganda)**

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

Vuylsteke, D, J.d'A.Hughes., F.M.Quin. J. Kubiriba, and W. Tushemereirwe

Project Objectives

To combat two disease problems (banana streak virus (BSV) and black sigatoka disease) that 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 Rationale

IITA and JIC have developed diagnostics for detection of the viral form of BSV. Although some additional research is needed for further refinement of the protocols, IITA has made good and relatively rapid progress in the adaptation of these diagnostics for routine use, to a point where it is possible to transfer these to a few candidate national programmes.

IITA has also made progress in their *Musa* breeding programme in screening and selection for field tolerance/resistance to BSV. There are now at least five *Musa* hybrids that are black sigatoka-resistant and registered germplasm in the public domain, which also display field tolerance/resistance to BSV. These hybrids are held by the INIBAP Transit Centre and were certified as satisfying the FAO Guidelines for Safe Movement of *Musa* Germplasm in 1995/6.

The project aims to establish a sanitation and germplasm delivery system in Uganda and Ghana.

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/resistance are:

Origin IITA: TMPx 4479-1
TMPx 7152-2
TMBx 1378
TMBx 5295-1
TM3x 15108-6

Origin FHIA: FHIA 1
FHIA 3

2. With technical backstopping from IITA, national programmes would use (TAS) ELISA and IC-PCR 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.

Progress

The Uganda project was started in August 1996 by introducing five IITA hybrids into the Kawanda tissue culture laboratory. 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. Four of the five hybrids are also believed to be tolerant or resistant 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 : 264
- TMPx 7002-1 : 505
- TMPx 7152-2 : 154
- TMBx 1378 : 254
- TMBx 5295-1 : 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). The IITA hybrid TM3x 15108-6, introduced directly from IITA, was included in 1997.

The 12 genotypes were further multiplied to a few hundred plantlets each (see Table) for nursery planting by August 1997. The nurseries were established by the NARO Banana Program staff in liaison with the relevant District Agricultural Officers of the Ministry of Agriculture in the District Farm Institutes of the target districts (Masaka and Rakai).

About 3000 plants were planted in the clean field nurseries in early November 1997. These field nurseries are used for further multiplication of clean suckers and not for production. Clean suckers of healthy nursery plants are being used to plant secondary nurseries in more locations in 10 districts and for distribution to farmers during the current planting season.

Table: Numbers of tissue culture-propagated banana plantlets established in the nurseries (Gatsby BSV project, Uganda)

Genotype	Location and planting date Masaka District Farm Institute, 5 Aug. 1997	Rakai District Extension Hq., 4 Aug. 1997	Total
Km 5	180	228	408
Grande Naine	192	245	437
Mbwazirume	60	52	112
TMPx 4479-1	180	179	359
TMPx 7002-1	210	208	418
TMPx 7152-2	240	224	464
TMBx 1378	210	102	312
TMBx 5295-1	210	210	420
FHIA-03	84	104	188
Total	1566	1552	3118

Extract from the Project 7 (Musa) 1996 Annual Report:

7.4.2. Clean and tolerant materials for BSV control in Uganda by D.V. - in collaboration with J.d'A.Hughes, F.M.Q. J. Kubiriba, W. Tushemereirwe

A strategy for the control of banana streak virus (BSV), based on the deployment of virus-free plants and/or virus-tolerant genotypes in infected areas, has been developed within the framework of a NARO/ESARC project in Uganda, supported by the Gatsby Charitable Foundation. *In vitro* cultures of selected landraces have been initiated and virus-tested cultures of tolerant/resistant IITA hybrids (TMPx 4479-1, TMPx 7152-2, TMBx 1378, TMBx 5295-2) were introduced from INIBAP in 1996. After rapid *in vitro* propagation at the NARO-Kawanda tissue culture facility, clean tissue culture plants will be planted in clean nurseries in Masaka and Rakai districts for sucker multiplication and distribution to farmers. Farmers will be urged to rogue diseased plants and to replant with the clean suckers of landraces and hybrids.

PROSPECTS FOR BREEDING FOR AGRONOMICALLY SUPERIOR MUSA WITH RESISTANCE OR TOLERANCE TO BSV

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Plantains and bananas are among the most important staple and commercial commodities worldwide. There is virtually no region in the world where banana fruits or derivatives are not consumed. Underlying this global trade is the global flow of genetic resources which has allowed breeders to successfully develop cultivars or hybrids with both wide and local adaptation. For example, black sigatoka is the most serious constraint of plantain production in Africa. A short term solution to this problem was the introduction of cooking bananas from Asia which were more resistant to this disease than the local plantain landraces. Meanwhile, the more long term strategy was to transfer black sigatoka resistance from diploid accessions introduced from South-East Asia and new hybrids possessing these genes were developed. The combined potential benefits from heterosis for yield and genetic control of the black sigatoka disease were estimated at US$6.2 billion added value per annuum for the African economy. Similarly, sources of tolerance to nematodes have been identified in the Latin American germplasm. Incorporation of these accessions in breeding schemes will result in substantial production gains for farmers in sub-Saharan Africa. In turn, access to improved germplasm by our research and extension partners in national programs necessitates routine multilocational evaluation trials, cultivar release, multiplication and distribution. Thus, sustained production of plantains and bananas in Africa largely depends on unrestricted flow of global germplasm.

Recently, banana streak virus (BSV; genus: Badnavirus) was identified as a new and serious constraint to the production of plantains and bananas (*Musa* spp. L) in some areas and has become a major threat for germplasm distribution worldwide. While progress has been made in understanding the epidemiology of the disease, little is known on specific host factors that may affect BSV expression frequency and severity in *Musa* germplasm. Nevertheless, one approach to controlling BSV could be through the selection and deployment of genetically resistant or tolerant hybrids, since natural variation has been observed for BSV occurrence and severity in *Musa* accessions. In this regard, 36 varieties of diverse genetic background (Table 1) were evaluated at three geographical locations in Nigeria following a north-south gradient in rainfall and soil fertility characteristics: Abuja (southern guinea savannah), Ibadan (forest-savannah transition), and Onne (high rainfall humid forest) (Table 2). Test genotypes were grown under sole cropping at all locations with and additional experiment under a multispecies alley cropping system at Onne, giving a total of four environments.

Data were collected on several agronomic characteristics and reaction to black Sigatoka and BSV. In addition, serological tests were carried out to determine the relative concentration of BSV antigens. The data were analyzed using an additive main effects and multiplicative interaction (AMMI) model allowing for dissection of variation into principal components.

Additive differences between the genotypes and environments were significant for time to flowering, bunch weight, number of hands, fruit weight and number, plant height and suckering behavior, and reaction to black sigatoka (*Mycosphaerela fijiensis* Morelet). Interaction between genotypes and environments was also significant for these traits and accounted for a substantial amount of the variation attributable to the treatment design. This interaction was essentially captured by the first axis of the PCA analysis for time to flowering, plant height, fruit number and fruit weight. However, the full model was required for bunch weight, number of hands and

suckering pattern, thereby indicating that genotype performance was least predictable for these variables across environments.

Significant differences were also observed for symptom incidence and relative concentration of BSV antigens among genotypes and across environments but there was no significant interaction between genotypes and environments for both traits. Lack of G x E effects indicates that screening for BSV can be done at one location with considerable assurance that the results would have predictive value for other locations. In this regard, symptom expression and relative concentration of BSV antigens in plant tissues were generally higher at Ibadan than at Onne. The lack of G x E effects also iincates that the potential for BSV expression is a genetically determined trait that is little affected by environmental conditions. Thus, the environment would therefore be a scaling factor rather than a modifying factor, which could have important implications for germplasm transfer but would simplify breeding efforts.

When phenotypic variance for BSV related traits was dissected into its components, there was little contribution of non-genetic factors to phenotypic differences among test entries. Non-genetic factors accounted for less than 5% of the variation in symptom expression and relative concentration of BSV antigens. In contrast, genetic effects accounted for 66.4 % of the variation in symptom expression and 62.6 % of differences in the relative concentration of BSV antigens. This indicates that both traits have high broad sense heritabilities. Furthermore, symptom incidence and the relative concentration of BSV antigens were positively correlated ($r=0.867^{**}$), suggesting that these traits may be different expressions of the same genetic factors.

Interestingly, similar levels of symptom expression were observed between some triploid plantains and their tetraploid offspring, e.g. Obino l'Ewai vs. PITA7 or Agbagba vs. PITA9, although the relative concentration of BSV antigens was higher in the offspring compared to the parents. Thus, ploidy level may may regulate the relationship between viral load and BSV symptom expression. Considerable differences were also observed for symptom expression of accessions which had similar relative concentration of BSV antigens. These data suggest that specific genetic factors (nature of integrated BSV sequences?) and ploidy level (number of integrants) may be key factors in the expression of BSV symptoms.

Table 1. List of entries evaluated in multilocational trials in Nigeria

Hybrid/Cultivar	Breeding #	Parentage
IITA hybrids:		
Plantains		
PITA-1	TMPx 548-4	OL x Calcutta 4 (C4)
PITA-2	TMPx 548-9	OL x C4
PITA-3	TMPx 5511-2	OL x C 4
PITA-5	TMPx 2796-5	BT x Pisang lilin (PI)
PITA-6	TMPx 4698-1	OL x C4
PITA-7	TMPx 1658-4	OL x PI
PITA-8	TMPx 7002-1	OL x C4
PITA-9	TMPx 1112-1	AgFrench Reversion x C4
PITA-11	TMPx 2637-49	OL x C4
PITA-12	TMPx 6930-1	OL x C4
PITA-14	TMPx 7152-2	Mbi Egome x C4
PITA-15	TMP3x 15108-2	TMPx 4479-1 x SH-3362
PITA-16	TMP3x 15108-6	TMPx 4479-1 x SH-3362
Cooking bananas		
BITA-1	TMBx 612-74	Bluggoe x C4
BITA-2	TMBx 1378	Fougamou x BB(I-63)
BITA-3	TMBx 5295-1	Laknau x C4
EMBRAPA and EMCAPA (Brazil) banana hybrids:		
EMB402	PV 03.44	Pacovan x C4
EMB403	PA 03.22	Prata Ana x C4
EMCAPA 602	Natural hybrid	cv. Oura da Mata
FHIA (Honduras) hybrids:		
Plantains		
FHIA-21	SH 3460	AVP-67 x SH 3437
FHIA-22	SH ?	AVP-67 x SH 3437?
Bananas		
FHIA-1	SH 3481	Dwarf Prata x SH 3142 cv. Goldfinger
	SH 3640	Dwarf Prata x SH 3393
FHIA-2	SH 3486	Williams x SH 3393?
FHIA-3	SH 3565	SH 3386 x SH 3320
FHIA-23	SH 3444	Highgate x SH 3362
INIVIT/INIFAT (Cuba) somaclonal variant of banana hybrid:		
** SH 3436-9	Highgate x SH 3142	Somaclonal variant of original SH 3436
Exotic Asian germplasm (female parents of IITA hybrids or as Cardaba in pedigree of FHIA-3):		
Bluggoe	cooking banana	
Cardaba	cooking banana	
Fougamou	cooking banana	
African plantain landraces (female parent of some IITA hybrids was OL):		
Agbagba (Ag)	Medium False Horn	
Obino l' Ewai (OL)	Medium French	
Dessert bananas (original introductions from Asia but currently grown in Africa):		
Valery	Dessert banana	
Yangambi Km 5	starchy banana	

Table 2. Some biophysical characteristics of test locations in Nigeria

Characteristics	Abuja	Ibadan	Onne
Geographical location	9° 16'N; 7° 20'E	7° 31'N; 3° 54'E	4° 51'N; 7° 03'E
Altitude (masl)	300	150	10
Annual rainfall (mm)	1303	1300	2400
Temperature (° C)	26 - 34	26.5	27
Radiation (MJ m^{-2} $year^{-1}$)	5846	5285	5060
Soil type	Ferris luvisol	Alfisol, slightly acidic	Ultisol, highly acidic

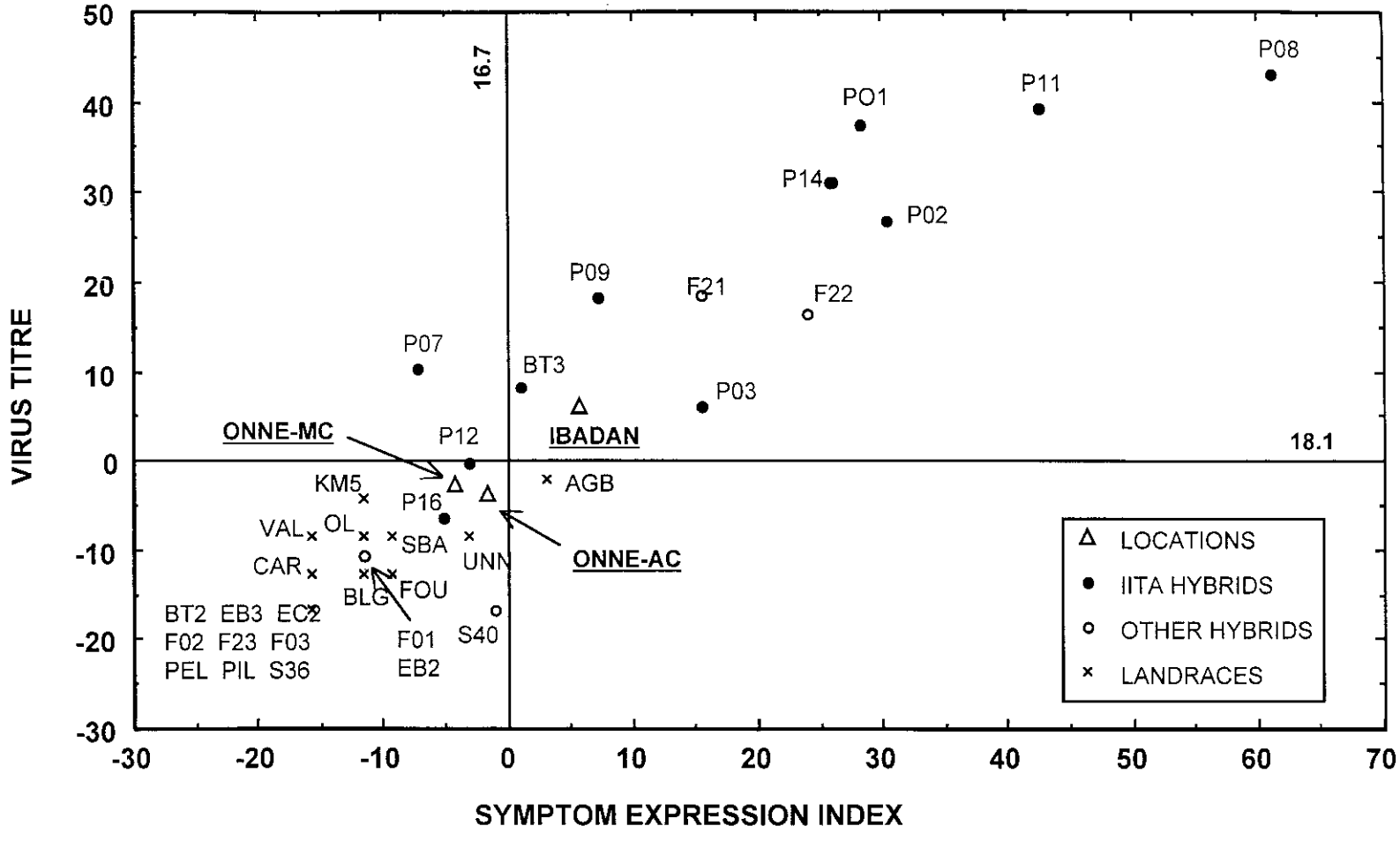

Figure 1. Biplot of BSV symptom expression and relative concentration of BSV antigens in *Musa* accessions evaluated in three environments in Nigeria.