International Institute of Tropical Agriculture

Crop Improvement Division

Activity Report and Work Plan 1993

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Root and Tuber Improvement Program

Cassava provides more than half of the dietary calories for over 200 million people in Sub-Saharan Africa (about half of the total population). It also contributes substantial protein, through consumption of the leaves. It is Africa's food insurance: it gives stable yields even in the face of drought, low soil fertility and low intensity management. It can remain in the soil until needed, spreading out food supply over time, helping avert the tragic "boom and bust" cycle of oversupply followed by famine. However, diseases (African cassava mosaic virus, bacterial blight) insects (mealybug, grasshoppers) mites (green spider mite, red spider mite) and nematodes take their toll, occasionally in epidemic proportions. Postharvest processing is laborious: the high water content of the root must be reduced quickly to avoid spoilage and high transport costs, and the cyanogenic potential could pose a health risk if roots are not properly handled before consumption and if the nutritional status of a population is poor.

IITA's founders invested in crop improvement on the premise that tropical food crops, had received insufficient research attention and would probably show a large response to dedicated efforts in crop improvement. This hope was borne out in cassava, as much higher yielding, pest-resistant varieties (particularly to bacterial blight and mosaic virus) were quickly developed and rapidly adopted by farmers wherever planting material became available.

Despite the proven track record in cassava improvement, many challenges remain. High levels of green mite resistance are not yet available. Most of our work so far has been in the lowland humid tropics, but germplasm adapted to other agro-ecologies is also needed - particularly the cool mid-altitudes and the dry savanna. Root dry matter and storability, cyanogenic potential and different flour qualities for various end-products need to be addressed. As always, increasing yield potential is a fundamental objective.

There are opportunities for breeding to contribute to increased production and decreased input requirements for yam. An erect (rather than spreading) shoot type might reduce or eliminate the high labor input for staking, and avoid some foliar diseases. Resistance to beetles, nematodes and foliar diseases could greatly stabilize production. However, progress so far has been quite limited because erratic flowering makes controlled crossing rather difficult. Even if flowering were under the breeder's control, knowing which crosses might lead to viable hybrids cannot easily be assessed because phylogenetic relationships are poorly understood. Currently, basic research on florigenesis and phylogeny is an important adjunct to the applied work on crop improvement.

T.1 CASSAVA GERMPLASM INTRODUCTION AND EVALUATION

Project rationale

Cassava is not native to Africa - it was introduced from Latin America in the 1500's. Naturally, only a sample of the total gene pool was introduced; many species and ecotypes, representing the broader gene base of the crop, remained in the center of origin. Provision of new genetic diversity from Latin America to Africa could bring in useful new traits and create new heterotic combinations that would produce higher yields.

Completed studies

Odega, N.T., S.Y.C. Ng and M.O. Akoroda (unpubl.). Study on the effects of culture media on cassava micropropagation.

Five different media formulations were used to study the growth of single node cuttings of several cassava clones. Media that supported best growth were MS and LS. The effects of different cytokinins (BAP, kinetin and 2-ip) at several concentrations (0, 0.01, 0.1 and 1 mg/l) on micropropagation rate of cassava were also studied. Parameters used for this study were number of nodes, number of leaves, survival rate and number of roots. There were different responses to the different cytokinins by the genotypes. In general, more roots were produced in MS medium than LS medium in all cytokinin treatments. However, cytokinins at 1 mg/l inhibited rooting in all clones studied. Number of nodes increased with the increase in kinetin concentration and was highest at 1 mg/l. This was not the case in BAP and 2-ip treatments. The higher number of nodes were obtained at 0.1 mg/l BAP and 2-ip treatments. The most suitable medium for rapid propagation of cassava was LS medium supplemented with 0.1 mg/l BAP.

Bokanga, M. (unpubl.) Cyanogenic potential of cassava : 1. Genetic variability and sampling methods for field trials.
Cassava is a cyanogenic plant which accumulates linamarin and lotaustralin in its tissues. The terminology of "cyanogenic potential" or "CNP" is proposed to refer to the concentration of all compounds that can be converted to cyanide. Analysis of 851 cassava genotypes indicated that the distribution of CNP in cassava roots is strongly skewed positive. Over 60 % of the genotypes had a CNP below 10 mg HCNeq/100g dry matter. Differences in CNP between roots and between leaves of the same plant, between plants of the same genotype, and between genotypes were significant at greater than the 1% level. The coefficient of variation of CNP of roots was much greater than that of leaves. There was no correlation between CNP of roots and CNP of leaves. Increasing the number of replications and the number of plants sampled per plot reduced the variance of the sample mean of CNP of roots. A sampling scheme of 3 roots per plant, 4 plants per each of 4 replications is recommended for field trials where the CNP of cassava roots is investigated.

Ekanayake, I., A. Dixon., R. Asiedu., and A. M. Izac. (In press). Improved cassava for inland valley agro-ecosystems: Proc of the fifth triennial symposium of the International Society of Tropical Root Crops - Africa branch, Kampala, Uganda 22-28 November, 1992.
A substantial impact on African agriculture can be made through research on crop improvement for the extensive inland valley agro-ecosystems (IVS) extending from the humid forest to the savanna zones in Sub-Saharan Africa. Systematic surveys of farmers have shown that cassava is a favoured upland crop for the IVS. Screening of cassava for IVS adaptation is a continuing activity undertaken by the Root and Tuber Improvement Program in collaboration with the Inland Valley Program at IITA. Elite clones TMS 85/00025, 63397 and 84/00003 with fresh weight tuber yield of 15.9t/ha, 13.3t/ha and 12.6t/ha respectively exceded the yield performance of local checks during a 6 month growing period. In addition, for cassava and other upland crops to be suitable crop components in this highly fragile agro-ecosystem, attention has to be given to a combination of improved genotypes with appropriate water control, weed control and fertility management. Research at IITA to date has attempted to address these short and long term production constraints.

Ntawuruhunga, P., Dixon, A., Bokanga, M. and Akoroda, M.
When cassava was planted at the end of the wet season dry matter content decreased between the fourth and eight month after planting and then sharply increased up to the 12th month. After the 12 month most of the varieties did not show any significant increases in dry matter content up to the 15th month. There was a significant decrease in dry matter content of TMS 30001 after the 12th month which may be attributed to the biodeterioration that was observed in this clone during the rainy season. Fresh root weights for all clones increased steadily up to the 12th month and then started decreasing gradually up to 15 month except for those of 30001 and 63397 that continued increasing up to 15th month. 91934 reached its optimum root weight at about the 9th month while 82/00661 increased its root weight sharply up to the 12th month before decreasing. The latter clone was also the top yielder (26 t/ha fresh tuber weight) at 15 months after planting. In terms of dry matter yield most clones generally showed a progressive increase up to the 12th month before decreasing. 30001 and 4(2)1425 increased their dry yield up to the 15th month. At the 8th month 91934 gave the highest dry matter yield among the clones but this dry matter yield subsequently increased at a slower rate up to the 12th month before levelling off. All the clones reached their maximum starch content at 12 months. The data indicated that the dry season had a profound effect and there was evidence of an extension of these effects on all the traits measured well into the early part of the rainy season.

Activities

T.1.1. Broadening the Germplasm Base
M.Porto, N.Q. Ng and R. Asiedu
True cassava seeds are being obtained from CIAT headquarters in Palmira, Colombia, and EMBRAPA in Brazil taking into consideration parental adaptation to different agroecologies. A number of IITA elite clones previously introduced to CIAT are also being used as sources of resistance to the African cassava mosaic virus (ACMV) in the introductions. National programs in Africa are also contributing true seed populations to IITA which serve as useful sources for adaptation and quality characteristics. Other seed populations used to initiate the breeding process include: 1) progenies from open and controlled pollination of polyploid parents; 2) interspecific hybrids and their backcross derivatives; and 3) hybrids among IITA elite genotypes.

During 1991-92, a total of 35,268 seedlings were evaluated in seedling nurseries established at Ibadan, Onne, Kano and Jos. Based on successive evaluations for resistance to the major pests and diseases and also on the basis of morphological and agronomic characteristics, 809 individuals were selected and advanced to clonal evaluation trials planted at 5 locations representing four different agroecologies.

At Ibadan and Onne severity levels of ACMV recorded in the Latin American germplasm were always higher than those recorded in the resistant clone TMS 30001 and slightly higher than those of TMS 91934. The incidence of the disease (percentage of individuals with symptoms on a family basis) recorded during 1991 and 1992 was also higher in the seedlings of Latin American origin, when compared with the levels observed in TMS 30001, but comparable to those measured in TMS 91934. ACMV was more severe at Ibadan than Onne. On a family basis, disease severity was more correlated with root fresh weight at Onne than at Ibadan. Correlation coefficients up to -0.68 were observed in November, suggesting a high influence of the disease on root yield. Although these data need further confirmation, it provide preliminary information on the effect of ACMV on fresh root yield, both on a single-plant and on a family basis, when using a large number of observations. The work is being repeated in the 1992-93 seedling nurseries and larger number of data pairs will be used to calculate the correlations.

In Kano access tubes were installed in the seedling nursery (SN) and clonal evaluation trial planted at Minjibir in 1991, in order to measure soil water content using a neutron gauge. Relative Water Content (RWC) measured at 145 cm was significantly higher in the Clonal Evaluation trial, indicating a lower volume of water extracted at deeper soil layers. Differences in RWC were also detected across families in the SN and across clones in the Clonal Evaluation trial. Changes in plant height measured in 10 seedlings per family and 3 plants per clone planted near the access tubes were measured and compared on basis of the slope of linear regressions calculated against time. Growth of seedlings was faster than that of clones during the dry period, probably due to the ability of the tap roots to reach deeper layers of the soil profile, when compared with the root system of the clones. During the recuperation period, however, the clones were able to grow at higher rates, probably due to the fact that the more voluminous superficial root system typical of vegetatively-propagated plants, was more efficient in absorbing water from the superficial layers of the soil profile at the beginning of the rainy season.

In 1992, 3,514 and 668 seedlings were transplanted at Ibadan and Onne, respectively. Incidences of ACMV, CBB, CAD, CM and CGM are evaluated on a monthly basis, as well as measurements of plant height to assess potential differences in growth habit and vigor. A total of 2606 and 2597 seedlings were transplanted at Kano (Minjibir Farm) and Jos, respectively, as a result of the 1992 seed introductions from CIAT. Evaluations for diseases (CBB and ACMV) and pests (CGM and CMB) are undertaken bimonthly. Plant growth and leaf retention are being monitored also every two months, in order to identify differences in initial vigor and growth under water stress. Seedlings now under evaluation at Ibadan, Onne, Minjibir and Jos will be harvested in 1993. The best individuals will be cloned and advanced to Clonal Evaluation trials in each agroecology. In cooperation with ESARRN and FAO, a lot of 13,863 cassava seeds representing 147 families with high potential of adaptation to mid-altitude and subtropical environments was sent to Malawi, where they were germinated under screenhouse conditions. The resulting seedlings will be transplanted into a seedling nursery and selections will be made on basis of their performance in that agroecology.

A shipment containing 50,000 seeds was introduced into Nigeria from CIAT in 1993 and will be germinated at the 4 locations in Nigeria. A lot of approximately 20,000 seeds obtained from crossings involving Brazilian parents adapted to semi-arid conditions will be also introduced in 1993. These seeds will be germinated and the resulting seedlings will be evaluated at Minjibir during 1993-1994. Quality aspects of the roots, especially the cyanogenic potential of the introduced material will be analyzed in collaboration with TRIP Biochemistry Section.

T.1.2 In vitro conservation and distribution

S.Y.C. Ng, R. Asiedu, N.Q. Ng and J.B.A. Whyte

Over 120 accessions of cassava germplasm collected from Nigeria and elsewhere were transferred from field collection to in vitro culture in 1992. Maintenance of the existing in vitro collections of cassava and wild *Manihot* sp. as well as transfer of field collections to in vitro culture will continue in 1993. In 1992, eleven cassava clones were certified by the Plant Quarantine Services (PQS) , Nigeria. Over 40 selected clones from Ibadan and Mokwa were regenerated from meristem culture and 15 clones were transplanted into the isolation room for virus indexing. These regenerated clones will be certified and distributed to the NARS. Cassava clones from different agroecologies both at UYT and AYT will be cleaned up in 1993 and distributed to NARS. Micropropagation and distribution of the 51 certified clones were the major focus in 1992. A total of 33 packages of cassava tissue culture materials were distributed to 16 countries in Africa and to our

collaborators in Australia, Denmark and Austria for collaborative research. Poor establishment of tissue culture materials is the bottleneck for the rapid dissemination of tissue culture materials to NARS. A series of experiments were carried out to better understand the major factors affecting plant establishment. Humidity and fungus invasion are major factors affecting plant establishment. Other factors studied were plantlet age and rooting hormone treatments. In 1993, studies will be continued with the aims of to improving the handling system and improving the establishment rate.

Other technical assistance to NARS in tissue culture will continue. In vitro germplasm collection of cocoyams and sweet potato will be progressively duplicated at the National Root Crop Research Institute (NRCRI), Umudike, Nigeria. In 1992, 34 accessions of cocoyam and 100 accessions of sweet potato were sent to NRCRI. Technical assistance was also provided to the Root Crops Research Program, Malawi, in setting up a tissue culture laboratory to serve the ESARRN member countries.

T.1.3 Measuring diversity in cassava

A. Dixon and R. Asiedu

In 1992, 342 IITA elite cassava germplasm developed for the Forest and Forest transition/Moist Savanna agroecology as well as 192 local germplasm accessions which are utilized in the breeding program were evaluated for morphological and physiological characteristics at four locations (Ibadan, Onne, Zaria, Niaouli). These elite clones have already been characterized for 26 shoot characters as well as pest and disease reactions. At harvest, in 1993 root characteristics and plant heights will be assessed. These data and differences in isozyme patterns will be subjected to principal component analysis. These techniques will be useful to identify specific genotypes accurately and thereby will enable assessment of the spread of improved clones on-farm, as a tool for impact assessment.

T.1.4 Cassava germplasm evaluation

A. Dixon, R. Asiedu, M. Porto, M. Bokanga, I. Ekanayake, C. Akem and Entomologist

The standard selection scheme for germplasm evaluation in the humid forest, forest, transition/moist savanna, mid-altitude and dry savanna agroecologies of Africa starts with a seedling nursery at one site within an agroecology in Nigeria. Selections from these nurseries are cloned and evaluated in successive trials - preliminary, advanced and uniform yield trials. With each evaluation, selection is made for performance with respect to ecological adaptation, reaction to pest and diseases and quality of the produce at each site. Evaluation for low cyanogenic potential is a high priority. At the uniform yield trial stage more than one locations is used as testing site within the agroecology. The selection scheme also provides for the progressive multiplication of planting material starting from one seedling in the seedling nursery. Evaluation and selection schemes were established in all breeding sites in 1992. Trials harvested in 1992 were subjected to selections and advanced to the next selection stage. So far many clones have reached the most advanced stage in the humid and sub-humid ecologies and are progressing in the mid-altitude and dry savana ecologies.

T.1.5 Evaluation at the Mbalmayo Station

A. Dixon, M. Bokanga and J. Ngeve

The characterization of cassava clones of IITA elite leading and breeding clones and cassava germplasm collection at the humid forest station in Mbalmayo was started in 1992 and will be completed and documented in 1993. A collection of local germplasm at Njombe was also duplicated at Mbalmayo and evaluation for possible utility in the breeding program is proceeding.

Morphological characterization (28 characters) and assessment of reaction to prevalent pests and diseases (ACMV, CBB, CAD, CM and CGM) as well as root quality traits like mealiness and cyanogenic potential were carried out on 556 genotypes of improved cassava germplasm (161 clones poundable and 378 clones non-poundable) as well as on the 105 local germplasm accessions collected in Cameroon and replanted in 1992 for characterization. The trial will be harvested in May, 1993 and evaluation for root characteristics will then be completed. Selections will be made for subsequent multiplication and future trials and the 161 poundable clones will be provided to the Cameroon National Root Crop Programs for national trials as agreed under the RRPMC project. Also 47 elite IITA cassava cultivars (in vitro) developed at the high rainfall station at Onne in Nigeria were established comprising 11 poundable lines and 36 non- poundable. 40 microtubers of 5 yam cultivars (TDr 608, 179, 747, 205, NDr/10) were planted. These will be rapidly multiplied in 1993 for evaluation under the Mbalmayo conditions. 6000 yams seeds of various families from poundable populations in Nigeria were also planted in a seedling nursery for evaluation.

T.1.6. Field performance of virus-free cassava.

A.O. Akano, R. Asiedu, S.Y,.C. Ng, H. Rossel and G. Atiri (Univ. Ibadan)

Earlier studies carried out in Ibadan during the main planting season have shown that the yield increase by planting cassava virus-free materials obtained through meristem culture was minimal. This might be due to the heavy ACMV pressure at Ibadan. In 1992, eight virus-free cassava clones were multiplied in vitro and established in three agroecologies (Ibadan, Onne and Zaria). The plantlet establishment rate ranged from 62 to 77%. The whitefly population was highest at Onne, followed by Ibadan whilst that in Zaria was low. ACMV symptoms were most prevalent and severe at Ibadan followed by Onne. No symptoms were observed at Zaria. These materials will be used to plant in a field trials at the three locations for evaluations in 1993. The same set of virus-free tissue culture materials will again be established in these ecologies.

T.1.7 Cyanogenesis in cassava: genetic variation, de novo biosynthesis in roots, and DNA probes for CNP screening

M. Bokanga, S.Y. Ng, J. Mignouna, B. Halkier and B.L.Moller (RVAU, Denmark)

Light grown seedlings rapidly lose the ability to produce active preparations of linamarin biosynthetic enzymes. Cotyledons of etiolated seedlings contain the quasi totality of the biosynthetic activity. However, they are covered with an endosperm which contains a very strong inhibitor of the biosynthetic activity. Contamination of cotyledons by the endosperm is unavoidable and leads to errors in the quantification of the biosynthetic activity. This research activity will investigate alternative ways of obtaining inhibitor-free preparations, such as embryo culture and callus/suspension culture from seeds and somatic tissues of cassava,in the search of a method to assess the biosynthetic activity in green cassava tissues.

There is evidence that the concentration of linamarin in cassava roots increases after the roots have been cut off from the plant. This activity will investigate whether the increase in concentration is due to a de novo biosynthesis in the root or whether it is due to the conversion of non-assayable translocated precursors to linamarin.

Several enzymes of the biosynthetic pathway of linamarin have been purified and sequenced by Prof. Møller's group in Copenhagen. The genes coding for these enzymes are being isolated. There is an agreement with Prof. Møller under which he will send us the genes to be used as DNA probes. The probes will be used to detect molecular differences between cassava genotypes of high and low cyanogenic potential. Such differences will be used as screening tools in the development of cassava lines of low or no cyanogenic potential.

T.1.8 Screening for flexible harvest time

A. Dixon, P. Ntawuruhunga and M. Bokanga

Cassava breeding programs have hitherto concentrated on breeding for high yield in terms of dry matter/unit area/time given a 12 month harvesting cycle, while farmers prefer cassava that would be harvested as early as 6 months (COSCA data) but at the same time want the flexibility of extended harvesting as much as up to 18-24 months. There is little knowledge on the optimum bulking and maximum dry matter/starch accumulation as well as deterioration in the ground after 12 months. One such study initiated in 1991 during the dry season with 10 elite clones to identify clones and breeding parents possessing these traits have completed 15 months of growth. A second season trial (wet season) started in 1992 with the same clones has just completed seven months of growth. The objective of the two seasons trial is to establish the effect of climate/ environment on dry matter accumulation over time. In addition, a new set of 35 improved clones just from the breeding program was planted in the 1992 wet season to assess bulking rate and preharvest root storability in order to make recommendations on their starch accumulation characteristics during germplasm delivery to NARS. This trial will be followed through for a 24 month growth period.

T.1.9 Cassava adaptation to inland valleys

A. Dixon, I. Ekanayake and M. Wouamane

Cassava is commonly grown in inland valleys on residual moisture after rice. Several elite cassava clones and standard varieties are being evaluated on an inland valley slope at Ibadan and a better understanding of the major constraints of this agro-ecology will be sought by comparing the effects of different water management practices, mulching, moisture supply and soil temperature on crop performance. A detailed soil-plant-atmosphere continuum study is underway using soil hydrology and crop physiology techniques.

Sixty elite cassava clones and two standard varieties popularly grown in the inland valley ecosystem were evaluated during the 1991/92 dry season at the C-hydromorphic area of IITA headquarters to evaluate their potentials under this ecosystem. The experiment was also conducted on the upland at Ibadan during 1992 rainy season to assess the relationship in performance in the upland and inland valley, given a six month growth cycle. In the first year, 60 clones were planted and harvested at 6 months. Records were taken on establishment, CMV, CBB and CGM, harvest index, weight of top, number of plants harvested, tuber numbers, fresh weight and size, poundability, dry matter contect and incidence of rots. 58 clones were replanted on the upland (2 clones dropped because of limited planting material) and harvested at 6 months. This trial was harvested in Dec. 1992. Records taken were similar to those of the first crop. Preliminary results indicate that there is sufficient genetic variability among our elite clones for adaptation to the inland valley ecosystem (IVS). Significant G X E interaction between upland and IVS was found for most traits measured but some clones showed adaptation to both ecosystems. There was an apparent increase in CMV scores in most clones when grown under IVS. Differential rotting of tuberous roots was observed in many clones. In the second year (Jan 1993), 54 clones were replanted in the IVS (6 clones were dropped on the basis of very poor establishment and unacceptable yield levels). Blocking took account of anticipated soil moisture conditions during the 6 months growing period as follows: dry dry, dry wet, wet wet, very wet wet. The clones will be evaluated again to assess genotype x environment interaction within this ecosystem.

Screening of cassava for adaptation to IVS will continue and will be extended to cover clones developed in the other agroecologies with the aims of finding the relationship between the performance of cassava in the uplands and in IVS within the same agroecological zone, the constraints to production in IVS and well adaptable high yielding clones for such systems.

T.1.10 Relationship between seed size and ploidy in cassava

V. Boateng, S.K. Hahn and S.Y. Ng

A preliminary study has indicated that the size of seeds resulting from open pollinated families of certain tetraploids may be related to ploidy in cassava. The embryos from the seeds of small, medium and large size groups will be cultured in vitro and ploidy levels of their seedlings will be examined in order to relate the seed size with ploidy. Embryo culture is necessary for this study because small seeds often do not germinate. The research results, if related, will enable breeders to screen cassava for ploidy in certain families at the seed stage, thereby enabling breeders to reduce space and time for cassava polyploid breeding.

T.1.11 Characterization of a mutant cassava clone using molecular markers

V. Boateng, S.K. Hahn and D.H. Mignouna

A non-pubescent cassava clone which is suspected to be a mutant of originally pubescent clone TMS 4(2)1425 has been identified. Crosses were made between non-pubescent and pubescent clones. The parental clones and their progenies will be subjected to RFLP and RAPD analysis to characterize the mutant using the molecular markers. The mutant to be characterized will be useful material for future genetic studies and genetic mapping in cassava. Since the pubescent character is a factor responsible for resistance to cassava green spider mite and mealybug, the markers will also be useful in terms of resistance to the pests.

T.1.12 Comparison of spontaneous sexual and asexual tetraploids, artificial tetraploids induced by colchicine, and their diploids.

S.K. Hahn and J. Udosen

Ten tetraploids with three differing origins – three spontaneous sexual tetraploids, two spontaneous asexual tetraploids, and five artificial tetraploids –have been identified. These tetraploids will be compared amongst themselves and with their diploids for their performance in the field. It is most fortunate in the sense that three types of tetraploids with different origins all have become available at IITA. With this experiment, it is anticipated that the potentials of the three tetraploid types will be estimated and that the results will be useful for formulating cassava polyploid breeding strategies.

T.1.13 Yield loss assessment and screening of cassava germplasm for resistance to the spiralling white fly

A. Dixon, I. Ekanayake and Entomologist

A potential threat to cassava as well as many other crops is the appearance of the apparent devastating spiralling white fly whose distribution has been noted in Ibadan, Lagos and Bauchi in Nigeria. To embark on a host plant resistance program for this pest, there is need to demonstrate the impact of this pest on root yields as well as leaves of cassava which are often consumed as a green vegetable in some African countries.

Experiments will be set up to evaluate such impact on cassava. From observations there seems to be apparent varietal differences among cassava genotypes growing in the fields of IITA, Ibadan. Thus there is a need to screen our cassava germplasm collection including local land laces to identify sources of resistance to this pest in case it turns out to be a key pest in cassava growing areas. An experiment will be initiated in 1993 to identify gene sources that could be used in the program.

T.2 ENHANCEMENT OF CASSAVA GERMPLASM POOLS

Project rationale

Utilizing new diversity effectively requires extensive intercrosssing and selection. The wide range of target agro-ecologies, farming systems, utilization patterns and consumer preferences for cassava in Sub-Saharan Africa dictates that a number of different recombining populations will have to be handled. Studies are underway concerning breeding procedures to maximize the efficiency of the recombination and selection processes, particularly as regards inter-ploidy crosses.

Completed studies

Dixon,A. and R. Asiedu (unpubl.). Mechanisms of resistance to Cassava Green Mite (CGM) in clonal hybrids of cassava

Fifty three cassava clones derived from the cycle 0 of the back-up population for CGM resistance were evaluated during 1992 to identify different mechanisms of CGM resistance or genes that relate to different mechanisms. The aim is to diversify CGM resistance and also combine different genes that control various mechanisms or the same mechanism of resistance. This may result in additive or epistatic interaction that will give higher levels of resistance in elite cassava populations. Results showed that different sources of resistance may have different mechanisms of resistance. Non-preference among the clones as measured by the difference in the number of females found between the test and control plant after two days of release were highly significant. The preference index ranged from -11.9 to 10.7 for the 53 clones. The best clones, selected for apparent field resistance based on damage rating, proved to be less preferred. Antibiosis, as assessed by the developmental biology (growth duration, adult life-span and fecundity) of CGM, was evaluated on the same 53 clones above as well as on the clone, 30001. There was significant variation among the clones only for growth duration of the mite (larva to adult) among the 54 clones. The relationship between CGM damage rating of cassava and pubescence was also assessed. The average damage rating of the 53 clones with various levels of field resistance to CGM and degrees of pubescence were evaluated biweekly under field conditions for 14 weeks during the dry season at Ibadan. At the start of the first data collection, the trichome density (no. of trichomes/mm^2) of the first fully expanded leaf of two plants per clone were determined in order to assess the degree of relationship between damage scores and trichome density. The results showed highly significant differences in damage scores for each date and across dates among the 53 clones and only a weak but significant negative linear relationship between damage scores and trichome density (correlation coefficient, r of -0.23 to 0.33). This suggests that there is more to resistance of cassava to green spider mite than just pubescence.

Dixon, A. (unpubl.). Effect of ploidy level on host plant resistance of cassava to CGM

The mechanisms of resistance to CGM of eight improved clones of cassava with various ploidy levels to green spider mite were evaluated for antixenosis and antibiosis. The Diploid and tetraploid levels of four cassava elite clones (8 clones) were evaluated in terms preference of CGM and antibiosis. Non-preference as measured by the difference in the number of females found between the test and control plant after two days of release on these clones was highly significant. The diploid clones 4(2)1425 (pubescent), 4(2)1425 (non-pubescent) and 30572 (non-pubescent) were significantly less preferred than their tetraploid counterparts. There was no difference for preference between the diploid and tetraploid clone, 91934. There was no significant difference in antibiosis (growth duration of mite: larva - adult) between the tetraploid clones and their respective counterpart except for the pubescent, tetraploid 4(2)1425 in which mites had a significantly longer growth duration than its diploid counterparts. The results have some implications to the cassava breeding program as field observations have shown that most polyploids growing at Ibadan field are very susceptible to CGM.

Dixon,A. and R.Asiedu (unpubl.). Genetic analysis of field resistance of cassava to CGM
A seven parent diallel experiment of first generation crosses including F1's and reciprocals involving 4 resistant and 3 susceptible clones of cassava to CGM was completed. After artificial infestation with infested cassava leaves, the damage scores taken at weekly intervals and those pooled across eight dates during the dry season showed significant difference among genotypes, parents and crosses. Taken at weekly intervals the comparison parents vs crosses, with which is a test for mid parent heterosis was not significant indicating a lack of mid-parent heterosis for damage score.

The genetic variation found among crosses for this trait was due to all genetic components, general combining ability (GCA), specific combining ability (SCA), maternal effects (ME) and specific reciprocal effects (SRE) suggesting that both additive and non-additive gene effects were important in determining this trait. GCA was the more important than SCA (ratio 5:1). The four resistance sources (TMS 4(2)1425, 42025, 61677, 91934) all had negative GCA effects for resistance as measured by damage score while all susceptible sources had positive effects for damage score. 42025 had the largest GCA effect while 4(2)1425 had the least among the resistant sources. The positive GCA effects of the susceptible sources (30001, 30572 and 30555) were roughly equivalent. The first generation crosses between the resistant and susceptible sources showed varying intermediate reactions and suggested a polygenic inheritance. The study indicated the damage score is an inherited trait but that the inheritance seemed to be complex and to a large extent depended on the source of resistance as was revealed by the significance of GCA, SCA, ME and SRE. Recurrent selection would be appropriate for accumulating the genes for this trait in both parents of the hybrids.

Bai, K.V., S.Y.C. Ng and S.K. Hahn (unpubl.). Megasporogenesis in Cassava.
The course of development of the embryosac was studied in the genotype TMS 4(2)1425. It was observed that the megaspore mother cell (MMC) or Embryosac mother cell,(EMC) undergoes meiotic division and the first meiotic division produces two dyad cells followed by the homotypic second division resulting in a linear tetrad of megaspores. Later, three megaspores of the linear tetrad degenerate while the chalazal megaspore enlarges and by means of three successive mitotic divisions, gives rise to an eight-nucleate embryosac. This type of development of the embryosac is referred to as "*Polygonum*" type. The eight nuclei become arranged in quartets at the micropylar and chalazal ends of the embryosac and three of the nuclei at the micropylar end become differentiated into cells which constitute the egg apparatus consisting of the female gamete or egg cell flanked by the two synergids. At the opposite end of the egg apparatus three of the four nuclei differentiate as the antipodal cells which soon degenerate. The two remaining nuclei - termed polar nuclei migrate from the opposite ends of the embryosac into the central region.

Bai,K.V., R. Asiedu and M. Bokanga. (Unpubl.) Calcium oxalate crystals in cassava and other Manihot species. I. Occurrence and distribution in different plant parts.
Calcium oxalate (CaOx) crystals in the form of druses, prismatics and raphides have been observed in cassava and other *Manihot* species. Their distribution vary from species to species and in different parts of the plant. Druses are the most frequent type and occur in the root cortex, cortex and pith of the stem and petiole; in the epidermis and mesophyll of lamina and parenchymatous ground tissue of the midrib; and in the connective tissue of the anthers. Their frequency varied in different regions and they were more frequent at the petiole base, lamina base and in midrib region. It was observed that the HCN content in different plant parts shows variation similar to the distribution of CaOx crystals. The midrib region of the lamina had the highest density of CaOx crystals and the HCN content was also highest in this region. Mid-region of the petiole had the lowest density of CaOx and the lowest HCN content.

Bai, K.V., R. Asiedu and A.G.O. Dixon (in press). Cytogenetics of Manihot species and interspecific hybrids. Proc. 1st International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, August 25-28,1992.
Using 12 of the 22 *Manihot* species available at IITA, interspecific hybrids were produced in 1991 and cytogenetic analysis was carried out in 1992. Chromosome pairing behavior at metaphase 1 in these hybrids showed differences in the extent of synapsis and chiasma frequencies. In the hybrids of cassava with the species, *M. epruinosa*, *M. esculenta* ssp. *flavellifolia* and *M. gracilis*, 18 IIs were observed at M-I and, a bridge and a fragment were frequently observed indicating an inversion at A-I. In hybrids with *M. gracilis*, 18 IIs or 17 IIs + 2 Is at M-I and a bridge and a fragment at A-I were common. The hybrids of cassava with *M. glaziovii*, *M. tristis*, *M. pohlii* and *M. tripartita*, differed considerably in chiasma frequency although they exhibited normal pairing of chromosomes as 18 IIs. Most of the hybrids between wild species exhibited chromosome structural differences by the presence of univalents or inversion bridge

and fragment or a quadrivalent from translocation. A bridge and a fragment were common in the hybrids between *M. tristis* x *M. glaziovii*, *M. tristis* x *M. brachyandra*, *M. epruinosa* x *M. brachyandra*, and a quadrivalent was frequent in the hybrids between *M. epruinosa* x *M. brachyandra*. Laggards at A-I were observed in the hybrids between *M. catingae* x *M. tripartita*. The average pollen stainability of these various hybrids ranged from 90% in hybrids of cassava x *M. tristis* to 33% in hybrids of cassava with *M. brachyandra* and from 12% in hybrids between *M. epruinosa* x *M. leptophylla* to 90% in hybrids between *M. tristis* x *M. tripartita* and *M. brachyandra* x *M. epruinosa*. Interspecific hybrids of cassava with the species *M. glaziovii*, *M. epruinosa*, *M. leptophylla*, *M. brachyandra* as well as the hybrid between *M. tristis* x *M. leptophylla* produced a small percentage (0.5% to 6.5%) of 2n or unreduced gametes. Preliminary observations on the pachytene chromosomes of *M. brachyandra*, *M. catingae*, *M. epruinosa*, *M. glaziovii*, *M. tristis*, *M. esculenta* and *M. leptophylla* showed that they differ with respect to the number of chromosomes with telochromomeres and the number of chromosomes with total heterochromatic short arm. *M. glaziovii* and *M. esculenta* have seven chromosomes with telomeres; *M. tristis* and *M. catingae* have six with telomeres, *M. epruinosa* has five and *M. leptophylla* has eight. The pachytene chromosomes in the interspecific hybrids also showed total synapsis with occasional terminal or interstitial non-pairing or two univalents. Even when they paired completely, small structural differences in the length, number of chromomeres, size of telochromomeres, etc., of the homoeologous partners could be discerned when the chromosomes were observed at late pachytene stage. However, they have general homology and can be considered as related.

Bai, K.V., S.K. Hahn and R. Asiedu (unpubl.) Asynapsis in interspecific derivatives of cassava (Manihot esculenta, Crantz)

Most interspecific hybrids in the genus *Manihot* exhibit almost regular chromosome association during meiosis. Complete asynapsis at metaphase I and pachytene has been observed in some of the derivatives from open pollination of hybrids between cassava and wild *Manihot* species. One (plant 194) out of eight seedlings from hybridization of TMS 30555 and TMS 87/00018-28 was asynaptic. Three out of 16 seedlings from open pollination of plant 197 ("normal-pairing" sibling of plant 194) were cytologically confirmed as asynaptic. Observations of abnormal chromosome behavior at anaphase suggest the feasibility of using these plants as sources of aneuploids, at least, through the partial female fertility exhibited.

Activities

T.2.1. Agroecology-based population improvement

A. Dixon, Pathologist and Entomologist

Recurrent selection steadily accumulates desirable gene combinations over time. Polygene accumulation also brings about more durable resistances that are difficult for pests and diseases to overcome. Recurrent populations are being developed and improved for the humid forest, forest transition/moist savanna, dry savanna and mid-altitude agro-ecosystems. Segregating families from diverse crosses are at various stages of evaluation and recombination for each of the agroecologies. For the humid forest, selection emphasizes tolerance to high soil acidity, low soil phosphorus and low solar radiation as well as resistance to cassava green mite (CGM), African cassava mosaic virus (ACMV), cassava bacterial blight (CBB) and cassava anthracnose disease (CAD). For the forest transition/moist savanna, soil constraints are fewer but selection for resistance to CGM, ACMV, CBB and CAD is still important. In the mid-altitudes, initial vigor and rapid growth under cold air temperatures as well as resistance to CBB, ACMV and tip die-back take priority. For the dry savanna, types are sought which survive under severe water stress, show high initial vigor and growth rate, and good leaf retention (stay-green) under drought; and secondarily, resistance to pests and diseases. For all agro-ecosystems these desired traits are sought in a background of good agronomic and eating quality. Selected superior individuals or families from any cycle of selection are candidates for advanced testing and distribution to NARS.

A comprehensive breeding system for cassava is being developed for population improvement. The method for developing the source populations involves the identification of heterotic groups through morphological and physiological characterization as well as biochemical fingerprinting (e.g isozyme analysis) of cassava germplasm. A predictive model of progeny performance from parental characteristics is pursued and parental selection is based on diversity and complementarity. Source populations are random mated to break up deleterious linkages and selection is based on both selfed lines and half-sibs and recombination based on selfed derived lines. The population improvement scheme involves the following steps:

(1) Self parents and collect half-sib seeds from source population;
(2) Grow S1 and half-sib seeds together;
(3) Evaluate half-sib progenies for breeding value of parents;
(4) Evaluate S1 progenies individually for pests and diseases, agronomic and tuber characteristics. Selected S1 progenies before pollination can be selfed for each family by sib-mating;
(5) Recombine S1 or S2 seeds of parent for the next cycle;
(6) At any cycle, line or halfsib progenies that are outstanding can be cloned for early generation testing following the normal selection scheme for cassava;
(7) S2-derived lines in S3 can be crossed to a tester and testcrosses evaluated for combining ability while the S3 lines are selfed;
(8) Selected S4 derived lines in the S5, on the basis of combining ability, trials can be crossed to make single cross hybrids. These can be evaluated in performance trials in multilocations in the target agroecologies.

T.2.2 Selfing and outcrossing in cassava

A. Dixon and R. Asiedu

Cassava is a highly heterozygous plant. Sib mating or selfing for one to a few generations should unmask deleterious recessive genes, and careful recombination can exploit both additive gene action and heterosis. Several hundred parental lines and plants from a broad-based and backup populations in various generations of selfing were selfed and crossed in 1992. Seeds from these selfings are being collected and will be evaluated. In 1992, selfed progenies of 6 and 3 parental clones from previous years (1990 and 1991 respectively) selfings (S2 and S3) were evaluated, selfed and crossed to produce test hybrids. These test hybrids will be evaluated in 1993 for their degree of heterosis to justify pure hybrid production. Comparison of single cross hybrids (true seed vs its clones) will be also investigated. Genetic male sterility has also been shown to facilitate efficient hybridization, and enhances hybrid production in several crops including cassava. Sources of genetic male sterility were selfed as well as crossed to improved IITA clones and will be subsequently backcrossed to recover the adapted background of the IITA material together with a reasonable frequency of male sterility to facilitate hybridization.

T.2.3 Back-up source populations for specific characters

A. Dixon, M. Porto and R. Asiedu

Three satellite back-up populations were set up as feeders to the agroecologically oriented broad based populations and a fourth one as a source of generating new polyploids and increasing the genetic base of the polyploid population. A half-sib family selection scheme of 25 families is being employed in improving a back-up source population for higher levels of CGM/CM resistance. Selected individuals or families from each cycle of selection will be used as sources of new cultivars at any stage in the selection program or incorporated into elite populations as breeding parents. Cassava improvement at IITA is also benefiting from introgression of exotic germplasm into IITA populations. Promising clones with specific characters identified from evaluation of introduced germplasm such as from IITA-CIAT germplasm exchange and African land laces from NARS are hybridized with IITA improved clones and populations. Cyclic evaluation is conducted for overall agronomic characteristics, disease and pest resistance and quality characteristics in special populations. Several introductions from African countries(Benin, Ghana, Sierra Leone, Uganda, and Nigeria) in the form of true seeds as well as local poundable clones collected in Northern Nigeria were evaluated and introgressed in respective agroecologies. The introductions are systematically converted to improved germplasm (as in the Sorghum Conversion Program in the U.S.) while retaining the vital adaptability traits, food quality characteristics and consumer preferences of the respective countries of origin in special populations. This back-up source population will undergo evaluation and will be developed primarily for low cyanide and mealiness of the boiled tuberous root. Selected individuals or families from each cycle of selection will be used as sources of new cultivars at any stage in the selection program or incorporated into elite populations as breeding parents. The improved seed populations will also be available for distribution to the respective NARS for final selection under their specific local conditions.

The genetic base of the polyploid population is narrow because the original set from which the population was set up was restricted and had limitations. A population of 2n gamete producers is being improved by recurrent selection at Ibadan in a bid to produce more polyploids and to diversify the genetic base of the polyploid population. In the 1992 seedling evaluation of this population, some new polyploids have been identified and confirmed cytologically by TRIP's cytogeneticist.

T.2.4 Inheritance studies in cassava

A. Dixon, R. Asiedu and C. Akem

Inheritance studies for selected traits such as mealiness of tuberous roots, carotene content, inner skin of tuberous root, petiole color and resistance to anthracnose disease is being investigated in order to elucidate their genetic control. This information will aid the plant breeder in formulating an efficient breeding strategy and increase his efficiency in incorporating these traits to elite material. A Ph.D. student (C.Fokunang) commenced work in 1992 on the evaluation and inheritance of cassava to anthracnose disease. He has investigated 20 *Colletotrichum* isolates from four states of Nigeria for virulence on some cassava cultivars and is also making *in vivo* and *in vitro* evaluation of cassava clones with the most virulent *Colletotrichum gloeosporioides* f.sp *Manihot* for selection of resistant, intermediate and susceptible cassava clones to be used for hybridization and inheritance studies.

T.2.5 Interspecific hybridization in cassava

R. Asiedu, K. V. Bai, M. Bokanga and M. Fregene

Interspecific hybridization of African-adapted cassava clones with wild *Manihot* species introduced from Latin America introduces useful genes and helps elucidate phylogenetic relationships. Resistance to green mite and high protein content of tuberous roots have been identified in some hybrid combinations. Cross compatibilities were established and numerous backcross progenies were generated for further evaluation. DNA studies of phylogenetic relationships are continuing (Ph. D. thesis). Interspecific hybrids involving 22 pairwise combinations (including reciprocals) of nine wild *Manihot* species are undergoing evaluation in the field. Crosses between cassava and *M. tristis* have produced hybrids whose roots contain up to 8% crude protein (dry matter basis). Out of 557 hybrids analyzed, 61 (or 11%) had protein content exceeding 3%. Amino acid analysis indicates that the crude protein increase is due to an increase in protein amino acid. However, the chromatogram carries a very large non-identified peak appearing about three minutes before any other amino acid.

In order to produce amphidiploids in interspecific hybrids of cassava involving five *Manihot* species viz., *pohlii, epruinosa, catingae, tristis* and *leptophylla* were subjected to colchicine treatment. About 30 stem cuttings from each combination were planted and the sprouting axillary buds were treated with 0.5% aqueous colchicine solution for 24–30 hours using the cotton wool plug method. The treated plants which show the characteristics of amphidiploidy are being planted out in the field. So far treatment was not effective in hybrids of cassava with *M. leptophylla*. With other species about 1 to 4 treated plants show signs of amphiploidy.

T.2.6 Cytogenetics of *Manihot* species and hybrids

K.V. Bai and R. Asiedu

Karyomorphology of the pachytene chromosomes was observed and in all the species so far examined, the chromosomes were found to be of differentiated type, each bivalent consisting of a centromere with proximal heterochromatic and distal euchromatic regions and were matacentric, submetacentric or acrocentric. In all the species three bivalents were associated with the nucleolus. The morphological characters that were used to identify the chromosomes included: (i) chromomeric pattern, (ii) position of centromere, (iii) presence of deeply stained knobs, (iv) presence of telochromomeres, (v) relative lengths of hetero-and eu-chromatic regions in the arms, (vi) presence of accessory nucleolus and (vii) association with the nucleolus. As such at least 10 out of 18 bivalents could be easily distinguished for each species using these criteria. There will be further analyses of karyotypes in *Manihot* species and hybrids and their pairing configurations at pachytene and metaphase I during 1993.

T.2.7 Cytological screening of progenies from cassava polyploids and 2n gamete clones

K.V. Bai, A. Dixon, R. Asiedu and S.K. Hahn

About 2,940 progenies from the controlled as well as open pollination of the polyploids were screened for ploidy status based on morphological characters and the ploidy was later confirmed either through tetrad analysis, pollen size and stainability or chromosome counts. Three asynaptic clones were identified among the progeny which are second generation derivatives from the cross TMS 30555 X TMS 89/00018-28. TMS 89/00018-28 is a triploid. This will be continued in 1993 for establishment of genetic inheritance of asynapsis and its usefulness for production of aneuploids. The screening of the progenies from the polyploids through visual observations on plant characters indicated few plants may be aneuploids. However, as these plants were weak, the majority did not flower and hence their ploidy could not be confirmed. A few plants which flowered were cytologically screened and were confirmed as diploids. Hence no new aneuploids could be identified. These studies will be continued to identify the aneuploids, if any, among

the progeny. A population consisting of about 174 plants derived from crosses between 2n gamete producing clones was cytologically screened for 2n gamete frequencies. About 15.53% of the population produced 2n gametes ranging from 0.11% to 13.64%. Besides, two triploid (2n=3x=54) plants were obtained from this population.

T.2.8 Induction of polyploidy in cassava

K.V. Bai and S.K. Hahn

With the objective to develop polyploids of the elite clones for the improvement of cassava at the tetraploid and triploid level, clones TMS 30001, TMS 63397, TMS 91934, and TME-2 were subjected to 0.5% aqueous colchicine treatment for 24 to 36 hours using cotton-wool plug method. Tetraploids (2n=4x=72) were obtained from these and their ploidy status was confirmed cytologically. Even higher ploidy was sought to determine the optimum ploidy level tolerable by cassava by treating clones TMS 84/00316 (2n=4x=72) and TMS 89/0018-28 (2n=3x=54) with 0.5% aqueous colchicine solution to produce octoploids and hexaploids respectively. Only in one plant from TMS 84/00316 was colchicine treatment successful leading to an octoploid. This plant lacked vigor and leaves were distorted indicating octoploidy may not be tolerated for normal growth.

T.2.9 Tetraploid population improvement

S. K. Hahn, A. Dixon and K. V Bai

In order to improve the cassava population at tetraploid level, twelve sexual tetraploids, two spontaneous tetraploids and four artificially induced tetraploids derived from respective improved diploids for unilateral polyploidisation were crossed onto seven improved diploids. In addition, tetraploids were crossed among themselves for bilateral polyploidisation. A total of 40,000 crosses were made, resulting in approximately 2,000 hybrid seeds. These seeds will be germinated in a nursery and the seedlings will be transplanted out in the field for morphological and cytological examination and for observation of important agronomic characteristics. Over 1,000 seedlings were raised from the hybrid seeds of 1991 and were evaluated for ploidy and other agronomic characteristics.

The selected seedlings will be advanced to clonal evaluation, and promising clones will be further advanced to preliminary yield trials, advanced yield trials, and uniform yield trials.

T.2.10 Genotype X Environment Studies

A. Dixon, R. Asiedu and J.A. Whyte

The stability and adaptability of yield, yield components and harvest index of improved cassava evaluated in multilocational trials for two years in Nigeria were assessed. The objective was to identify the most efficient selection procedure to circumvent the inverse relationship between total tuber root weight and dry matter content. Preliminary results indicated that different yield components show different degrees of sensitivity to environments. Root number showed the least sensitivity and is highly correlated with yield and dry matter content suggesting its use as an indirect selection parameter. The final year trial planted in 1992 will be harvested in 1993 and final data will be taken for a thorough G x E analysis. Stability and adaptability studies of yield and its components of new improved cassava genotypes developed in various agroecologies is continually assessed to identify those with high yield and stable performance as well as those with narrow and wide adaptation. The characterization of genotypes as well as environments, will continue in collaboration with the Agroclimatology Unit of RCMD, PHMD and TRIP physiologist. A data base will be constituted from all past and future multilocational trials for a modelling approach to understanding the adaptation of cassava in different environments and the prediction of an ideotype cassava for specific environments.

T.2.11 Mechanisms of CGM resistance

A. Dixon, I. Ekanayake, M. Porto, V. B. Mbuyongha and N.E. Nukenine

Identification of different and new mechanisms of CGM resistance is sought to diversify resistance sources and pyramid different mechanisms to give higher levels of resistance in elite cassava populations. Selected clones from apparently field resistant clones will be retained for further screening and studies on their mechanisms of resistance in the screenhouse and laboratory in 1993. The interactions of drought stress and resistance to CGM are also being investigated through studies on plant water relations, stomatal behavior and other morphological and leaf tissue characteristics. The effectiveness of predators as a function of host plant resistance of cassava to green spider mite (CGM) is monitored, with a view to determining which level of resistance is appropriate for integrated control. Phytoseids are periodically released and the influence of phytoseid activity on CGM damage is investigated in terms of the mite

population and dynamics on clones with various levels of resistance. Investigations of the interactions (competition and succession) that may exist between CGM, CM and RSM as a function of host plant resistance both in the laboratory and in the field are being carried out. Some physical factors (leaf trichome size, orientation and density, cell density in different leaf layers, wall thickness, etc) and chemical factors (tannins, trypsin inhibitors, N, K, Mg, P, starch, sugars, lipids and amino acids) in relation to CGM varietal resistance in 11 cassava clones to CGM is being carried out. CGM, CM, and RSM population dynamics on all eleven clones including their respective damage scores, chemical analysis, leaf histology and trichome studies are monitored and will continue in 1993. Competition studies as well as the effect of trichome density and size in CGM developmental biology will commence in 1993 for one year. The study will elucidate resistant factors indispensible to accurate breeding for resistance to CGM as well as integrated pest management. Two Ph.D. students are involved with these activities.

T.2.12 Field evaluations of cassava plants regenerated through somatic embryogenesis

S.Y.C. Ng and O.J. Adeniyi

About 100 plantlets were regenerated via somatic embryogenesis from five cassava clones. These plantlets will be multiplied, established and planted out in the seedbed/field together with their mother plants. They will then be evaluated based on the morphological characters and biochemical markers to detect somaclonal variation.

T.2.13 Molecular markers for ACMV resistance in cassava

M. Bonierbale (CIAT), J. Thome (CIAT), M. Mignouna (IITA),
R. Asiedu (IITA), H. Rossel (IITA), S.Y. Ng (IITA) and M. Porto (CIAT-IITA)

A joint work involving IITA and CIAT will start in 1993 with the objective of linking molecular characterization with ACMV resistance. Seeds of progeny CM 7857, a cross between IITA's mosaic resistant clone TMS 30572, and CIAT's elite clone CM 2177-2 (resistant to whiteflies and mealybugs), obtained at CIAT, will be germinated *in vitro* at IITA in order to allow both institutions to correlate resistance screening to ACMV and molecular characterization. This will involve: 1) Establishment of 150 clones, *in vitro*, by culture of the embryos at IITA's Tissue Culture Laboratory. 2) Propagation and maintenance *in vitro* for use in: (a) exposure to ACMV under field conditions at Ibadan, for identification of individuals with different levels of resistance; (b) maintenance of duplicates in vitro and DNA characterization at IITA; (c) shipment of duplicates of susceptible and resistant individuals to CIAT, where DNA characterization and field testing will be made. Field data to be generated at IITA will be compared with the results of DNA characterization of the same clones at CIAT and IITA, hoping to identify polymorphisms linked to ACMV resistance.

T.3 PHYSIOLOGY OF ADAPTATION IN CASSAVA

Project rationale

A better understanding of the underlying mechanisms of physiological adaptation of cassava to a range of agro-ecosystems from the humid forest, forest transition, moist savanna, dry savanna, semi-arid and mid-altitude agroecologies aims to strengthen the selection and adoption process of the improved cassava clones. Physiological studies on cassava emphasize the adaptation of cassava clones to the different abiotic stresses experienced in each of these ecologies; in the humid forest zone - tolerance to low solar radiation, soil acidity and low soil phosphorus and from the transition to semi-arid zone - tolerance to drought varying from mild to severe stress, in the inland valleys - adaptation to early season wet/mid season drought/late season wet soil stresses and in mid-altitudes – tolerance to low temperature. In addition, tolerance to abiotic and biotic stress interactions are also addressed. Development and refinement of screening methods and evaluation of clones is done through multi-locational testing and with the use of traditional growth analyses. This work is complemented with controlled environment studies and analytical techniques and covers both technology generation and knowledge generation. Data sets will also support the validation of cassava growth models. Development of NARS capacity to conduct physiological research towards a goal of increased and sustainable production levels is expected.

Completed studies

Akparobi,S.O., Ekanayake,I.J. and M.O. Akoroda (unpubl.) Effect of different temperature regimes on the early growth of four improved cassava varieties. Senior author's MS thesis. Dept. Agronomy, Univ. Ibadan.
Studies on the effect of different day/night temperature regimes (10/6, 15/10, 25/15, and 35/25°C in Convirons and ambient 33/22°C) on the early growth of four IITA improved clones indicated high sensitivity of cassava growth to low temperatures. Growth retardation in terms of plant height and leaf formation and size were observed at low temperatures. The 10/6C regime was the most unsuitable. Other temperature combinations below 25/15C also resulted in poor sprouting of stem cuttings while the highest sprouting rate was noted at 35/25C. TMS 30572 gave the best performance at lower temperatures with reference to number of leaves and fibrous roots produced. Four IITA elite genotypes were relatively poorly adapted to low temperatures at the early growth phase.

Daraja,Y.B., I.J. Ekanayake and M.O. Akoroda. (unpubl.) Effects of mulching, water stress and quality of planting material on the establishment and early growth of cassava (*Manihot esculenta* Crantz). Senior author's M.Sc. thesis. Univ.of Ibadan
Field and greenhouse experiments were conducted at IITA Ibadan to investigate effects of water deficit, mulching and quality of planting materials on growth and establishment of elite clones (IITA improved clones adapted to moist savanna zone – called Mokwa selections, and those with 'broad adaptation') and local clones from Kano (Sudan savanna). Mulching positively influenced the growth of all clones with no mulch X clone interaction. During early establishment stage significant clonal differences were observed among clones. Combined effects of water deficit, dehydration of planting materials, and a high evaporative demand led to poor sprouting of all clones indicating the importance of quality of cuttings and management factors. Significant three way interactions also indicated the possibility of selecting clones with adaptation to water stress and dehydration of cuttings. Water stressed plants also had subsequent poor growth rates compared with the well watered treatment. Clone TMS 82/00062 had superior growth as regards to water deficit tolerance, plant height, leaf number, stem number and total biomass production.

Kapinga, R.E., J.A.I. Omueti and I.J. Ekanayake(unpubl.) Intercropping of cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) in the semiarid zone of Tanzania. PhD thesis of senior author, Agronomy Dept., Univ. Ibadan.
Two intercropping studies were conducted at Ukiriguru, Tanzania, during 1989/90 and 1990/91 seasons to identify suitable plant type and optimum plant density for the intercropping of cassava and sweet potato. Four cassava plant types-erect tall, semi-erect tall, spreading medium tall and spreading short were compared. Four cassava densities, 6,666; 10,000; 13,333 and 20,000 plants/ha were also compared after a basal fertilizer application of 60N-30K-30P kg/ha. Cassava and sweet potato in the mixtures competed strongly for mineral N,P,K,Ca and Mg especially in the fertilized plots. In general intercropping reduced the growth and yield of cassava and sweet potato. The response of cassava plant type was different to that of intercropped sweet potato. Semi-erect tall and spreading medium tall cassava types gave reasonable yields of both crops with LER of 1.53 and 1.44 for cassava and sweet potato. Cassava densities had significant effects on intercropped cassava and sweet potato. Cassava yields increased with an increase in density up to 20,000 plants/ha in the mixture, however sweet potato yield decreased with an increase in cassava density. Highest gross yield were obtained from the mixture with 20,000 plants/ha of cassava followed by 13,333 plants/ha. These gave total LER of 1.52 and 1.48 respectively. In both studies the addition of fertilizers increased cassava yield by 35% while sweet potato yield increased by 48%.

Simwambana,M.S.C.,I.J.Ekanayake and T.Ferguson (unpubl) Effects of environmental factors on flowering of four cassava clones (*Manihot esculenta* Crantz). Senior author's PhD thesis. Dept. Crop science. Univ. West Indies, St. Augustine, Trinidad.
Environmental factors which influence the flowering habit of two local clones TME1 and TME2 were compared with that of two IITA clones TMS 30555 and TMS 91934 at two sites, Ibadan (7.43'N3.90'E) and Ubiaja (6.39'N 6.20'E). The TME locals flowered at Ubiaja but did not flower at Ibadan. TMS 30555 behaved as a photoperiodically neutral clone which flowered profusely at any time depending on the soil moisture availability. However the sex of the flower varied during the season. TMS 91934 was a moderately flowering clone with a shortening day length requirement. Floral induction of TME's were regulated by several factors such as minimum night temperature, air humidity, solar radiation and soil moisture in addition to the photoperiod. Irrespective of the planting date, TME's flowered during the

second year after planting, as the days shortened from August onwards. At Ibadan the second year crop of TME2 produced first and second branching with no flower buds while TME1 had first branching. Tests of soil type at the two sites indicated that both Ibadan and Ubiaja soil types induced flowering in TME's under Ubiaja conditions suggesting that the difference in flowering behavior in the two sites could not be attributed to the soil alone. Other methods of flower induction such as girdling induced flower bud development in Ibadan for TME2 but was irregular. Studies on the critical minimum temperature to induce flowering showed a delay in flowering as the temperature decreased to 20C and 15C. 15C night temperature was lethal to most of the TME's. Studies also indicated that a high minimum relative humidity influenced profuse flowering in TME in Ubiaja. Induced high humidity conditions in Ibadan produced branching in TME's. A reduction of total solar radiation from 100% to 40% normal induced a decline in the flowering rate of TME's.

Osiru, D.S.O., O. Osonubi and S.K. Hahn (1992). Varietal response to drought stress in cassava (*Manihot esculenta* Crantz). In: Akoroda, M.O. and O.B. Arene. Proc. 4th Triennial Symposium of ISTRC-AB, Kinshasa, Zaire, 5-8 Dec. 1989.

Two sets of experiments are described in which IITA's improved cassava varieties were evaluated for their performance under drought stress. In one set, three improved varieties (TMS 30572, TMS 91934 and TMS30555) and a local cultivar, Odongbo, were grown in pots under four watering regimes of early stress (ES); late stress (LS); stress throughout (ST) and no stress (NS). ST and ES significantly reduced the number of tubers per plant, tuber size and total fresh tuber yield in all varieties bur LS had almost no effect on these components. The effect of stress was greatest on TMS 30555 and Odongbo. Both varieties had lower Drought Response Index under increasing stress suggesting less tolerance to drought. TMS 30572 and TMS 91934 accumulated more dry matter in the feeder roots, and had higher LAI even under stress. In the second set of experiments, 10 varieties were evaluated under field situations for stomatal conductance, transpiration rates and xylem pressure potential in dry and wet seasons. Diurnal stomatal conductance and transpiration rates had a similar pattern during both seasons; with peaks at about midday for all varieties. In the dry season, diurnal stomatal conductance and transpiration rates of TMS 30572 was 50% less than those of TMS 91934. But in the wet season, TMS 30572 showed 160% higher diurnal stomatal conductance and transpiration rates than TMS 91934. Xylem pressure potential had no clear pattern. We suggest that the extent of feeder root dry matter accumulation and the ability to maintain higher LAI under drought stress are important criteria when selecting varieties for drought tolerance.

Activities

T.3.1 Screening for drought tolerance

I.J. Ekanayake,M. Porto and M. Bokanga

The savanna has potential for expansion of cassava cultivation, but existing germplasm was developed for the forest environment where drought risk is less. Cassava is a relatively drought tolerant species, but prolonged drought will reduce yields. Field trials during the dry season at Ibadan and at the Minjibir Station, Kano and Zaria (dry savanna) were initiated in 1992 to study the effect of drought at different growth stages on development and tuberous yield. Response to drought of wild *Manihot* species, cultivars and breeding lines are being compared. Detailed physiological studies are conducted on some accessions to elucidate the mechanisms of tolerance to drought in association with their growth pattern and stomatal behavior (continuing studies); further analysis of photosynthetic potential is also envisaged. Diploids, triploids and tetraploids were compared for their water relation traits based on diffusive resistance and transpiration, where significant differences were noted in different environments (Ibadan, Mokwa and Zaria) and among pubescent and non-pubescent variants. Preliminary data showed that significant differences exist among and within the different wild *Manihot* spp. for diffusive resistance and transpiration rates. Elite clones were analyzed for their chlorophyll content, stomatal density and stomatal function to determine their potential tolerance to drought. Drought screening in Kano and Zaria are based on growth and development and water use patterns.

The growth analysis experiment will be repeated this year. A field screening of poundable clones grown without supplemental irrigation will be done at Minjibir. In addition, with the collaboration of the biochemist, a survey of local clones grown by farmers in the dry savanna will be done to investigate their quality traits such as cyanogenic potential (CNP), bitter compounds, poundability and preferences. The same clones will be grown in Mingibir and Ibadan to retest their performance.

T.3.2. Adaptation to water deficits

M. Porto and I.J. Ekanayake

Complementary studies in the area of drought stress physiology of cassava are being carried out at Minjibir station in order to assess the feasibility of using differences in the fibrous root system of cassava as a selection criterion for drought tolerance. Preliminary studies initiated in 1990 and conducted in Ibadan showed large differences among the 10 genotypes tested. The experiment now underway at Minjibir will also provide information on the effect of the depth of the water table on growth and yield. This will enable breeders to select adequate evaluation sites for screening for drought resistance as a function of the availability of water in the soil profile. A similar study is under way where rooting depth is assessed based on water extraction patterns and specific fibrous rooting patterns.

T.3.3 Screening for cold tolerance

I.J. Ekanayake, M. Porto and M. Bokanga

The mid-altitudes could grow more cassava, but low temperatures lead to slow growth, slower tuber bulking and delayed maturity. There is a dearth of knowledge on cold tolerance in cassava. The temperature regime of the mid- altitudes are simulated in controlled environments (using the Convirons at IITA), complemented by field studies at a mid altitude site (Vom, near Jos). Adaptation, growth habits and dry matter partitioning are compared in 12 clones at Vom (clay soil with <.09%TN, <2% P and pH 5.3) by making detailed growth analyses. Clonal differences were noted for sprouting ability in Vom where most of the elite clones bred for adaptation to moist savanna and transition had better sprouting than the local with TMS 30572 having the highest sprouting. Growth rates and water relations parameters are evaluated at different growth phases and seasons. All test clones show retarded growth due to low temperatures compared with the location for which they have optimal adaption. The influence of different day/night temperature combinations (4 regimes with temperatures varying from 10 to 35C) were also tested on the early growth and survival of a series of cassava clones in Convirons. A visual score was refined to monitor the leaf survival. Clonal differences were significant. A 10C regimen was apparently lethal for growth after the third week. Cold tolerance studies this year will emphasise the tissue level damage and membrane integrity, and photosynthetic potential under cold temperature regimes and will encompass a PhD thesis.

T.3.4. Screening for tolerance to low light intensity

I.J. Ekanayake and A. Dixon

Cassava in the humid forest agro-ecological zone is grown under low light levels due to cloudy and overcast skies and particularly during its early life cycle as an intercrop component. Photosynthetic efficiency and light use efficiency of cassava elite clones will be tested under differentially shaded conditions during its early ontogeny. In addition to the photosynthetic rates, photosynthetic capacity will be computed using growth analysis in Onne and Ibadan. Efficacy of these different parameters will be compared to select desirable screening tools. Pot tests will be done to examine the photosynthetic acclimatisation of cassava when transferred from low light to high light conditions and vice versa. A PhD student at UI will examine the growth, water relations and photosynthetic rates of cassava intercropped with maize in alley cropping systems where available light levels differ.

T.3.5. Flowering in cassava

I.J. Ekanayake, M. Simwambana, S. Jagtap and T. Ferguson.

Shy flowering habit is a deterrent to hybridization in some of the local cassava clones and those introduced from Latin America. A major effort was made last year to understand the climatic, edaphic and physiological factors which cause flowering to occur at Ubiaja but not at Ibadan for the local genotypes TME1 and TME2. Although data generated (refer to completed studies - Simwambana et al) help us to obtain a better understanding of the control mechanisms of flower induction, our efforts to date to induce flowering in these clones in Ibadan through environmental manipulatory actions have achieved only first and second order branching and perhaps aborted flower formation (the after needs confirmation). Effect of photoperiod X temperature interactions on the induction of flowering was observed at northern and southern latitudes. TME's in Vom (cool site) did not branch or flower although have been observed to flower in 1989 with irrigation (Osiru, per. comm.). TME1 was observed to have irregular branching and aborted flower formation by November in Zaria. In Onne (4.85N) TME1 had first level branching. Multilocational flowering observations will continue. Field data generated on flowering are being used to validate and improve a growth model(with Agroclimatology Unit) as determined by climatic factors which can then be used to predict flowering dates for different geographical locations. A series of studies will be conducted to

induce flowering through stresses such as girdling, grafting and with exogenous hormone applications (early observations by Osiru et al showed negative results). In addition, in collaboration with the biotechnology unit, flowering regulation by a critical low night temperature and photoperiod regimes will be studied.

T.4 FOOD QUALITY OF ROOT AND TUBER CROPS

Project Rationale

Many of IITA's elite cassava clones have yields far exceeding the yield of local varieties. The greatest challenge for the adoption of the improved clones is the performance of their leaves and tuberous roots in the postharvest system. To select for a plant type which would be readily acceptable by farmers and consumers, breeders need to know the root and leaf characteristics associated with postharvest handling and processing. Issues of cyanide in cassava are of paramount importance for NARS. An understanding of mechanisms of detoxification during the various modes of processing is necessary for making the right choice when introducing new cassava varieties in a location with specific modes of cassava processing. It is also necessary to clarify the relationship, if any, between the cyanogenic potential of cassava roots and their taste because the experimental data available from our past research and the information found in the literature do not support a direct link.

Completed studies

Tiamiyu, K.K., O. Tewe and M. Bokanga (unpubl. MSc. thesis). Metabolizable energy and protein quality of cassava leaf meal

Rat feeding trials were conducted to determine the metabolizable energy and protein quality of cassava leaf meal dried without and after pounding. The control diet contained casein as the nitrogen source. Treatments consisted of substituting casein with either one of the two cassava leaf meal (pounded or non-pounded). The data indicate that there was no statistically significant difference in the biological values of cassava leaf protein and casein. There was no statistically significant difference in their metabolizable energy . The feed intake was significantly less on the pounded cassava leaf meal than on the other diets, and the weight gain was slightly lower. The data indicate that cassava leaf meal can be used as a nitrogen source in animal nutrition.

Moibi, J.A., O. Tewe and M. Bokanga (unpubl. MSc. thesis). Influence of varying levels of acetone cyanohydrin on the performance and biochemical indices of albino rats

Two fourteen day trials were conducted to evaluate the effect of 0, 1000, 2000, and 3000 ppm levels of dietary acetone cyanohydrin on the performance and biochemical indices of the rodents. In the two trials the presence of cyanohydrin in the diets did not cause a statistically significant ($p > 0.05$) reduction in both feed consumption and growth rate. The change in feed efficiency and protein efficiency ratios were also not statistically significant ($p < 0.05$). Dietary cyanohydrin significantly ($p > 0.05$) affected the serum thiocyanate concentrations. Urinary thiocyanate increased proportionately with dietary cyanohydrin levels. Serum urea was also significantly influenced ($p > 0.05$) by the dietary treatment in both trials, while serum total proteins, serum albumin, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) were similar in all groups Results showed that the toxic effect of acetone cyanohydrins was restricted to its influence on thiocyanate concentrations and that the performance of rats was not affected ($p < 0.05$).

Eggleston, G., P.E. Omoaka and D.O. Ihedioha, 1992. Development and evaluation of products from cassava flour as new alternatives to wheaten breads. *J. Sci. Food Agric.* 59: 377-385.

Egg white with margarine, and xanthan gum, have been used to produce alternative breads from cassava flour fortified with 20% soy flour. All the additives increased the amount of air entrapped in the cassava batters at the mixing stage, as indicated by their lower batter densities. The lower the batter density the higher was the maximum gas retention volume attained in the 60 min fermentation stage. However, final loaf volume also depends on the stability of the batter; egg white primarily acts as a stabilizer. Margarine, and more significantly egg white, reduced the extent of starch gelatinisation and solubilisation in the bread. Breads made with locally available egg white and margarine are acceptable to Nigerian consumers and have very good keeping qualities.

Eggleston, G., P.E. Omoaka, and A.U. Arowshegbe. Flour, Starch and Breadmaking Quality of Various Cassava Clones. Part I - Composite Breadmaking. Submitted for publication in the *Journal of the Science of Food and Agriculture.*

Bread was made using a straight dough baking process from a local soft wheat flour substituted, at 10, 20, 30 and 40% levels, with flour from nine different cassava (*Manihot esculenta*, Crantz) clones. The cassava flours' physico-chemical properties, including starch quality, were measured and related to dough rheology, bread volume and crumb characteristics. Breadmaking quality at 10 and 20% substitution levels was reliably predicted only from the cassava flour diastatic activity. Flours with diastatic activities, i.e. above ~ 145 mg of maltose, had deleterious breadmaking effects. Baking absorption effects were more critical at the 30 and 40% levels. Cassava flour diastatic activity was highly dependent on the moisture contents of their respective tuberous roots and affected the extent of starch gelatinisation in the bread crumbs.

Eggleston, G., P.E. Omoaka, and A.U. Arowshegbe. Flour, Starch and Breadmaking Quality of Various Cassava Clones. Part II - Alternative (Wheatless) Breadmaking. Paper submitted for publication in the *Journal of the Science of Food and Agriculture.*

Alternative (wheatless) cassava breads were made with cassava flour from eight different cassava (*Manihot esculenta*, Crantz) clones fortified with 20% soy flour, using margarine and egg white, or xanthan, as additives. Bread specific volumes were reliably predicted only from the diastatic activity and maximum paste viscosity of the cassava flour. Flours with diastatic activities above 145 mg of maltose, and indirectly low maximum paste viscosities, produced dense, pudding-like structures with completely gelatinised crumbs. The flour diastatic activity was dependent on the moisture content of the freshly harvested tuber; above a critical threshold moisture (around 75%) content the specific air intakes of the loaves decreased sharply. Extent of starch gelatinisation and solubilisation did not critically affect loaf volumes but affected crumb structures.

Eggleston, G. and R. Asiedu (in press). Effects of boiling on the texture of cassava clones: A comparison of compressive strength, intercellular adhesion and physio-chemical composition of the tuberous roots. Tropical Science.

A highly significant relationship was found between the compressive strength of 8 boiled cassava (*Manihot esculenta* Crantz) tuberous roots and their intercellular adhesion, as measured by a retained dry weight method, suggesting that compressive strength is a measure of cell separation. In general, compressive strength is related to amylopectin content, intercellular adhesion strength and moisture levels. The addition of calcium ions to the boiling water progressively increased compressive strength, with a concomitant reduction in dry weight loss and water absorption, until a saturation point was reached. For 7 improved clones, moisture level was inversely related to compressive strength. One local Nigerian cassava variety, Isunikankiyan, demonstrated markedly different physico-chemical characteristics to the improved clones: it had the lowest compressive strength with a relatively low moisture level, and its endogenous calcium content was 10 fold higher and less tenaciously held. It is suggested that other compositional and/or structural differences are involved in determining the final texture of boiled Isunikankiyan. These findings indicate that cooking (boiling) behavior of cassava depends on several rather than one single parameter and may be regarded as the result of several complex processes.

T.4.1 Testing of a simple method for linamarase preparation

M. Bokanga, NARS scientists

The bottleneck for adoption of the methodology for determination of the cyanogenic potential of cassava is the availability of the enzyme linamarase. The commercially available enzyme is expensive. Existing methods for preparing the enzyme require a refrigerated centrifuge which is usually not available for NARS. A simpler method for the preparation of the enzyme without using a centrifuge has been developed and will be tested by NARS scientists in Benin, Sierra Leone, Uganda, Tanzania, and Malawi.

T.4.2 Cassava processing and detoxification

M. Bokanga, S. Essers (WAU, Netherlands), N. Mlingi (TNFC, Tanzania) and H. Rosling (Uppsala U. Sweden)

It has already been established that the endogenous enzyme linamarase plays an important role in the detoxification of cassava during processing. It is becoming increasingly evident that acetone cyanohydrin, the hydrolytic product of linamarin, is the compound responsible for food intoxication attributed to cyanide in cassava. The non-enzymatic decomposition of cyanohydrins, the activity of hydroxynitrile lyase (the

enzyme which decomposes cyanohydrins) and the effect of processing conditions on these processes will be investigated. To probe the contention that insufficiently processed cassava may be at the origin of many reported endemic cases of intoxication, the effect of the degree of processing on the removal of cyanogens in cassava roots will be investigated.

T.4.3. Nutrient supply and cyanogenic potential

J.H.Bradbury (Australian National Univ.) and M. Bokanga

In addition to the current work on cyanogenic potential under water stress, controlled experiments are being conducted in the greenhouse using pots containing fixed amounts of nutrients to assess the effect of the nutrients on the cyanogenic potential.

T.4.4 Bitter compounds in cassava roots

M. Bokanga, and J.H. Bradbury (ANU, Australia)

It is often assumed that varieties with high cyanogenic potential taste bitter, and they are referred to as "bitter varieties". However, there are varieties that have a high cyanogenic potential without the bitter taste. The bitter compounds of cassava have been extracted and concentrated. Their chemical structure will be determined using the techniques of mass spectrometry and NMR spectroscopy.

T.4.5 Assessment of the food quality of root and tuber crops

M. Bokanga, F. Nweke, O. Tewe (U.I.), A. Larbi (ILCA, Ibadan) and N. Poulter (NRI)

Samples collected as a part of COSCA Phase III will be shipped to IITA for analysis of their food quality. This study will make it possible for the first time to compare cassava foods from various parts of Africa. Based on the information available from the COSCA study and from other sources of literature, a protocol for evaluating the food quality characteristics of fresh cassava roots and leaves associated with postharvest handling and processing will be carried out. The evaluation will include morphological (size, shape, color), physical (texture) and biochemical parameters (nutrient content, cyanogenic potential, shelf life) of cassava roots and leaves. Both human and animal nutritional aspects will be investigated where roots and leaves are an important food source.

Yam elite breeding lines will be assessed for their food quality characteristics. Tests for food quality traits will consist of texture evaluation after boiling, starch quality, protein content, polyphenols content and enzymatic browning.

T.4.6 Assessment of cassava flour quality for bread making

M. Bokanga, and J.A. Delcour (KUL, Belgium)

Previous research has indicated that cassava flour diastatic activity and maximum paste viscosity are good indicators of the flour's bread making potential. A high performance methodology for the determination of maximum paste viscosity has been set up. The methodology for diastatic activity remain to be improved. Various handling and processing conditions will be evaluated as to their effect on diastatic activity and maximum paste viscosity in order to derive the optimum conditions for producing flour with maximum bread making potential.

T.4.7 Food systems analysis of the competing use of wheat and cassava products in Africa

Economist, M. Bokanga

The consumption of bread and other wheat products in non-wheat-producing countries is increasing, causing a drain on their budgets. Several years ago, Nigeria banned the importation of wheat into the country thereby providing potential wheat substitutes with great opportunities to replace wheat. The ban on wheat imports has now been lifted. Does cassava products, particularly cassava flour, cassava starch and the newly developed cassava bread have a chance to be competitive on the market place? From a technical point of view, these products could easily be utilized in many industrial processes. In particular, cassava starch has excellent characteristics compared to starches from other sources. This study which is part of the special project on the utilization of cassava bread for baking bread will look at the constraints in the utilization of cassava products, particularly cassava flour and starch, and at the prospects of the utilization of cassava as a substitute for wheat.

T.4.8 Postharvest biodeterioration of cassava roots

M. Bokanga, and I.J. Ekanayake

Cassava roots are known to undergo a natural discoloration (possibly within 24 hours) once they are cut from the plant. There seems to be some variability in the susceptibility to this biodeterioration. A method will

be developed to assess the rate of biodeterioration of cassava roots and to identify those varieties that are less prone to deterioration. Biochemical differences between highly susceptible and more resistant genotypes will be established and used to develop biochemical screening tests.

T.4.9 International workshop on the safety of cassava as food and feed

M. Bokanga, Coordinator

Two international workshops convened by IDRC in 1973 and in 1982 have focused on the goitrogenic effect of the cyanogens in cassava. Since then, new attention on cassava-related food intoxicants has been aroused by the occurrence of several outbreaks of a paralytic disease named "konzo" in several parts of Africa, and numerous reports in the Nigerian press of alleged intoxications with sometimes fatal incidences from cassava consumption. There is increasing evidence that konzo may be due to cyanogen intake from poorly processed cassava roots. On the other hand, a workshop convened in Nigeria in 1989 failed to find sufficient evidence that the reported intoxications were actually due to cassava consumption. The controversy continues unabated. Since the 1973 and 1982 workshops cassava has gained importance. Production has been steadily increasing despite increasing reports on negative health effects. Research on cassava has received increased attention so much that a Cassava Biotechnology Network has been created. Research on cyanogenesis is high on the priority list of the Network although there is no consensus on what direction this research should take. A large amount of information on the existing modes of cassava processing has been generated and a modest experience in the dissemination of improved cassava processing technology has been built up. It is time now to assess the state of the knowledge that has been accumulated, to determine what we really know about cassava utilization and what needs to be done to avoid toxicity problems, even in those areas where cassava is just being adopted. It is time to take stock of the evidence in health implications of cassava cyanogens, not only the physiological and epidemiological data, but also the magnitude of the problem and the social context in which this occurs. It is time to assess our understanding of the cyanogenic potential of cassava, the agroecological factors that influence it, and the impact of the various processing methods on the safety of cassava food products. The workshop will aim to channel information between researchers, development workers, policy makers and aid organizations. It will aim to establish appropriate procedures for assessing the cyanogenic potential of cassava and cassava products and for reporting cassava-related food intoxications, and to set criteria to be used in the safety evaluation of cassava and cassava products.

T.5 YAM IMPROVEMENT

Project rationale

IITA's mainstream yam improvement activity is modest, in accordance with the Strategic Plan of 1989-2000. The effort focuses on conserving and characterizing germplasm, identifying useful new clones and distributing these for testing to national programs. Genetic variability in yam has been little-utilized because making crosses is difficult. Firstly, flowering is erratic and asynchronous for most species, and this seems to be strongly influenced by the environment. A second reason is that yam ploidy is complex and little understood. Breeders need to know more about floral biology, chromosome numbers, and understand genomic compatibility among yam accessions in order to plan and implement successful hybridization schemes. Focused, strategic studies to break some of the barriers in yam hybridization are being addressed.

Completed Studies

Ng, S.Y.C.(unpubl.) Production of yam (*D.rotundata*) minitubers

A system for production of yam virus-free minitubers from virus-free plantlets was established using five yam clones. About 2,000 yam minitubers can be produced in an area of 72 m^2 in a screenhouse with sterile soil. Average number of tubers produced per plant ranged from 1 to 2 and average tuber weight per tuber ranged from 26g to 62g depending on the genotypes.

Activities

T.5.1. Cytogenetics of *Dioscorea* species and hybrids

K.V. Bai and R. Asiedu

In 1992 meiotic chromosome pairing was observed in genotypes of *D. rotundata, D. bulbifera* and *D. alata.* In *D. rotundata* the chromosomes paired as bivalents and 20 IIs were observed at diakinesis and M-I. Pollen stainability for 124 genotypes ranged from 1.27% to 99%. In *D. alata*, the chromosomes paired as bivalents and 20 IIs were observed at diakinesis and M-I. The pollen stainability for 9 genotypes ranged from 5% to 82%. In *D. bulbifera* meiosis was observed in two of the seven male clones that flowered. The chromosomes paired as trivalents or trivalents + bivalents + univalents. In few PMCs of genotype 92/1205, the entire complement of chromosomes paired as trivalents (20IIIs). In genotype 92/1187, a maximum of 12 IIIs were observed giving associations such as 12.III + 10 II + 4.. The high trivalent frequency indicates that these clones are triploids. Meiosis in these clones is highly irregular resulting in high pollen sterility (from 0% to 5.8% pollen stainability). To study pollen fertility, apart from stainability tests, pollen grains were germinated on various concentrations of sucrose or sucrose + boric acid. Pollen from genotypes of *D. alata, D. rotundata* and *D. dumetorum* recorded 18 to 35% germination in sucrose solutions. In *D. rotundata* pollen tubes as long as 120 µ could be observed in about 8 hours when germinated on 5% sucrose solution. In the other two species pollen tubes length ranged from 50-90 µ over a similar period. Chromosome numbers were also determined for several genotypes of *D. rotundata, D. alata* and *D. bulbifera, D. rotundata* and *D. alata* had 2x = 40 whereas *D. bulbifera* showed 2x = 60 chromosomes. The foregoing activities will be continued in 1993 to establish more basic information for guiding the choice of parental genotypes and species for yam breeding.

T.5.2 Optimum pollination period in yam species

K.V. Bai and R. Asiedu

Time of anthesis in male and female flowers, anther dehiscence in male flowers, stigma receptivity in female flowers and the duration of pollen viability/stigma receptivity are important factors in hybridization of yams. Observations were made in 1992 on the time of anthesis and anther dehiscence for five species of yams viz: *D. bulbifera, D. dumetorum, D. cayenensis, D. rotundata.* and *D. alata* planted at Ibadan. In *D. bulbifera* the anthesis time was about 08.00h am and anther dehiscence around 09.00h; in *D. dumetorum* anthesis is about 11.00h and anther dehiscence about 11.30h; in *D. cayenensis* anthesis is around 10.30 and anther dehiscence about 11.15h; in *D. rotundata* anthesis was around 10.30h and pollen liberation around 11.50h and in *D. alata* anthesis was about 13.40h and pollen liberation about 14.00h. The pollen grains are whitish in *D. bulbifera* and *D. cayenensis*, yellowish in *D. rotundata* and *D. dumetorum* and deep yellow in *D. alata.* The pollen grains were sticky and therefore cling to each other. Two species of thrips were found inside the flowers during anthesis which are the probable pollinating agents. A mild fragrance is emitted by *D. rotundata* and *D. cayenensis* during anther dehiscence and while the pollen remained viable; the fragrance is stronger in *D. alata* and lasted for about 3-4 hours. It may be taken as indication of pollen viability and stigma receptivity. These observations will be valuable in 1993 for setting up intra- and interspecific hybridization schemes which will confirm optimum pollination times and cross-compatibilities.

T.5.3 Gender expression in *D. rotundata*

V. Chikaleke, R. Asiedu and I. Ekanayake

Most accessions of *Dioscorea* species are male, female or non-flowering. A few genotypes of *D. rotundata* have a tendency to be monoecious in some years and unisexual in others. Selections from the 1992 crossing block will be re-evaluated in 1993 to check consistency of gender and to investigate probable influences on gender expression in selected genotypes of factors such as sett size, tuber portion, planting date and fertilizer application. This will also offer the opportunity to re-assess the reported relationship between gender and tuber yield.

T.5.4 Yam germplasm characterization

N.Q. Ng, R. Asiedu, I. Ekanayake, A. Dixon, M. Bokanga and NRCRI

Our yam collection includes many accessions from several species. Distinct criteria for identification are needed. A total of 1000 accessions (including 64 from NRCRI, Nigeria) were assessed for their agrobotanical, morphological, biochemical, physiological and food quality characteristics in 1992 through a tri-locational activity (Ibadan, Abuja, Uturu) jointly run between TRIP, GRU and NRCRI. Selections from this preliminary investigation will be subjected to further evaluation in 1993. The remaining accessions in

the germplasm collection will be characterized this year. Time to physiological maturity of *D. rotundata* and *D. alata* will be investigated for different maturity classes of elite clones. Influence of yam tuber age on dormancy, shelf life and time to flowering in association with physiological maturity and dormancy will also be studied.

T.5.5 Yam germplasm evaluation

R. Asiedu, C. Akem, G.Orkwor (NRCRI), Q. Ng and D. Florini

Selections from 1991 seedling nursery and intermediate yield trials of *D. rotundata* were bulked and 468, 976 and 240 genotypes, respectively were evaluated at Umudike (forest zone), Ibadan (transition zone) and Abuja (moist savanna zone) in single row plots. Data was collected on flowering, tuber characteristics, reactions to diseases (foliar necrosis, viruses) and pests (leaf and tuber beetles, nematodes). Selections at each location will be set up in 1993 as a preliminary yield trial at that location. Multilocational uniform yield trials of *D. alata* (16 genotypes) and *D. rotundata* (35 genotypes) conducted at the three locations will be repeated this year. Collaborative evaluation of introductions and local genotypes of yam will be conducted with NARS colleagues in Guinea-Conakry, Ghana, Cameroon, Equatorial Guinea, Rwanda, Uganda, Zambia and Malawi.

These countries are among the national programs in Africa that have received *D. rotundata* genotypes from IITA in the past. A few have made collections of their local yam germplasm as well. Additional introduction will be made this year into these countries. Ghana and Cameroon will be assisted to complete their local collections. All available introductions will be evaluated together with the leading local varieties.

Performance of yams are affected by a host of insects (e.g mealybugs and beetles, *Heteroligus* spp.), nematodes (e. g *Scutellonema bradys, Meloiodogyne* spp. *Pratylenchus brachyurus*), leaf spots (e.g. *Cercospora* spp.), viruses and tuber rots (e. g *Botryodiplodia theobromae, Fusarium* spp. and *Aspergillus niger*). Damage by some of these agents can be erratic in breeding plots owing to poor distribution of causal organisms and complex synergistic interactions. More efficient screening methodologies will be developed for evaluation of germplasm for reaction to causal agents of some of the major pest problems, foliar necrosis, nematodes, and tuber rots. This will involve student thesis projects under supervision of colleagues in PHMD and University of Ibadan.

T.5.6 Yam germplasm distribution

S.Y.C. Ng, R. Asiedu, G. Thottappilly and N.Q. Ng

Eighteen packages of yam virus-free plantlets were distributed to NARS in 12 countries in Africa and Dominican Republic and a collaborator in Austria. Over 2,000 yam minituber were produced in screenhouse and were distributed to NARS in nine African countries. Another four selected yam clones were certified by PQS, Nigeria. An additional eleven selected clones were regenerated from meristem culture. These will be transplanted for virus indexing, certification and eventually distribution. More selected breeding lines and leading Nigerian farmers' varieties will be subjected to virus clean up. The newly certified clones will be rapidly multiplied both for distribution to NARS as well as for minituber production in the screenhouse.

T.5.7 Effect of plant age on yam meristem culture

S.Y.C. Ng

In 1992, a study on the effects of plant age on yam meristem culture was carried out using two selected clones of *D. rotundata* and two of *D. alata*. Preliminary results indicate that meristems obtained from two month old plants gave better response, followed by three month old plants. This study is still in progress and will be repeated in 1993.

T.5.8 Yam in vitro microtuberization and mass propagation from node cuttings

S.Y.C. Ng and S.H. Mantell (Wye College, UK)

Previous studies in *D. rotundata* indicated that sucrose at 3 to 5% in the presence of kinetin induced microtuber formation. Some of these microtubers sprouted after few months of storage. The germinated microtubers were successfully established in seedbeds and grown to maturity. Nevertheless, the system for the production of microtubers needs further study in order to increase the production rate as well as the germination rate. In this study, the effects of different growth regulators (singly or in combinations) on *in vitro* tuberization and germination of microtubers of three yam species, *D. rotundata, D. alata* and *D. cayenensis* will be carried out.

A series of experiments will be carried out to determine the best medium for rapid propagation of *D. rotundata, D. alata* and *D. cayenensis*, using node cuttings. Factors under investigation are different media formulations, combinations of cytokinins and auxins, and carbon source.

T.5.9 Field performance of virus-free yam

S.Y.C. Ng, H.W. Rossel and I. Ekanayake

Preliminary results obtained in 1992 indicated poor survival rate of tissue culture plantlets in the field due to sensitivity to water stress. Minisetts from virus-free minitubers showed less rotting than the field tubers and had higher establishment rates. There was no significant difference in terms of tuber weight per plant and tuber weight per plot between minisetts from field tubers and minitubers though data showed that minitubers has higher tuber weight per plot. Tuber weight per tuber, number of plants harvested and tuber weight per plot were significantly lower in tissue culture plantlets. This study will be continued in 1993 including microtubers.

T.6 COLLABORATION WITH NARS AND TRAINING

Project rationale

Collaborative research schemes help identify regional research priorities, catalyze information exchange, preclude duplication of research efforts, and increase the transfers of technologies among partners. Making use of the comparative advantage of NARS to conduct certain types of research benefits both NARS and IITA. Feedback on IITA technology from NARS is invaluable in the continuous refinement of our own research strategies. Training strengthens the research capacity of NARS.

Activities

T.6.1 International testing of cassava

A. Dixon, R. Asiedu, S.Y. C. Ng, J.B.A. Whyte and NARS (West and Central Africa)

Improved cassava clones developed for various target agro-ecosystems are distributed to NARS as *in vitro* plantlets. Performance is evaluated in multilocational trials in collaboration with NARS. The performance of ten such clones was evaluated in 1989/90 and 1991/92 growing season. Results for two years of testing indicate superior performance of TMS 30572 and 4(2)1425 in Ghana, Togo, Benin and Nigeria and TMS 50395 and TMS 91934 in Ghana and Nigeria. In Nigeria the varieties responded differently to varying levels of soil fertility. TMS 4(2)1425 gave a yield improvement of over 60% on application of 60kgN 30kgP and 90kgK while TMS 30572, TMS 50395 and NR/41044 responded far less (21%, 15%, and 18% respectively). These last three gave economic yields on low fertility soils and are amenable to low input systems. These identified varieties in the various countries are undergoing on-farm testing and are undergoing multiplication and distribution to farmers under the EEC/IITA-OFAR project. The Ghana National Root Crops Program has recommended these clones (TMS 30572, 4(2)1425, 91934 and 50395 as well as 30001) to the National Committee for Seed Release. Another set of 19 clones established and multiplied in these NARS have completed their first year of testing in 1992. These clones were also established, multiplied in Sierra Leone and Guinea Conakry for multilocational testing in 1992. In 1992, a third set of 16 low cyanide, poundable clones (mealy) were established and multiplied in Benin, Togo, Ghana, Sierra Leone and Guinea Conakry, Gambia, Cameroon and Guinea Bisau for international testing in 1993. In 1992, improved seed populations comprising 320 seeds representing 482 families segregating for CGM, CMV, CBB, high yield and dry matter and cooking quality (culinary quality, yellow roots, etc) were distributed to 16 countries in Africa (Malawi, Angola, Republic of Benin, Sierra Leone, Guinea, Uganda, Gambia, Zaire, Niger, Lesotho, Mali, Cameroon, Mozambique, Sao Tome and Cote d' Ivoire) for selection under their local conditions. In Nigeria, in 1992 269 elite IITA cassava breeding lines (95 adapted to Forest/savanna transition zone; 130 adapted to the humid forest and 44 adapted to the moist savanna) as well as several thousands of improved pest and disease resistant seed populations were provided to NRCRI, Umudike for evaluation and use in their breeding program. At the request of other NARS in 1992, technical assistance was given in the establishment of *in vitro* cassava and yam to facilitate our efforts of germplasm transfer and realization of more impact of our technology outside Nigeria. Five large consignments with 300 to 400 plantlets each comprising 50 cassava clones were delivered to Cameroon, Gambia, Niger, Burkina Faso and Cape Verde. Apart from normal technical backstopping of root crop programs of NARS, technical support to

root crop projects agreed upon under RRPMC, IITA/EEC-OFAR, ESARRN and the Ghana Smallholders Rehabilitation Project and CORTIS will continue in 1993.

T.6.2 National cassava trials in Nigeria

A. Dixon and NRCRI staff

Under the auspices of the Nationally Coordinated Research Project (NCRP) on cassava, we work with the National Root Crop Research Institute (NRCRI) of Nigeria in the evaluation of the performance of improved cassava clones nation-wide. Ten IITA improved clones were tested alongside NRCRI improved clones in 16 diverse environments for a second year in 1992. The first year (1991) result of this trial showed that TMS 82/00058, TMS 81/00110 and TMS 81/01635 were the top yielders across all the locations and they performed better than the popular check variety TMS 30572. Eight new clones have been nominated and distributed to NRCRI for the first testing in the 1992 trials. Clone TMS 91934 recommended to the National Accelerated Food Production Program (NAFPP) for multiplication and mini-kit trials in 1992 (pre-release stage) is being evaluated in farmers fields. Also four elite clones selected by NRCRI from seeds populations received from IITA were recommended to the NAFPP for the 1993 miniket trials. A third set of new elite clones (low cyanide and poundable) has been nominated for the 1993 trials and is being multiplied for testing under NCRP in 1993.

T.6.3 Tropical Root and Tuber Crops Bulletin

A. Dixon and H. Mutsaers

The scientific quality of the Tropical Root and Tuber Crops Bulletin was improved upon and greater emphasis was put into encouraging more NARS to utilize this medium for flow of information among NARS and between IITA and NARS in root and tuber crop improvement and production research in Sub-Saharan Africa. Two issues of this reconstituted forum of communication among Root and Tuber Crop Researchers in Africa were produced and were distributed to all root and tuber crops researchers in the NARS in 1992. Two issues will also be produced in 1993.

T.6.4 Training Courses

M. Ajayi, I. Ekanayake, M. Bokanga, S.Y. Ng, J. Whyte, A.Dixon, J. Gulley and NARS

As a result of the decentralization of training, two group training courses have been scheduled for 1993 which emanated from the previous Root Crops Research and Technology Transfer course of IITA. These two courses were selected based on their importance and needs of the NARS, for example the Post-Flask Management course has been found to be very important due to the inability of many NARS in managing tissue culture materials received from IITA. Scientists will be involved in preparation and execution of the courses and preparation of training materials. Courses are: 1. Rapid Multiplication and Post-Flask Management of Root Crops; Gambia, July 26-August 6, 1993. The general objective is to strengthen knowledge and skills of research technicians on rapid multiplication techniques and management of tissue culture materials. The course will draw participants from anglophone West Africa. 2. Rapid Multiplication and Production of Root Crops; Republic of Congo, September 6-October 1, 1993. This course aims to provide basic knowledge and skills in root crops production to individuals responsible for establishing, managing field research and providing extension services to farmers. The course participants will be drawn from countries in Central Africa.

The Postharvest course which normally covers four main areas of postharvest food technology: socio-economic aspects, biological constraints, food technology, and equipment design and maintenance will also be conducted in 1993 with emphasis on Food Technology. The course covers the general principles of postharvest technology, – chemistry, biochemistry and microbiology of food deterioration, biology and control of storage pests, drying methods, storage and processing of IITA mandate crops. The overall goals are to improve skills for postharvest food crop research and to stimulate postharvest research in Africa.

T.6.5 Yam Monograph

I.Ekanayake and G.C. Orkwor

Preparation of a monograph which reviews the past and ongoing research information base on the major *Dioscorea* species is underway with the collaboration of several IITA and NRCRI scientists. Most of the draft contributions have been received to date. Completion of a pre-publication draft is expected by the end of 1993.

T.6.6 Graduate training

1) Characterization of elite genotypes of yam, cassava and sweet potato. A.Efisue (M.Sc. thesis) and R. Asiedu.
2) Gender expression in *Dioscorea rotundata* . V.Chikaleke (M.Sc. thesis) and R. Asiedu.
3) Sprouting ability in cassava and interspecific hybrids under different moisture regimes. Manga (M.Sc. thesis) and R. Asiedu.
4) Effects of environmental factors on flowering of cassava (*Manihot esculenta* Crantz). M.Simwambana (Ph.D. thesis) and I. Ekanayake. See T.2.11.
5) Intercropping of cassava (*Manihot esculenta*) and sweet potato in semi arid zone of Tanzania. R. Kapinga (Ph.D.thesis) and I. Ekanayake.
6) Effect of reciprocal grafting on cyanide content in cassava. M.Makame (Ph.D. thesis) and S.K. Hahn.
7) Spontaneous polyploids in cassava: identification, agronomic characteristics, response to biotechnology. V.Boateng (M.Sc.thesis) and S.K.Hahn.
8) Evaluation of virus-free cassava clonal materials. A.O.Akano (Ph.D.thesis), R. Asiedu and S.Y.C.Ng.
9) Investigations of physiological maturity and dormancy on tuber shelf life and time to flowering of yam. B.Aighewi (Ph.D. thesis), R.Asiedu and I.Ekanayake.
10) Evaluation of first generation crosses among resistant and susceptible parents for their reaction to cassava anthracnose disease. C. N. Fokunang (Ph.D thesis), A. Dixon and C. Akem.
11) Host plant resistance to cassava green mite and its interaction with drought stress and predator effectiveness. V. Mbuyongha (Ph.D thesis), A. Dixon and I. Ekanayake.
12) The effect of competition on and the physical and chemical bases of cassava resistance to *Mononychellus tanajoa* Bondar. E. N. Nukenine (Ph.D thesis) and A. Dixon.
13) Cassava adaptation to the inland valley ecosystem. W. Mbele (Msc. thesis) and A. Dixon.
14) Comparative studies on yield physiology and quality aspects of cassava grown in the lowland and upland. M.T.Lahai (Ph.D. thesis) and I. Ekanayake.

T.6.7 Collaboration with CTCRI, UWI and ROTREP on yam research

R. Asiedu, S. G. Nair(CTCRI), T. Ferguson (UWI) and R. Dadson (ROTREP)

The Central Tuber Crops Research Institute, India, and the University of the West Indies have long experience in yam research. Collaboration with them involves mainly exchange of germplasm and information but will be strengthened to enable joint approaches at tackling global or regional concerns such as foliar necrosis of water yams (*D. alata*). Collaboration with the Root and Tuber Research Project (ROTREP), Cameroon offers good opportunities for use of ROTREP tissue culture laboratory for virus elimination and indexing of improved root crop genotypes for regional distribution; maintenance and exchange of germplasm; training; and development of various new technologies relevant to root crop production and research.

T.7 ESARRN Network

Project rationale

The East and Southern Africa Root Crops Research Network (ESARRN) brings together cassava researchers in the region and links them to IITA and other relevant organizations for technical assistance. Network efforts focus on coordination, training, germplasm development, postharvest technology and integrated pest management. The current phase ends Sept. 30, 1995.

Activities

T.7.1 Regional research coordination

M. Alvarez, J. Ikeorgu and NARS (East and Southern Africa)

To effectively utilize limited root/tuber research resources in the East and Southern Africa region, research planning and evaluation is coordinated among the region's NARS. Forums used include: steering committee meetings, head of program meetings, root crops collaborators meeting, root crop symposia, scientist exchange visits, technical monitoring tours and annual research reviews. Information on research activities is disseminated through production of proceedings of the meetings, and a twice-yearly newsletter.

T.7.2 Training

J. Ikeorgu, M.N. Alvarez and NARS (East and Southern Africa)

Inadequate trained manpower is a major constraint to root crops research in the region. In country training for technicians, extension staff and farmers is being emphasized through on -farm trials and technology transfer efforts, particularly in the areas of rapid multiplication of planting material, socioeconomics, and plant protection.

T.7.3 Characterization and diagnosis

M.N. Alvarez, F. Nweke and NARS (East and Southern Africa)

The absence of detailed baseline information on root and tuber crops in Eastern and Southern Africa hinders the research planning and evaluation of progress. Surveys and field trials are being conducted to correct this situation. Additionally, the COSCA project collected valuable data in Malawi, Rwanda, Burundi, Zambia and Kenya. Efforts will be made in 1993 to complete the surveys and reports.

T.7.4 Broadening the germplasm base

M. N. Alvarez, M. Porto, R. Asiedu and NARS (Eastern and Southern Africa)

The Eastern and Southern Africa region encompasses diverse agroecosystems in accordance with its highly variable topography, rainfall and soils characteristics. A broader genetic base is needed for cassava so that an array of clones adapted to the different environments can be identified. TRIP-Ibadan has sent out useful seed populations and tissue culture materials for this purpose. These are being evaluated. More will be imported, both in seed and tissue culture form. More crossing blocks will be established to increase the volume of hybridizations. National programs in Malawi, Rwanda, Uganda and Zambia are capable of generating improved seed populations. Materials generated by NARS will be distributed for evaluation.

T.7.5 Evaluation & distribution of germplasm

J. Ikeorgu, M. Alvarez and NARS (East and Southern Africa)

Promising germplasm selections are evaluated for yield, disease and pest resistance and consumer acceptability. On-farm trials including economic analyses are being intensified, as is the multiplication of elite materials so identified. Some good selections identified from germplasm sent by TRIP Ibadan are currently being multiplied for distribution to farmers.

T.7.6 Postharvest technology

M. Alvarez and R.Sauti (Malawi)

Root and tuber crops are less well-known in Eastern and Southern Africa as compared with West and Central Africa. More efficient methods of processing and utilization of these crops need to be identified to make them appealing to more consumers, and attractive for increased production, including a commercial scale. This should result in increased demand and hence incentive for farmers to increase production. Surveys are underway to characterize traditional processing and utilization methods and identify their constraints.

Simple processing equipment been imported from IITA-Ibadan for evaluation. A processing facility established in Malawi will be used as a base for new product development and evaluation.

T.7.7 Technology transfer

J. Ikeorgu, M. Alvarez and NARS (East and Southern Africa)

Improved root and tuber crop technologies will have no impact if they are not transferred to farmers. Multiplication and distribution of improved germplasm will receive emphasis in 1993. Additional multiplication sites will be established. More in-country training courses for farmers and extension staff will be organized in 1993. Field days, demonstrations, and preparation of extension documents will be done by NARS.

Maize Improvement Program

Maize is in high demand in Africa for human consumption as a starchy base in a wide variety of gruels, porridges and pastes, as well as a fresh vegetable ("green maize", or corn on the cob). Industrial demand (brewing, livestock feed, starch corn oil) is also growing. Often intercropped with cassava in the forest, its contrasting growth cycle increases the overall system's efficiency of nutrient capture and use, and smooths out peaks in the time course of labor input and food output for the enterprise. In the moist savanna sorghum/millet belt and in the mid-altitudes, the nitrogen responsiveness of maize has stimulated a boom in its cultivation for cash-crop sale to distant urban markets as well as to diversify local diets. This dramatic expansion is having important "ripple" economic benefits throughout the savanna area.

Africa's predominantly smallscale, home labor-based farmers appreciate the low labor requirement of maize cultivation: spacing is wide, minimizing the work involved in sowing, and creating room for intercrops and for easy movement during weeding; its vigorous, rapid vertical growth makes it highly weed-competitive; stooping to harvest is unnecessary; and the cobs form an ideal storage unit as-is. They also value the crops' pest resistance: ears are protected from birds, rodents and insects by husks; and open-pollination ensures genetic heterogeneity, allowing rapid gene reassortment in response to new pest challenges, thus preventing severe epidemics. Marketing of maize is also simple: the tough dry grains are easily handled, transported and stored during retailing.

In view of the importance of maize in African farming, IITA has mounted a maize improvement effort since 1972 focussed upon West and Central Africa. It has scored many notable successes, such as the creation of open-pollinated germplasm yielding 30-100% more than local varieties under a range of smallscale farmer management systems; hybrid germplasm adding another 15-25% yield increment; the first practical, large-scale system for breeding resistance to maize streak virus - work which earned IITA the CGIAR's highest accolade, the King Baudouin Award, in 1986; high and stable resistance to rusts, blights and downy mildew; increasing resistance to *Striga*; and the incorporation of all these traits into a range of maturity, agro-ecology and grain-type classes to fit the diverse needs of producers and users. These accomplishments, and the wide spread of the materials on-farm were in large part achieved through collaboration with national research scientists. Many mechanisms were (and are) being used to strengthen NARS capacity to develop, utilize and transfer improved technologies to farmers.

M.1 MAIZE IMPROVEMENT FOR THE SAVANNA

Project rationale

Maize cultivation in the moist savanna has expanded dramatically over the past 20 years for various reasons. The environment is favourable with abundant sunshine and adequate seasonal rainfall (800-1200mm) followed by low humidty after the rains which assists natural drying. North-South road construction (better market access), the availability of fertilizers (often subsidized) and more responsive varieties, largely developed by IITA, and World Bank funded Agricultural Development Projects also contributed. High and stable yield potential to maximize profitability in this system remains a central priority for research. Pressure on governments to reduce fertilizer subsidies is increasing drive production costs up and might cause farmers to cut back on maize in favor of lower-input, lower-output crops, reducing overall regional food production. To avoid this decline, increased internal generation of N in maize cropping systems and improved nutrient recovery by maize must be achieved. Although improvements in cropping systems will be most important to attain these goals, breeding a maize plant that is more efficient in capturing soil N can complement this. Drought is also a major abiotic constraint in the savanna which tolerance/resistance breeding can help combat. A priority biotic production constraint of the savanna which appears highly amenable to tolerance/resistance breeding is the parasitic weed *Striga*. Additional important pests (although less important than in the forest) against which breeding has proven highly effective include streak virus, lowland and highland rusts and blights, stalk rots and storage insect pests. All these resistances/tolerance need to be combined in an adapted genetic background including different maturity durations and grain quality characteristics desired by different producers and consumers.

Activities

M.1.1 Breeding for high yield in the savanna

S.K. Kim, J.G. Kling, J.M. Fajemisin, B. Badu-Apraku, M. Esseh-Yovo, C. Thè (IRA, Cameroon) and M. N'Kashama (PNM, Zaïre)

The "comprehensive breeding system" base populations TZL Comp. 3 and 4 (full-season) and TZE Comp. 3 and 4 (early maturing) now form the core of both our OP and hybrid improvement efforts for both savanna and forest ecologies (see "Completed studies CID Activity Report and Workplan 1993"and see details of their improvement in M.2.1) . Additionally and specifically for savanna OP improvement, new experimental varieties from two high-yielding savanna materials, Pop 29-SR and TZUT-SR will be tested in preliminary trials in 1993. Emphasis in selecting the families has been on ear rot resistance (screened at Ikenne), so the new cycles should be better for a key weakness of this germplasm. Full-sib families from TZUT-SR C6 will be screened in Ikenne and Cote d'Ivoire. For the southern, wettest part of the savanna, work on an extra late-maturing population (135 days) will be resumed in 1993. Since the crop will mature closer to the end of the rainy season, drying and storage problems should be reduced, although it is not yet clear if such a variety will fit well into the farming systems in other respects. There has been a request from Nigeria to convert population 49-SR to yellow grain color to get an intermediate-maturing yellow dent, which commenced in 1992. Hybrids selected in 1992 will be tested for grain in the southern, central and northern (driest) parts of the savanna (Mokwa/Abuja, Samaru/Funtua, and Bagauda) in 1993. Hybrid 9021-18 STR (a new version of 8321-18) is particularly promising, with high yield performance and stability across testing sites. It will be re-tested. New hybrids will be recommended for the Nigerian National Zonal Trials in 1993. For the past two years, new "inter-agro-ecosystem" (mid-altitude x lowland) hybrids have been multilocationally tested in both mid-altitude and lowland ecologies. A fourth combining ability study of temperate x tropical crosses is underway using IITA tropical inbreds TZi 3 and TZi 15 and temperate inbreds Mo17 and B73. as testers The University of Hawaii and Iowa State University participated in testing these crosses in their contrasting environments. Topcrosses with Togolese lines and West African based traditional germplasm will also be made by our visiting collaborative scientist to identify best combiners.

M.1.2 Breeding for nitrogen use efficiency

J. G. Kling, H. A. Akintoye, S. K. Kim, J. Fajemisin and P. Salle

Feasibility studies have been ongoing since 1989 to determine whether genetic diversity exists within IITA's main populations for improving N use efficiency (NUE) and to understand the physiological mechanisms associated with tolerance to N stress. As part of this effort, a graduate student (H. Akintoye) is investigating the effects of heterosis ("hybrid vigor") on nitrogen use efficiency. Single crosses, double crosses and F2 progeny derived from four inbred parents were compared for the second time under varying N fertility levels (0, 70, 140, and 210 kg N/ha) at Ikenne, Mokwa and Samaru in 1992. This data will be analyzed and summarized in 1993. Research elsewhere indicated that "prolific" varieties (more than one ear per plant) may be more nitrogen-efficient, presumably due to their greater "sink strength" (greater mobilization of assimilates to the more numerous ear shoots). They may also be more productive than nonprolific varieties in maize-legume intercrop systems in the savanna where light interception is not limiting for the maize crop. Efforts are underway to introduce prolific genes from a variety of sources into regionally adapted germplasm. Selections have been made for prolificacy, streak resistance, lodging resistance, and ear rot resistance. Selected lines have been intercrossed to produce prolific composites. S1 families from these composites underwent multilocational testing for the first time in 1992. Selected families will be recombined in 1993. The best material will be compared with semiprolific populations from CIMMYT in 1994 under varying fertility levels to determine their usefulness.

M.1.3 Root growth and nitrogen use efficiency

J.G. Kling, W. Horst, G. Weber, H. Heuberger, and S. Oikeh

Studies have shown that 30-70% of soil nitrate is never captured by the maize crop. A collaborative project (GTZ-funded) with Prof. Dr. W. Horst began in 1992, to study the ability of varieties with more vigorous, extensive root systems to capture more nitrogen from the soil, and the possibility of developing seedling screening methods for improved root systems. Two postgraduate students will begin their field research in 1993.

M.1.4 Breeding for drought tolerance

B. Badu-Apraku, J. Fajemisin, S. K. Kim, D. Hema and J. G. Kling

Drought tolerance (DT) has been a major objective of IITA breeders in SAFGRAD since 1980, leading to the development of early (95 days) and extra-early (80 days) maturing, DT germplasm. Experimental varieties from selected Pool 16 DT C3 full-sib families will be tested multilocationally by IITA staff and SAFGRAD collaborators in drought-prone areas of Nigeria (Bagauda and Minjibir), Burkina Faso, Cote d'Ivoire and northern Cameroon. Full sibs were generated from Pool 16 DT C4 under streak pressure. In 1991, crosses were

made between Pool 16 DT and: Pool 16 Sequia, the early fraction of Tuxpeño Sequia and La Posta Sequia. These crosses were evaluated in 1992 and the best families were introgressed into Pool 16 DT . DT Comp. Early generated from 8 DT local and improved cultivars was crossed with a streak resistance source in 1991 and was recombined and selected under drought in 1992. Ten DT experimental varieties were formed in 1990 from TZDT-W and TZDT-Y populations and were tested in 1992. Analysis of data from a study of the genetic control of drought tolerance using generation mean analysis of diallel crosses among DT and susceptible inbreds were completed in 1992 (Ph.D. thesis of D. Hema). Seven of the F1 crosses included in this study have shown outstanding yield performance under drought conditions for three years in Nigeria and Burkina Faso.

M.1.5 Breeding for *Striga* tolerance/resistance (STR)

S. K. Kim, J. G. Kling, J. Fajemisin, A. Diallo, B. Badu-Apraku. D. Berner, V. Adetimirin, C. Thè (IRA, Cameroon), M. Esseh-Yovo (DRI, Togo), P.Y.K. Sallah (CRI, Ghana) and L. Akanvou (IDESSA, Cote d'Ivoire)

Over 5,000 genotypes are screened annually in fields artificially infested with *Striga hermonthica*. Within Nigeria, IITA scientists continued to screen for *Striga* tolerance/resistance at Mokwa, Abuja, Samaru, Minjibir and Jos in 1992. STR breeding trials are also conducted collaboratively with NARS in Cameroon, Togo, Ghana and Cote d'Ivoire. Genetic uniformity and STR level of selected varieties and populations will be upgraded. Emphasis in 1992 and 1993 has been placed on one early (TZE Comp. 5) and one late (TZL Comp. 1) savanna-adapted populations in order to rapidly attain OP varieties with moderate resistance comparable to the currently available hybrids. PASCON (Pan-African *Striga* Control)/FAO Network has requested 15 tons of STR seeds of maize for NARS.

M.1.6 Screening diverse germplasm for *Striga* resistance

J. G. Kling, L. Akanvou (IDESSA) and D.K. Berner

About 500 American landraces from CIMMYT's germplasm bank and 500 African landraces held at IITA have been increased and are being evaluated under *S. hermonthica* infestation at Abuja. Infestations were optimal in 1992. In an experiment with 3 replications comparing 62 African Landraces which had shown promise in previous trials, highly significant variation was observed for plant ratings and striga emergence. Landraces that show few or mild symptoms of striga attack and/or little or no striga emergence are being selfed and reselected in order to isolate new resistance genes, and are also being recombined for further selection and introgression into adapted STR populations. Efforts are also underway to backcross resistance genes from *Zea diploperennis* into resistant and susceptible IITA inbred lines.

M.1.7 Screening for resistance to *Striga aspera* and *S. asiatica*

S. K. Kim V. Adetimirin, M. Esseh-Yovo, J. Iken, S. Lagoke, and D. Berner

Germplasm tolerant/resistant to *S. hermonthica* may not be tolerant/resistant to *S. aspera*, another widespread species. However, IITA has done little work on *S. aspera* in the past. Seeds of *S. aspera* were collected near Bagauda (ICRISAT Station) and testing will start in 1993. This work will follow on previous studies that found resistance to *S. hermonthica* also to be effective against *S. asiatica* but not *S. aspera*. Verification trials in 1992 failed due to lack of infestation. Further trials are planned to evaluate the stability of STR across striga species. *S. asiatica* work has been done in collaboration with Togo and possibly at a new Nigerian site near Abeakuta.

M.1.8 Genetics of resistance to striga (inbreds and hybrids)

V.O. Adetimirin , S.K. Kim and M.E. Aken'Ova

Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) each of crosses between two resistance and susceptible inbred lines have been assessed under *S. hermonthica* infestation at Abuja and Mokwa for a second time. A total of 16,000 plants in both locations have been individually observed for *Striga* resistance. Generation mean analysis will be used to determine mode of gene action and numbers of genes involved. Further work will continue in 1993 with the testing of F_3 families and the determination of estimates of heritability. Genetic studies for resistance to *S. aspera* and *S. asiatica* will be initiated in 1993.

M.1.9 Inheritance of *Striga* resistance in OP's

L. Akanvou (IDESSA), J. G. Kling and M.A.B. Fakorede

Studies of genetic variances and heritability of resistance to *S. hermonthica* in OP populations began in 1992 (Ph.D. thesis). Design I crosses (full-sib families within half-sib families) will be generated and

evaluated under *Striga* infestation in 1993. Selected families will be recombined and evaluated the following year, permitting a comparison of realized heritability with estimates of heritability from the Design I experiment. This study will provide valuable information concerning the potential for increasing levels of STR using population improvement methods.

M.1.10 Effect of maturity group on *Striga* resistance

L. Akanvou (IDESSA), D. Berner, J. G. Kling, A. Diallo, J. Fajemisin, and M.A.B. Fakorede

Varieties representing a range of maturity groups (between 85 and 125 days to maturity) will be screened under *S. hermonthica* infestation to determine if time to maturity can provide an escape mechanism for maize under field conditions. Experiments will be carried out in Nigeria and Cote d'Ivoire.

M.2 MAIZE IMPROVEMENT FOR THE FOREST

Project rationale

Maize is traditionally an important food crop in the forest, commonly intercropped with cassava to benefit from their complementary growth cycles, canopy architectures and nutrient requirements. Some is consumed as a fresh vegetable ("green maize", an important cash crop) early in the season while some is left to mature, then stored as dry grain for gradual consumption or sale. The long rainy season and constant high humidity is condusive to fungal diseases and stem/cob boring insects, and inhibits grain drying, leading to storage pest problems. Nutrient-poor acid soils and low insolation limit growth and yield. Breeding in this unfavourable environment has nonetheless been successful in increasing on-farm yields by 30-100%. Resistance breeding has proven to be a highly effective approach for the control of fungi and viruses, and is showing promise against stem borers. The outlook for increasing storage pest resistance is also bright.

Completed studies

Kim, S.K. and Yoon, S.T. (unpubl.). Performance of hybrids vs OP's across environments.

Hybrid and OP germplasm were compared (14 vs. 6 varieties respectively) across five sites spanning both forest and savanna in Nigeria; results are being analyzed. Preliminary results indicate that high plant density (6.6/m2) reduces yield in the forest, but increases it in the savanna. Average yields of 3 hybrids and 3 O.P. varieties over 200 trials since 1985 showed that hybrids yielded 19% (white grained) and 21% (yellow grained) over O.P. varieties.

Activities

M.2.1 Breeding for high yield in the forest

J.G. Kling, S.K. Kim, C. Thè (IRA, Cameroon) and M. N'Kishama (PNM, Zaïre)

Field observations in 1991 and 1992 indicated that the reciprocal composite populations TZL Comp 3 and 4 (late-maturing) and TZE Comp 3 and 4 (early maturing), developed for the "comprehensive breeding system", have high yield potential in both forest and savanna environments. S1 and S2 testcrosses of TZE Comp. 3 C1 and TZL Comp. 4 C1, respectively, will be tested in both the forest and savanna in 1993. The best lines across sites will be recombined to generate the next cycles of selection, whereas the most elite lines within each ecology will be used to create experimental varieties for the forest zone and the savanna. New cycles and experimental varieties from TZE Comp. 4 and TZL Comp. 3 will be evaluated in preliminary yield trials in 1993. Fifteen promising hybrids were selected at Ikenne in 1992; yield testing of these and hybrids selected for downy mildew (10 hybrids), *Eldana* (3), and *Sesamia* (6) resistance will be tested again in 1993. TZL Comp 3, TZL Comp. 2, TZE Comp 3 and TZE Comp 4 have been used as testers for some Togolese breeding lines and temperate inbreds. These top crosses will be evaluated in 1993 for heterotic group studies..

M.2.2 Resistance to downy mildew

J.G. Kling, S.K. Kim, K.F. Cardwell and M. Omidiji (IAR&T)

Downy mildew (DM) is a devastating disease on late sown crops in parts of Nigeria and Zaire, and is spreading rapidly through the forest zone. Genetic sources of high DM resistance are available, but conversions of varieties adapted to Africa vary in degree of resistance achieved. Streak-resistant conversions of the original DMR varieties from Thailand (Suwan 1 and Suwan-2) showed about 10% susceptible plants under artificial infestation at Akure in 1991 and 1992, whereas varieties derived from

those sources ranged from 10-50% susceptible plants. Improvements in screening methods in Akure in 1992 included direct infestation of pregerminated seeds for spreader rows, resulting in over 90% infection in 6 ha. of trials. Methods for infestation of seedlings in dew chambers will be utilized for the first time in 1993, which should permit the rapid purification of existing DMR varieties and year-round screening. A genetic study of DM resistance was continued in 1992. Major emphasis was given to screening S1 lines from the comprehensive breeding system in 1991 and 1992 to ensure that basic breeding pools have a high level of resistance. This work will continue in 1993, along with the purification of existing DMR varieties. DMR inbred lines were selected in 1992. Now we have hybrids which confer high resistance to both DM and maize streak virus (MSV). Collection of maize from four DM infected states in collaboration with the Maize Association of Nigeria (MAAN) showed that only half of the maize grown by farmers carry DMR. MAAN and State Agricultural Development Projects (ADP's) have distributed DMR seeds to farmers. The Nigerian Federal Government launched a Task Force project combating DM in Nigeria in 1992. This will be a top priority in IITA/Nigeria collaboration.

M.2.3 Green maize studies of hybrid vs. OP varieties

S.T. Yoon and S.K. Kim

We have studied the relations between planting space, nitrogen levels and green maize yield with several hybrid and O.P. varieties for the last two years (1989-1992). A total of 60 kg N/ha and 75x30 cm for row and hill spacing gave the highest economic return. We will evaluate planting time relative to the start of the rain in the forest ecology. Trials will be conducted with 10 green maize varieties and 3 planting times (10 days interval) at 2 sites (Ibadan and Ikenne) under 60 kg N/ha level.

M.2.4 Studies on yield components

S.T. Yoon and S. K. Kim

Information on major yield components of leading varieties of maize is important to develop an ideal morphotype. We are summarizing yield components of 20 varieties (hybrid and O.P.) tested for the last four years. Any missing or insufficient data will be taken in 1993.

M.2.5 Fresh maize and cassava intercropping in the forest ecology

S.T. Yoon, S.K. Kim and A.G. Dixon

Maize and cassava are two of the most widely grown food crops in the forest ecology. Continuous cropping of either one of the two crops in the same soil disturbs sustainability of crop production and monocropping is prone to risk such as crop failure from drought and price fluctuations. Hence most small scale resource poor farmers grow several different crops. Cassava takes one and half year for harvesting but it is the most suitable crop because of its sustainable production under low input. Fresh maize can be harvested within 70 days of the onset of the first rainy season. Therefore, fresh maize-cassava intercropping can be viable economic package to increase farmers' income as well as sustainability and maize-cassava intercropping uses space and time efficiently. The research aims to identify more suitable maize varieties for the maize-cassava intercropping system. Trials of fresh maize-cassava intercropping will be carried out with 8 maize varieties (5 hybrid and 3 O.P) and 1 cassava variety (TMS 30572). Maize varieties included are ranged such as plant height (short/tall) and leaf morphology (erect/open canopy). Sole crops of both maize and cassava will be included as controls.

M.2.6 Green maize yield and weed control when intercropped with melon

S.T. Yoon, S.K. Kim, I.O. Akobundu and B.A. Gbadamosi

Weed infestation in maize cultivation is severe in the forest ecology. Manual weeding and herbicide use are expensive. Melon can supress the weeds and it forms a lower storey crop relative to maize. Studies on the intercropping of maize with melon will be conducted at IITA, Ibadan with 6 varieties of maize (3 hybrid and 3 O.P.) and one local variety of melon. Maize will be sown at 75cm and 25cm between and within rows respectively, while melon will be intercropped between maize rows, with 60kg N/ha.

M.2.7 Comparison of streak screening methods

M. Esseh-Yovo, S. K. Kim, and J. Kling

The seedling cage infestation method developed by the senior author doubled streak infestation on maize plants which were grown during the first and second rainy seasons. The difference between the seedling cage and direct field infestation were not significant during the third harmattan season. This study also confirmed the outstanding performance of near immune type of streak resistance in TZMi 301.

M.2.8 Resistance to MSV

M. Esseh-Yovo, S.K. Kim and J. Kling

In 1992, research covered: i) Genetic study of resistance to MSV through Hayman diallel analysis, ii) Transfer of streak resistance into Togolese, temperate inbred lines and into some IITA SR inbreds selecting for a near immune response, iii) Efficiency of different selection schemes for MSV resistance improvement, and iv) Improvement of the level of MSV resistance of some West African traditional varieties (Togo, Benin, Nigeria, Mali, Cameroon).

M.2.9 Breeding for resistance to the pink stem borer

J.G. Kling, S.K. Kim, O. Gold, and N.A. Bosque-Pérez

Two populations developed for resistance to *Sesamia calamistis*. continue to be improved under artificial infestation (TZBR Sesamia-1 and TZBR Sesamia-3). An experiment in 1992 showed TZBR Sesamia-3 to be more resistant than the best available OP variety and comparable to the best available hybrids. Plant vigor appears to have a strong effect on resistance. In 1992, S1 lines from TZBR Sesamia-1 C1 and their testcrosses were compared to determine if there is a good correlation between inbred and non-inbred test materials. This study will be completed in 1993. A total of 224 inbred lines were infested artificially during the 1991/92 C-season with *S. calamistis*. Infestation was high and 9 lines which showed better resistance than TZi 4 were identified. TZi 4, which was previously selected as resistant, was segregating. Hybrids as well as synthetics resistant to *Sesamia*. can be developed if a high level of infestation can be routinely achieved on a large scale. A genetic study for development of synthetics and hybrids resistant to *Sesamia*

M.2.10 Breeding for resistance to the African sugarcane borer (*Eldana saccharina*)

J.G. Kling, S.K. Kim, O. Gold, and N.A. Bosque-Pérez

Three populations developed for resistance to *E. saccharina* continue to be improved using artificial infestation of spreader rows (TZBR Eldana-1, -2, and -3). Experiments in 1991 and 1992 showed that significant improvements had been made in resistance to ear damage through selection. Although two of the populations showed consistently less ear damage than the resistant hybrid check in both years of testing, the differences were not significant. Poor growing conditions on the West Bank at IITA in the second rainy season for the past two years may have reduced the effectiveness of the breeding trials. Hence, trials will be conducted on the East Bank in 1993, late in the first rainy season. A total of 200 lines, 600 hybrids and test crosses were tested under spreader-row infestation on the West Bank in 1992. All materials were planted in late August, but levels of infestation in the spreader rows were not adequate to get sufficient insect pressure on the test rows indicating that the infestation technique should be improved. Seed production of five diallel crosses for resistance to *Sesamia* and *Eldana* were carried out late in the first rainy season on the East Bank. Materials were highly infected by *Eldana* under natural conditions. A genetic study for *Eldana* resistance was initiated as Ph.D thesis (Mr. Gold). With the 1992 selected hybrids, an *Eldana* resistance trial was planted in East Bank and had high natural infestation. Three hybrids showed an average 20% yield advantage and higher resistance to ear *Eldana* and rot than TZBR-*Eldana* population.

M.2.11 Stem borer resistance on-farm

H. Tijani-Eniola (Univ. of Ibadan),S.K. Kim, and H. Mutsaers

Some of IITA's maize hybrids appear to be resistant to *S. calamistis* and *E. saccharina* under artificial infestation at IITA, Ibadan. The relevance of these findings to realistic farm conditions needs to be determined. Researcher-managed trials using typical farmer practices were conducted in the second season at Ayepe, Nigeria, 1992. The most outstanding variety was 8644-27 which was moderately resistant/tolerant to downy mildew, borers and streak.

M.2.12 Breeding for weevil resistance

J.G. Kling, D.K. Kossou, S.K. Kim and N.A. Bosque-Pérez

Storage pest damage is a serious problem in the forest. Research has shown that longer, tighter husks help protect maize cobs from invasion by weevils. Many improved maize populations have poorer husk cover than traditional varieties, so selection for good husk cover is now routinely practised in all populations, and reasonable progress is being achieved. Hard, flinty kernels are also more resistant to weevils, but some forest zone consumers prefer floury (soft endosperm) maize varieties because they are easier to mill and give higher flour yield. Thus, grain hardness is not a widely useful resistance mechanism. Methods for

controlled infestation of ears with and without husks have been worked out and will continue to be applied on forest-targeted populations and hybrids in 1993.

M.2.13 Screening inbreds against *Sitophilus* weevils

D.K. Kossou (Univ. of Benin), C.G. Yallou and S.K. Kim

Inbreds and hybrids were grown in Benin to test for resistance to *Sitophilus* weevils under farmer storage conditions (with husk and grain on cob, in bins). However, the trial failed due to drought. Similar trials will be conducted with Benin NARS scientists in 1993.

M.2.14 Improving post-harvest properties of maize for Benin

C.G. Yallou, D.K. Kossou (Univ. of Benin), J.G. Kling and A.E. Okoruwa

Although IITA varieties have been shown to outyield traditional varieties by a large margin, Benin farmers prefer the ease of milling, small seed size, high flour yield and storage properties of the traditional variety 'Gbogbe' (pers. comm. M. N. Versteeg). This collaborative project aims to transfer the Gbogbe grain quality into the higher yielding, agronomically superior background of TZSR-W-1, while simultaneously improving the latter's husk cover to better protect the grains from weevils. Half-sib families from Gbogbe x TZSR-W-1**2 were made in Ibadan and sent to Benin for field trials in 1992, but reliable data were not taken due to drought. Seeds from families with good husk cover have been grown out and recombined to produce new families for testing in 1993. These will be screened in Benin for husk cover and weevil resistance and selected families will be further evaluated in Ibadan for milling quality. Some preliminary results from on-farm trials in Benin and quality tests in Ibadan suggest that it may be necessary to introgress additional local germplasm into the population to give it the desired quality attributes.

M.3 MAIZE IMPROVEMENT FOR THE MID TO HIGH ALTITUDES

Project rationale

Mid-altitudes are areas from 800 to 1500 meters above sea level, while high altitudes are those above 1500 masml. Maize is important in the mid- to high altitudes of Zaire, Cameroon, Togo, Nigeria, Guinea-Bissau and Guinea-Conakry, as well as in Eastern and Southern Africa. Yield potential of this environment is higher than in the lowlands, because cooler temperatures extend crop duration and phenology. Cooler temperatures also inhibit breakdown of soil organic matter, so these soils often have higher organic matter content and greater CEC relative to soils of lower altitudes. Different diseases and pests are found in the mid-altitudes to which lowland germplasm is susceptible: northern blight (*Exserohilum turcicum*) Rust (*Puccinia sorghi*), *Diplodia* ear and stalk rot, and *Phaeosphaeria* and *Cercospora* leaf spots. *Busseola fusca* is a stem borer common to this zone. Constraints in common with the lowlands include streak virus, drought and soil acidity. Lowland germplasm grows poorly in the mid- to high altitudes. Different germplasm bases are needed for these environments, and have been created based on materials from the East and Southern African mid-altitudes. The high yield potential of the mid-altitudes opens a special opportunity to exploit hybrid vigor.

Activities

M.3.1 Mid-altitude breeding in Nigeria

S.K. Kim and R. Olafare (UTC Seeds)

Three testing sites on the plateau of Nigeria were used in 1992: Segau (900m amsl) in Kaduna State, Vom-WAMCO farm (1,300m amsl) and UTC -Tenti farm (1,350 mamsl) in Plateau State. Among the OP's, population improvement of TZEMSR received emphasis; 1992 was the first year of full sib testing. Due to substantial rainfall (1800mm) UTC Farm at Tenti, the trial failed. However, the same materials were tested at Bagauda and Kaduna where excessively dry growing conditions occurred. Experimental varieties will be formed for TZEMSRDT-W (IITA early maturing mid-altitude streak resistant, drought tolerant). The highlight of mid-altitude maize research in 1992 was achieved in Segau, Kaduna State (900 mamsl). Over 500 new hybrids were tested. The highest yield was 14.7 t/ha with hybrid 9117-18 (a three-way cross). Several hybrids yielded over 12 t/ha. UTC, Kaduna ADP, Maize Association of Nigeria (MAAN) and IAR-ABU in collaboration with IITA, Pioneer and UAC Seeds held a one-day farmers' field day on 26 September, 1992. The UTC Seeds plan to produce hybrid seeds in the Segau-Saminaka area, commencing 1993. Several outstanding hybrids from Cameroon and Nigeria bred materials at WAMCO showed root and stalk lodging resistance. *Diplodia* infection at WAMCO in 1992 was less than in 1991. Trials will

continue at all three testing sites in 1993. Inbred and hybrid development continues, deriving inbreds from the TZMSR population. Since the Cameroon NCRE project is winding down (see M.3.2), efforts are underway to integrate its germplasm into the Nigeria-based program. The best-performing materials identified in 1991 from Cameroon and holding displaying *E. turcicum* and *Diplodia* stalk/ear rot resistance in Nigeria in 1992 were test-crossed with inbred testers TZMi 101 and 407 to classify them into the heterotic groups of the Nigeria germplasm base. Comparative yield testing for yield of hybrids from both countries will continue for a second year in Nigeria in 1993.

M.3.2 Mid- to high altitude breeding in Cameroon

N. Beninati (IITA-NCRE), M. Ndioro and I. Tabi (IRA), and J. Foko (Univ. Centre Dschang)

Maize is the dominant staple food crop in the Western Highlands of Cameroon, the most heavily populated part of the country (25% of the total population), accounting for 60% of the country's maize production. The NCRE highlands breeding project which has been improving maize for this zone since 1984 came to an end in 1992 due to the unexpected departure of N. Beninati. Five trial locations are currently utilized in the Western Highlands: Foumbot (1,000m amsl), Babungo (1,200m amsl), Bambui Station (Mfonta site, 1,300m amsl), Dschang (University Centre Dschang, 1500m amsl) and Mbiyeh (MIDENO Station, 2,100m amsl). Maize is also a new high potential crop in the Central Highlands (Adamaoua Plateau); an additional site is located there, Mbang Mbirni (1,200m amsl). Half-sib family selection is underway in an acid tolerant population (ATP), and high altitude population (HAP). Several populations used as germplasm sources are also being maintained or improved in the mid-altitudes e.g., Ecuador 573 and Kitale 2 from Kenya, Kasai from Zaire, Populations 90 and 92 from Tanzania, and Populations 32 and 43 from CIMMYT. Streak resistance conversion is underway for Shaba and Kasai. The "comprehensive breeding system" used in Cameroon has produced both high yielding OP synthetic varieties and hybrids. The latest synthetics, Syn 4 to Syn 6 show substantial yield increases over previous material (about 10%). Inbred and hybrid development continues with selfing from populations, crosses, and reciprocal synthetics. Inbreds and hybrids are in preproduction testing by Pioneer Hi-Bred International.

M.3.3 Lower limit mid-altitude maize for Togo

M. Esseh-Yovo and S.K. Kim

A small area in western Togo (Dayes and Gbadi-Nkugnan sites) approaches mid-altitude (700-800 mamsl) and lowland varieties do poorly there. IITA's mid-altitude O.P variety, Across 87 TZMSR, mid-altitude hybrid 8535-23 and EV49-SR (CIMMYT/IITA) showed good performance.

M.4 MAIZE GRAIN QUALITY

Project rationale

Little research has been done worldwide on maize grain quality, since maize is mainly an animal feed in developed countries. This modest project is only three years old. The first priority has been to try to identify the major parameters associated with "grain quality" for the most common end-uses in Africa. Working hypotheses have been developed and are being tested. Once these parameters are defined in specific physico-chemical terms, rapid screening techniques for them will be worked out. These will then be applied to investigate the genetic control of these parameters, and to breed varieties that are acceptable for various end-uses in a high yielding, improved maize genetic background.

Activities

M.4.1 Environmental effects on maize grain quality

A. Okoruwa and J.G. Kling

A study was undertaken in 1991 to determine how the environment influences physical and chemical properties of maize grain. Grain from eight varieties grown in large plots at three locations (Ikenne, Mokwa, and Samaru) in Nigeria in 1991 were analyzed in the lab in 1992. 1992 study materials from the three locations will be evaluated for grain quality and milling properties this year.

M.4.2 Rapid test for milling performance

A. Okoruwa and J.G. Kling

Pilot scale dry milling tests for maize grain were developed in 1992. These methods will be applied and further modifed in 1993.

M.4.3 Improving traditional hand milling

B. Assa Kante (IER, Mali), A. Okoruwa and J.G. Kling

Maize is more difficult to hand-mill than millet and sorghum and this inhibits its adoption in some areas. A collaborative project with IER, Mali was initiated in 1991 to evaluate varietal differences in ease and effectiveness of hand-milling, and to develop simple modifications of the traditional maize pounding method which are less labor intensive. Data will be analyzed and summarized in 1993. This work will be further developed as part of a Ph.D. thesis (Assa Kante).

M.4.4 Oil content of whole vs. dehulled maize

A. Okoruwa and J.G. Kling

Oil content of some maize varieties before and after dehulling and milling have been measured. This is to determine if the oil content of whole maize grain is a good indicator of oil content of dehulled maize flour, an important parameter for storability and brewing quality. Results will be analyzed and summarized in 1993.

M.4.5 Inheritance of grain oil content

J.G. Kling and A. Okoruwa

Results in 1991 revealed that oil content of many varieties is higher than desired for most dry milling and storage purposes. Considerable genetic variation was found, and some evidence suggested only a few genes control this characteristics such that selection for lower oil should be possible. Inheritance will be studied using a diallel cross of inbred parents of hybrids known to span a range of oil content values.

M.4.6 Evaluating floury conversions

A.E. Okoruwa and J.G. Kling

Floury grain conversions of some dent and flint varieties were increased in 1992 under uniform conditions and will be evaluated for grain quality and dry milling properties. This study will compare these conversions with traditional varieties to ascertain whether they are acceptable for pasting and related properties desired for some food products.

M.4.7 Potential of maize tortillas in Nigeria

V.A. Obatolu (IAR&T), A.E. Okoruwa and J.G. Kling

This project has been ongoing since 1991. Further work to be carried out includes i) demonstration of tortilla-making/consumer acceptability survey at selected schools, ii) evaluation of maize varieties (10) for tortilla-making quality, iii) determining the most acceptable form of tortilla presentation and iv) storage studies.

M.4.8 Fortification of maize tortillas with soybean

S.M. Osho , R. Abiodun (soybean util.), and A.E. Okoruwa

In Mexico and Central America, maize tortillas are consumed as unleavened flat breads, but they are low in protein. The potential of soybean-fortified maize tortillas will be investigated.

M.4.9 Improving post-harvest properties of maize for Benin

D.K. Kossou, C.G. Yallou, J.G. Kling and A.E. Okoruwa

See section M.2.15. Families selected in the field for husk cover and in the lab for weevil resistance in 1993 will be evaluated and selected for dry milling properties in 1994.

M.4.10 Disease-resistant popcorn varieties

S. K. Kim and J. G. Kling

Popcorn is a popular snack food. Popcorn germplasm has been crossed to streak resistance sources, to create an OP population. Characteristics that will be improved are disease resistance (MSV, rust, and ear rot), popping expansion, palatability and yield potential. Inbred popcorn lines developed from these pop-SR lines could be combined to form a high-performance OP synthetic.

M.5 COLLABORATION WITH NARS AND TRAINING

Project rationale

A major goal of IITA is to strengthen NARS research capacity. Additionally, IITA's research effectiveness can be increased through collaboration with national scientists, extending our perspective and experience, our range of sites, and our linkage to agencies that can transfer technologies to farmers. For all these reasons, collaborative activities with NARS and integral to much of the research already described, consuming about 30% of MIP's budget. Activities highlighted below are explained in more detail under the relevant agro-ecosystem project.

Activities

M.5.1 SAFGRAD Network, terminal phase

B. Badu-Apraku

Since the mid-1970's, the Semi-Arid Food Grains Research and Development (SAFGRAD) Network has stimulated collaboration in maize improvement research among NARS for mutual benefit and complementarity. It also provided technician training and a forum for exchange of results through workshops. Collaborative research produced valuable germplasm that is in advanced testing and release stages in several countries. MIP backstopped SAFGRAD in all these activities. Officially ended on Dec. 31, 1991, SAFGRAD Phase II has been extended at a reduced activity level to March 31, 1993. During the extension, the top priority is to assess impact from the previous phases. A USAID-appointed specialist consultant has assisted in this study. A modified project focussing on an impact-driven network is being proposed.

M.5.2 International germplasm trials

B. Badu-Apraku, J.G. Kling, S.K. Kim, J. Fajemisin and S. Adewunmi

International trials, assembled in collaboration with SAFGRAD, are distributed to all interested NARS. They include both "near-finished" and "source" breeding material. NARS entries as well as IITA and CIMMYT/IITA entries are included. IITA collates and analyzes data returned by NARS and sends them results including cross-site analysis to illuminate stability of performance across environments.

M.5.3 Fitting varieties to environments

J.G. Kling, A. Akalumhe, S. Jagtap, J. Fajemisin , A.O. Diallo and S.K. Kim

A collaborative project with the Agroclimatology Unit of RCMD to characterize and map the regions of adoption of elite maize varieties continued in 1992. A second year of testing of 60 OP varieties in Nigeria, Cote d'Ivoire and Burkina Faso was completed. Data are being summarized and fact sheets for each variety will be prepared in 1993 for distribution to NARS.

M.5.4 Usefulness of IITA germplasm in Togo

M. Esseh-Yovo (DRA, Togo), S.K. Kim and J.G. Kling

Our 1992/1993 visiting collaborative scientist has examined the potential of IITA germplasm for maize improvement in Togo. Transfer of *Striga* and streak resistance into Togolese material was initiated. Combining ability of IITA inbreds with Togolese varieties has been determined. Mid-altitude materials will be scrutinized for 600-800 mamsl areas in Togo. Farmers' reactions to IITA material will be studied on-farm in Togo. Streak resistance of traditional West African varieties is being upgraded, and IITA vs. Togolese streak virus challenge methods have been compared with farmers' method during rainy seasons.

M.5.5 Adoption of improved maize in Nigeria

M. Adenola, J. Iken (Maize Assoc. of Nigeria); J. Akinwumi,M. Fakorede, S.K. Kim, F. Nweke

Improved maize varieties yield 30-100% more than traditional varieties and resist the major diseases of the region. Because of these attributes and the efforts of seed multiplication and extension agencies over the years, it is believed that they have been widely adopted. However, objective data are needed to validate this impression. A 2.5 year study modeled on the COSCA format has gathered information on adoption and farmers' preferences and practices. Group interviews will be held in about 300 villages and collected seed samples returned to IITA for confirmation of varietal identity.

M.5.6 Savanna Station, Cote d'Ivoire

J.M. Fajemisin, A. Diallo (CIMMYT), S.K. Kim, J. Kling, B. Badu-Apraku, K. Goli and A. Koffi (IDESSA)

Efforts ongoing since 1987 to start-up activities at the Station will continue again in 1993. Current issues are: finalizing administrative and technical agreements; planning Station development; installation of a streak resistance screenhouse, setting up *Striga* lab and some routine breeding trials.

M.5.7 Collaborative research

1) Breeding for *Striga* resistance /tolerance - S. K. Kim, J. Kling, J. Fajemisin, B. Badu-Apraku, C. Thè, P.Y.K. Sallah and M. Esseh-Yovo. See M.1.5.
2) Evaluation of usefulness of IITA germplasm for maize improvement in Togo. M. Esseh-Yovo, S. K. Kim and J. G. Kling. See M.5.4.
3) Breeding for resistance to downy mildew - J. G. Kling, S. K. Kim, K. F. Cardwell, J. Iken and M. Fakorede. See M.2.2.
4) On-farm testing of stemborer resistant maize hybrids - H. Tijani-Eniola, University of Ibadan; S. K. Kim and M. Mutsaers. See M.2.11.
5) Breeding for husk cover and weevil resistance - J. G. Kling, D. K. Kossou, S. K. Kim and N. A. Bosque-Pérez. See M.2.12.
6) Traditional maize processing in Mali - B. Assa Kante, A. E. Okoruwa and J. G. Kling. See M.4.3.
7) Potential of maize tortillas in Nigeria - V. Obatolu, A. E. Okoruwa and J. G. Kling. See M.4.7.
8) Collection of local varieties in Guinea and Nigeria - N.Q. Ng, F.L. Guilavogvi, El Sanonssy Bay, J. G. Kling and S.K. Kim.

M.5.8 Graduate training

1) Genetic control of *Striga* resistance in inbred lines - V. Aditimirin (Ph.D. thesis), M.E. Aken'Ova and S. K. Kim. See M.1.8.
2) Genetic study to determine potential for improving resistance to *Striga hermonthica* using population improvement - L. Akanvou (Ph.D. thesis), J. G. Kling, D. K. Berner and M. Fakorede. See M.1.9.
3) Breeding for nitrogen use efficiency - H. A. Akintoye (Ph.D. thesis), J. G. Kling and E.O. Lucas. See M.1.2.
4) Root growth and nitrogen use efficiency - H. Heuberger (Ph.D. thesis), S. Oikeh (Ph.D. thesis), J.G. Kling, W. Horst, G. Weber. See M.1.3.
5) Genetic study of stem borer resistance/tolerance in inbred lines. - O. Gold (Ph.D thesis), M.E. Aken'Ova and S.K. Kim. See M.2.9 and M.2.10.

M.5.9 Training course

B. Berner, S.K. Kim, J.G. Kling, G. Weber, S. Lagoke (IAR, FAO) and A. Awad (FAO).

A *Striga* resistance breeding and infestation technology course will be offered to African national scientists. The course will be held jointly with FAO Pan-African Striga Control Network (PASCON). - Coordinator, Prof S. Lagoke, IAR-ABU, Zaria, Nigeria.

Grain Legume Improvement Program

L.1 COWPEA BREEDING PROJECT FOR SAVANNA ECOLOGIES

Project Rationale

Cowpea [*Vigna unguiculata* (L.) Walp] is an important food legume in the tropics. Being a warm weather crop with drought tolerance, it is grown in the semi-arid and the low rainfall regions of tropical Asia, Africa and South America. It is particularly important in the savanna region of Sub-Saharan Africa where it originated and forms an integral component of various cropping systems often grown in mixture with cereals without many inputs. Of the world total of 7.7 million hectares under cowpea, Africa alone accounts for about 6 millions hectares. Cowpea is especially valued in the cereal based farming systems of the African dry savanna because protein-rich plant biomass is in short supply there. Green leaves, green pods, green peas and dry grain are all consumed by people. Cattle production is a major activity in the dry savanna, so in many cases the fodder value of cowpea is as important as that of its grain. Major constraints in cowpea production are insect pests, low soil fertility, drought and heat, parastic weeds (*Striga Alectra*) and fungal, bacterial and viral diseases, due to which the average grain yield of cowpea is low ranging from 100 to 300kg/ha.

During the last 2 decades , a number of early maturing high yielding, disease and insect resistant varieties with diverse seed and plant types have been developed by IITA for pure cropping. With proper management and 2-3 insecticidal sprays these varieties can yield about 2 tonnes/ha within 60-70 days. Over 45 countries have released improved varieties from IITA for general cultivation. However, due to non-availability or uncertain availability of insecticides, most of the small-scale farmers, particularly in Africa, are unable to use chemicals for crop protection. The need for spraying insecticides arises from the lack of availability of germplasm lines with a high level of resistance to post-flowering insects such as thrips, *Maruca* the pod borer, and pod sucking bugs. This has limited the spread of improved varieties and the majority of the small scale farmers continue growing cowpea in traditional intercropping systems with little or no inputs. For improving traditional cowpea varieties, IITA established a sub-station at Kano, Nigeria in 1989 which represents agro-ecologies and cropping systems of several Central and West African countries. The rationale for improvement is based on the premise that by scientific expliotation of genetic variability in cowpea germplasm for better growth in poor soils, efficient use of nutrients and moisture, shade and drought tolerance, better competing ability with cereals and resistance to disease and insect pests, it would be possible to improve traditional cowpea varieties for higher yield potential for both grain and fodder. Preliminary work began in 1989 while the station was being established. Systematic research started from 1990. The general strategy is to (1) study major production systems and their constraints, (2) screen cowpea germplam lines and local varieties for desirable traits and (3) develop a sound breeding programme to combine these traits into improved varieties which will fit in traditional systems and give greater yields than local varieties. In addition, IITA is exploring biotechnological possibilities for developing resistance to flowering and post-flowering insects This approach is being pursued in collaboration with advanced laboratories abroad.

Completed Studies

I.D.K. Atokple, B.B. Singh and A.M. Emechebe 1993. Genetics of resistance to *Striga* and *Alectra* in Cowpea. (manuscript under review).

Inheritance of resistance to *Striga* and *Alectra* in B301, IT82D-849, SUVITA-2 and IT81D-994 were studied and a test of allelism was conducted. The results indicate that the genes for resistance to *Striga* in B301, IT82D-849 and Suvita-2 are different from each other. However, the genes in B301 and IT82D-849 are either very tightly linked or multiple alleles at the same locus because no susceptible plants were observed in F2. The data further revealed that the gene for *Striga* resistance in IT82D-849 is derived from Emma 60, a germplasm line from Uganda. The genes for resistance to *Alectra* in B301 and IT81D-994 are also different from each other.

B.B. Singh and I.D.K. Atokple 1993. New sources of resistance to *Striga* in cowpea (manuscript in preparation).

Presently B301 and IT82D-849 are being used as sources of resistance to *Striga* in cowpea. Therefore, to diversify the genetic base for resistance, 1600 germplasm lines comprising 800 from West Africa and 800

from East and southern Africa were screened to identify additional sources of resistance to *Striga*. These lines were planted in the *Striga* sick plot and 6 weeks after planting only 158 lines were found free from *Striga*. The remanent seeds of these were planted in the screen house in pots inoculated with *Striga* seeds to eliminate the possibiliy of escapes in the field. Of these, only 19 lines remained free which were again tested using inoculated pots with 4 replications. All the 19 lines remained free from *Striga*. Interestingly, all of these lines are from East and southern Africa, B301 and Emma 60 also come from East and Southern Africa where there is no *Striga*. All the lines from West Africa were highly susceptible except for a few which showed delayed emergence and less *Striga*.

B.B. Singh and S.F. Blade 1993. Genetic variability for yield performance of cowpea under intercropping with millet. (Manuscript in preparation).
Over 1200 new breeding lines were screened for yield performance when intercropped with millet in 1989, 1990 and 1991. A great deal of genetic variability was observed and the best 18 lines were then tested at 3 locations using 2 local checks in 1992. Most of these lines had grain yields of over 300kg/ha and were significantly better than the local varieties. The best among these are IT89KD-457, IT90K-261-3, IT89KD-391, IT84D-666 and IT89KD-307 which will be used as parents in the breeding program.

B.B. Singh and A.M. Emechebe. 1993 Registration of improved *Striga* resistant cowpea breeding lines.
Several new breeding lines have been developed which combine resistance to aphid, bruchid, thrips, *Striga* and *Alectra* along with several diseases. Of these IT90K-59 and IT90K-76 have done well in multilocation trials.

Blade, S.F., D.E. Mather, B.B. Singh and D.L. Smith, 1992.
Evaluation of yield stability of cowpea under sole and intercrop management in Nigeria. Euphytica 61: 193-201.
IITA cowpea lines evaluated in this study yielded similarly to traditional cowpea lines in Nigeria, but exhibited high yield potential when tested under improved management.

Activities

L.1.1 Study of cropping systems
Gerben van Ek, Jantien van Ek (VSO), B.B. Singh, S.F. Blade, D. Florini, H. Bottenberg, Sule Gaya (KNARDA)
Cowpea is primarily grown in mixtures with cereals like millet and sorghum in the northern savanna and with maize and cassava in humid and sub-humid zones. The cropping systems are diverse and not only differ from region to region but also from farmer to farmer. Therefore, in the past, general field surveys were conducted in Nigeria, Benin, Togo, Ghana, Burkina Faso and Niger Republic to identify major cropping systems involving cowpeas. A quantitative study was initiated in 1991 on 14 selected farms where cowpeas intercropped were in Minjibir and Gezawa local government areas of Kano State. This survey continued in 1992 on 42 farms. A 20m x 20m block is marked in each field and detailed notes are taken on field history, land preparations, crops and varieties planted, dates of planting, planting densities, planting patterns, diseases, insects, maturity, harvesting and yields of grain and fodder. This permits the quantitative description of different systems and their constraints which will help sharpen the research focus. Preliminary observations indicate that farmers intercrop two types of cowpea varieties, – one for grain and one for fodder, in alternate rows with millet and/or sorghum in the same field. Both varieties are photoperiodic and have spreading growth habit but the grain type has short duration and is planted earlier than fodder type. Principal constraints seem to be low population density, competition with cereals, diseases and insect pests and late maturity but the main yield reducing factor for cowpea grain is the pod borer, *Maruca*. The project will continue for one more season.

L.1.2 Cropping systems in the dry savanna
A. Moutare, M. Nouhou (INRAN), S. Blade, B.B. Singh, D. Florini and H. Bottenberg.
A preliminary survey of cropping systems was done around Niamey and Maradi in Republic of Niger which is the second major country of cowpea production. A few selected farms at both sites will be studied in the 1993 crop season. Component crops in the mixture, planting densites, spacing, planting dates, disease, insect pests, yield of grain and fodder of each component will be determined. This will expand our understanding of production constraints in dry savannas.

L.1.3 Collection and evaluation of local varieties

B.B. Singh, and M. Ishiyaku

A total of 36 local varieties from northern Nigeria were evaluated for agronomic characteristics in replicated trials at Kano, Gumel, and Samaru using 3 improved local type breeding lines as checks. All the varieties were photoperiodic and had spreading growth habit but some were earlier maturing than others. The early maturing ones were normally grown for grain and the late ones for fodder. There was considerable variability and heterogeneity for seed type and other agronomic characters. All local varieties were highly susceptible to aphid and performed poorly compared to the improved local type varieties which combine resistance to aphid, bruchid and thrips. This trial will be repeated for one more season.

L.1.4 Breeding value of local varieties

B.B. Singh, Sanusi Gaya, S. Blade and NARS scientists

Local vareities differ from region to region, and vary widely for seed type, maturity and photoperiod sensitivity. To improve local varieties or to develop local-type varieties we need to better understand their genetic diversity. Farmers' fields in northern Nigeria were visited in 1991 and 1992 crop seasons and local varieties were collected. These had a mixture of many types of seeds which have been separated and will be evaluated. In 1993, farmers' fields around Maroua (Cameroon), Kamboinse (Burkina Faso), Bamako (Mali), Niamey and Maradi (Niger), and Sokoto and Maiduguri (Nigeria) will be visited and seeds of local varieties collected and evaluated in collaboration with national scientists. Interesting materials will be used in the breeding program.

L.1.5 Local variety x improved line crossing

B.B. Singh, D. Florini, H.W. Rossel

The best available local varieties or local-type varieties (spreading, photoperiod sensitive, late) were crossed with improved breeding lines to incorporate resistance to the major diseases and pests into the traditional plant type. F_1 plants were raised in the screenhouse and segregating populations were grown in the field. Selected progenies are being tested and selected for resistance to viruses, aphid, bruchid, *Striga* and *Alectra* as well as for the traditional plant growth habit.

L.1.6 Farmers' participatory evaluation of advanced breeding lines

Sanusi Gaya, S. Blade, B.B. Singh, D. Florini, H. Bottenberg and E.C. Odion (IAR)

Breeding varieties for high input management is satisfactorily done at experiment stations. However, screening varieties for low input farming on experiment stations may not be relevant because soil fertility levels and management practices are so diverse among farmers. This activity involves farmers at the penultimate stage of breeding. About 200g seeds of advanced lines were given to 36 selected farmers at Minjibir Local Government Area to be grown using their own management regime together with their preferred local varieties. Each farmer had 2 lines (one grain type and one fodder type) and the same varieties were given to four farmers. The plots were monitored by researchers for agronomic performance, and farmers were asked to advise on the strengths and weaknesses of the new lines. The reaction of farmers was quite good and they were eager to try some of the varieties again. This project will continue.

L.1.7 Multilocation testing of improved photoperiod sensitive varieties under various intercropping systems.

S. Blade, B.B. Singh, D. Florini and H. Bottenberg

A number of photoperiod sensitive cowpea lines have been developed which look like traditional vareities but have added resistance to aphid, bruchid, and thrips and a shortened time to maturity. These were evaluated in different cropping systems at different locations (on and off-station) using traditional varieties as checks. A considerable genetic differences were observed and genotype x location and genotype x cropping system interactions were obtained. This will continue in the coming crop season.

L.1.8 Multilocation testing of advanced lines

B.B. Singh, M. Ishiyaku, D.F. Florini, S. Tarawali (ILCA), A. Moutare (INRAN), M. Nouhou (INRAN), J. Detongnon (Maroua)

Advanced breeding lines are grouped in several trials and relevant ones are tested annually at Kano, Samaru, Ibadan, Abuja, Gumel, Maiduguri, Niamey, Maradi, and Maroua. Six types of trials are conducted: (1) Early-maturing (60-70 days) erect and semi-erect lines for grain (as opposed to fodder) (2) Intermediate maturing (70-80 days) erect and semi-erect lines for grain (3) Intermediate maturing (75-85 day) spreading

growth habit lines for grain. These were derived from crosses of traditional local photoperiod sensitive varieties with high yielding improved lines in an attempt to combine the plant habit of the former with the aphid, bruchid, thrips and disease resistance of the latter. The lines are evaluted under three management regimes: pure crop with 2-3 sprays, intercrop and pure crop without sprays (4) Dual purpose (fodder + grain) derived from local x improved crosses in the 85-90 day class, to replace farmers' 100-120 day varieties which yield little grain and are subject to terminal drought risk (5) Late maturing (100-125 days) cowpea varieties and other selected fodder species, tested in collaboration with ILCA at Kano and Kachia without any fertilizer or insecticides, mainly for increased fodder yield but grain yield is also measured (6) New *Striga* resistant breeding lines, tested at two locations in Kano. Some entries have complete resistance to both *Striga* and *Alectra* and some have moderate to high resistance to one or both. Their growth habit ranges from erect early to spreading photoperiod sensitive and late. Several promising breeding lines were selected from each group in 1992 trials and these will be further tested in 1993.

L.1.9 Selection methods for intercropping performance

B.B. Singh

An experiment was initiated in 1991 to determine whether selection for intercrop and performance in segregating populations must be done under intercropping, or can also be done in pure cropstand which is easier. Two F_2 populations were planted in pure crop (with 2 sprays) and in intercrop without spray. Individual F_2 plants were selected from each population. The respective F_3 progenies were again planted in 1992 in pure and intercrop and desirable F_3 plants were selected. This will continue until the F_6 generation after which the selected lines will be evaluated for intercrop performance.

L.1.10 Screening for intercropping performance

S.F. Blade, B.B. Singh, and D. Florini

All new breeding lines (over 300) in advanced and preliminary trials were planted intercrops with millet in 2 replications without insecticide sprays and evaluated for grain and fodder yield along with local varieties as checks. Much variability was observed for both grain and fodder yield in 1990 and 1991. The best lines were tested in larger plots in 1992. The most promising ones will be further tested in 1993 at several locations.

L.1.11 Pest resistance breeding of local varieties

B.B. Singh, Bottenberg, D. Florini and A.M. Emechebe (IAR)

Resistance to pests will be incorporated into traditional local varieties, which are readily accepted by farmers. This will stabilise their production while attempts will be made to develop new varieties with higher yield potential. In consultation with national program scientists, two or three best traditional varieties from Burkina Faso, Cameroon, Mali; Republic of Niger and Nigeria will be selected as recurrent parents in a backcrossing program involving IT90K-59 and IT90K-76 as donors for resistance traits. The work began in 1992 and TN5-78 from Republic of Niger, 58-57 from Senegal and Kanannado and Borno local from Nigeria were crossed to IT90K-59 and IT90K-76.

L.1.12 Screening for field resistance to insects

B.B. Singh and H. Bottenberg

All the breeding lines included in advance and preliminary trials (over 300) are evaluated under pure cropping without insecticide protection. Large differences in insect damage were noted in 1991 and 1992 and grain yield ranged from nil to over 600kg/ha with high fodder yield. Selected lines will be further tested in 1993 using larger plots and several locations to reconfirm these results.

L.1.13 Screening for insect resistance

B.B. Singh and H. Bottenberg

Screening for insect resistance is done using a combination of field incidence and laboratory screening. Incidence of thrips, aphids, pod borer (*Maruca*) and pod sucking bugs is noted in monocrop trials without insecticide. Artificial infestation with aphids and bruchids is done in the laboratory. Many lines show combined resistance to aphid; bruchids and thrips but little or no resistance to *Maruca* and pod sucking bugs. The available thrips resistance is also low but noticeable in the field. It has been obseved that lines which possess even this low level of thrips resistance plus less susceptibility to *Maruca* and pod bugs produce some grain without insecticide spray therefore, efforts are being made to select for this combination of "resistance". This is a contining longer term project.

L.1.14 Screening for disease resistance

B.B. Singh, D. Florini. H.W. Rossel, A.M. Emechebe (IAR)

Entries from the advanced preliminary trials are screened at hotspots for different diseases (scab, (Septoria), leaf spot and black spot, blight (Ascochyta), bacterial blight, ashy stem blight etc). Screening for virus resistance is done at Ibadan in screenhouses. Entries showing combined resistance to virus, bacterial blight and other diseases have been identified. These will be further tested and used in breeding. This is also a continuing longer term project.

L.1.15 Additional sources of pest resistance

B.B. Singh, D. Florini, N.Q. Ng, A.M. Emechebe(IAR) and A. Moutare (INRAN)

Resistance is available for *Striga, Alectra*, scab, *Septoria* bacterial blight and ashy stem blight in elite breeding lines. However, there is a need to identify additional sources of resistance in case new strains arise. A large number of healthy (virus and pathogen free) germplasm lines will be screened each year for the next 4 years to identify additional sources of resistance and study their genetic control. Screening will be done at Kano, Samaru and Niamey. The first set of 1600 lines were screened at Kano and Samaru in 1992 and several new sources of resistance to *Striga, Alectra Septoria* and scab have been identified. Additional lines will be screened in 1993.

L.1.16 Breeding for combined resistance to different viruses

B.B. Singh and H.W. Rossel

TVU401 was found to be completely resistant to a number of viruses. This line has been crossed with improved breeding lines such as IT90K-59, IT90K-76 and eight others to incorporate multiple virus resistance into these breeding lines. F_1 and F_2 progenies will be grow in 1993.

L.1.17 Inheritance of blight resistance

B.B. Singh and D. Florini

Bacterial blight and ashy stem blight are two major diseases in savanna ecologies. A few sources of resistance have been identified. A genetic study was initiated to elucidate the nature of inheritance of resistance to these diseases which will facilitate their use in the breeding programme. Crosses were made involving bacterial blight resistant parents; for ashy stem blight the resistant lines need to be reconfirmed before they are used in genetic studies.

L.1.18 Genetic studies in cowpea

B.B. Singh and M. Ishiyaku

To support the ongoing breeding program, basic genetic studies on male sterility, resistance to nematodes, plant characteristics – black spot seed type, plant growth habit and photoperiod sensitivity are in progress. A number of male sterile and other mutants are being maintained.

L.1.19 Screening against *Striga* and *Alectra*

B.B. Singh and A.M. Emechebe (IAR)

Over 300 new breeding lines were screened in 1991 for *Striga* and *Alectra* resistance in the field and screenhouse with known resistant lines like B301 and IT82D-849 as checks. The objective was to identify additional sources of resistance so that a program for breeding for horizontal resistance could be initiated. A number of lines showed high levels of resistance and these were tested further in 1992. Fourteen lines showed moderate to complete resistance to *Striga* at Kano. These will be tested at several locations across West Africa in 1993.

L.1.20 Genetics of *Striga* and *Alectra* resistance

B.B. Singh, I.D.K. Atokple and A.M. Emechebe (IAR)

Systematic genetic studies were conducted to elucidate the allelic relationship between the genes conferring resistance to *Striga* in B301, IT82D-849 and Suvita-2 and IT81D-994. The genes for *Striga* resistance in B301, IT82D-849 and Suvita-2 are different from each other. Similarly, IT81D-994 has a different gene for *Alectra* resistance than the two present in B301. These lines are now being crossed to the new sources of resistance identified during 1992 germplasm screening to continue further tests of allelism.

L.1.21 Effect of resistant lines on the *Striga* population over time

B.B. Singh and I.D.K. Atokple and K.F. Cardwell

An experiment was initiated in 1990 in which resistant and susceptible lines along with fallow and intercrop treatments are planted in the same plot year after year. The *Striga* population is assessed by planting "windows" of a susceptible variety and also by counting *Striga* seeds in the soil. The experiment will extend over 4 years to ascertain the effect of resistant varieties and other treatments in reducing the *Striga* population.

L.1.22 *Striga* yield loss assessment

B.B. Singh and I.D.K. Atokple

A set of isogenic lines have been developed which differ with respect to *Striga* resistance. The performance of the these lines was studied with different levels of *Striga* infestation to assess yield reduction due to *Striga* in 1992. The experiment will be repeated in 1993.

L.1.23 Possibility of new biotypes of *Striga gesneriodes*

B.B. Singh, Remi Adeleke, S.R. Voudouhe (Benin Rep.) and A. Lane (Long Ashton)

In 1990 there was a report of a strain of *Striga gesneriodes* in Benin Republic that could overcome the resistant of B301. Observations in 1991 supported this finding but were not conclusive. Further testing in 1992 showed about 10% plants of B301 and about 30% plants of IT82D-849 as susceptible. The seeds obtained from *Striga* plants attached to B301 and IT81D-849 plants are being used in pot culture experiments to test against B301, IT82D-849 and derived lines to a confirm whether or not there is a new strain.

L.1.24 Screening cowpea varieties for higher leaf yield and quality for human use.

B.B. Singh, Jantien van Ek, Hajia Aliu (KNARDA), Y.W. Jeon, L. Halos, S. Das-Gupta (UNICEF)

Cowpea leaves are widely used as spinach in East and Southern Africa and a few region in West Africa. Quality analyses have shown upto 27% protein in leaves on dry basis which is similar to that in grains. Preliminary cooking trials have shown that cowpea leaves can be accepted for human consumption in northern Nigeria and possibly in other West African countries. Therefore, a number of improved and local varieties will be evaluated for leaf yield as well as cooking quality in 1993. The main rationale for this project is based on the fact that insects cause low grain production in cowpea but do not affect the leaves. If cowpea leaves can be used for human consumption, both fresh and as dehydrated, cowpea can be a major source of protein in the dry savannas where several inferior kinds of leaves are used.

L.1.25 Breeding cowpea for grain quality

B.B. Singh, S. Nielsen (Purdue Univ.) and M. Bokanga

Considerable genetic variability has been observed for protein content (22 to 32%) and cooking time (21 to 62 min.) in cowpea. Therefore, a breeding program has been initiated to develop cowpea varieties with higher protein content and reduced cooking time. A few crosses have been made and more will be done in 1993.

L.1.26 Cowpea performance under irrigation

B.B. Singh and S.F. Blade

Several countries in the savanna region have developed irrigation facilities where wheat or vegetables are grown in the dry season. Cowpea can be an alternative crop. A trial consisting of 10 cowpea varieties was planted at 3 dates: January 19, January 31, February 20, 1991 at Wudil, Kano State in collaboration with Hadejia-Jamaare River Basin Authority to identify cowpea varieties suitable for planting in fields where wheat could not be planted on time. A few varieties such as IT84S-2246-4 yields had grain of about 2 tonnes/ha. The trial was repeated in 1992 and IT84S-2246-4 and other lines derived from it, had grain yields of over 1.5 tonnes/ha. The selected varieties will be tested in agronomy trials for maximum grain yield potential and new breeding lines will be evaluated in variety trial.

L.1.27 Screening cowpea varieties for Rice Fallows in Fadamas

B.B. Singh, B.N. Singh (WARDA), Remi Adeleke

WARDA'S cropping system program has shown interest in evaluating new cowpea varieties in rice fallows on residual moisture with little or no insecticide protection. Even if 300-400kg/ha grain yield can be obtained without spraying, it will have tremendous scope for the hydromorphic fadamas of West and Central Africa. Therefore, a joint experiment with WARDA was initiated. Sixteen early maturing newly

developed breeding lines were planted in rice fallows at Ibadan in a split plot design with zero and one spray treatments. The promising varieties will be tested across several locations in 1993.

L.1.28 Screening cowpea varieties for harvest and post-harvest characteristics

B.B. Singh, Y.W. Jeon, L.S. Halos, Sule Gaya (KNARDA)

Harvesting, threshing, cleaning and storage of traditional cowpea grain is labour intensive due to multiple picking, hard pod walls, and manual threshing which also reduce seed quality. Improved varieties will be compared with traditional varieties for these characteristics and new breeding lines will be screened.

L.1.29 Breeding for drought tolerance

B.B. Singh and T. Terao (TARC)

The drought tolerant lines selected following field and pot culture screening are being crossed to improved breeding lines. The segregating populations will be used for genetic studies as well as for developing new breeding lines combining drought tolerance with multiple disease and insect resistance.

L.1.30 Induced genetic variability for resistance to *Maruca*

B.B. Singh and H. Bottenberg

None of the cowpea germplasm lines are resistant to *Maruca* which is the major pod insect pest in cowpea. *Vigna vexillata,* a wild relative is resistant but cannot be crossed to cultivated cowpeas. Therefore, a mutation breeding approach is being initiated to induce resistance to *Maruca*. Ten cowpea varieties were irradiated with 20 Kr and 30Kr gamma rays through the courtesy of International Atomic Energy Agency (IAEA). These will be followed through M_1, M_2 and M_3 generations and screened for resistance to *Maruca*.

L.1.31 Ecological analysis of interaction in a millet-cowpea intercrop

T. Terao

This study is planned to investigate the effect of millet on cowpea. Evaluation of plant density and date of planting of millet on the cowpea micro-environment will be determined. Canopy structure (using layer harvesting), light penetration into the canopy and quantitative analysis of root distribution will be conducted.

L.1.32 Variation in photosynthetic adaptation of leaves in low light conditions

T. Terao

Cowpea grows under reduced levels of light in a cereal intercrop. To evaluate shade adaptation wild cowpea lines from four climatic zones ranging from forest to sahel savanna and two improved (erect and prostrate) cowpea lines will be grown to identify which type is most adapted to low-light conditions. Shade adaptation screening techniques and investigation of what regulates the shade adaptation response will be carried out.

L.1.33 Analysis of on-farm physiological constraints

S.F. Blade and T. Terao

In co-operation with local farmers, measurement of light interception and canopy structure will be done with the five major cropping systems identified in the Kano region. Moisture and nutrient status of crops will also be quantified.

L.1.34 Analysis of water stress

T. Terao

Drought resistant lines will be studied to identify the specific mechanism of tolerance, and biochemical and molecular biological methods will characterize specific plant responses under drought conditions (protein, nRNAs).

L.1.35 Evaluation of organic/inorganic fertilization in savanna cropping systems

S.F. Blade and T. Terao

Due to the complete removal of above ground biomass in traditional cropping systems, additional nutrients must be returned to the soil to guarantee sustained crop production. The role of farmyard manure and inorganic fertilizers were assessed in 1992 on both sole cowpea and a millet-cowpea intercrop. Crops will be planted on the identical experimental plots in 1993 to assess yield and nutrient status.

L.2 WIDE CROSSING FOR INSECT RESISTANCE IN COWPEA

Project Rationale

Pod borers (*Marcuca* spp.) and pod sucking bugs (PSB) are major pests of domesticated cowpea (*Vigna unguiculata* ssp. *unguiculata*) and commonly cause substantial losses in grain yield. Some wild subspecies of *unguiculata* and some other wild species eg *V. vexillata* display moderate to high levels of resistance to these insect pests. Therefore wide crossing between cowpea and its wild relatives offers a way of transferring insect resistance into the domesticated crop.

Completed studies

N.Q. Ng, 1992. Wide crossess of *Vigna* food legumes. Page 75-80. In: Thottappilly, G., L. Monti, D.R. Mohan Raj, and A.W. Moore (eds.). Biotechnology: Enhancing Research on Tropical Crops in Africa. CTA/IITA co-publication. IITA, Ibadan.

Many successful wide crosses among Asiatic cultivated *Vigna* species and between them and a wild species, *V. trilobata*, have been reported. However, despite many attempts to cross the African cultivated *Vigna* species with wild *Vigna* species and the Asiatic cultivated *Vigna* species, there has been no success as yet. Nevertheless, crosses between cultivated and wild subspecies of *V. unguiculata* were fairly easy and their hybrids are fertile. Studies conducted at IITA have shown that crossing barriers between *V. unguiculata* and several African wild *Vigna* species were due to abortion of young embryos and pollen-pistil incompatibility. With more basic research on reproductive biology and tissue culture, and a larger number of crosses among species and genotypes, there will be a greater chance of success for wide crosses between *V. unguiculata* and wild *Vigna* species which possess some very desirable traits for the improvment of this cultivated species.

Thottappilly, G., S. Padulosi and N.Q. Ng. Screening for virus resistance in wild cowpea (*Vigna unguiculata* (L.) Walp.). In: Joint cowpea Biotechnology workshop Bari (Italy), June 29 - July 1, 1992.

Germplasm of about 350 accessions of wild *Vigna unguiculata* collected from all over sub-Saharan Africa were tested against three viruses: cowpea yellow mosaic virus (CYMV), cowpea mottle virus (CMeV) and cowpea aphid borne mosaic virus (CAbMV). The objectives were to characterize the germplasm accessions, to identify sources of resistance, to investigate if there is any difference in reaction to these viruses among the varieties within this species and to determine if the resistant germplasm accessions have a specific range of geographical distribution. The results have shown that a total of 22 accessions were found to be resistant to CYMV, 38 accessions to CMeV and 147 accessions to CAbMV. There is no specific correlation between geographical origin of the accessions and their resistance to viruses. It appeared that the resistant accessions are randomly distributed all over Africa.

Ng, N.Q., J. Apeji and S.Y.C. Ng (unpubl.). Crossability of cowpea with *V. vexillata* and embryo rescue of putative hybrids.

Previous research on crosses between cowpea and *V. vexillata* revealed abortion at the globular stage. Attempts were then made to make many crosses in the hope of obtaining a successful hybrid between the two species. Out of 11,771 pollinations made, only 183 'hybrid pods' remained on the peduncles for more than five days. Very few of these young seeds had embryos that were big enough to be excised. These were apparently accidental self-seeds, as those that could be rescued by embryo culture were no different from mother plant. Any chance of getting hybrids seem to rely on ovule or pod culturing or other inovative approaches, such as protoplast fusion and manipulation of ploidy level.

S. R. Schnapp, L. W. Kitch, G. O. Myers, R. A. Bressan P. M. Hasegaw. Interspecific hybridization between *Vigna* species using in vitro culture to rescue abortive hybrid embryos.

An interspecific hybrid between *V. oblongifolia* and *V. luteola* is reported. Fertile hybrid plants were obtained by embryo rescue and confirmed by DNA fingerprinting.

S. R. Schnapp, A. Del Giudice, F. Saccardo, L.W. Kitch, N.Q. Ng, S. Padulosi and P.M. Hasegawa. Embryo development and interspecific incompatibility between *V. vexillata* and *V. unguiculata*.

Activities

L.2.1 Cowpea Wide Cross and Biotechnology Research Working Group

N.Q. Ng, G. Myers, S. Schnapp, L.E.N. Jackai, G. Thottappilly, S. Padulosi, H. Mignouna, P. Petrilli, S.Y.C. Ng, external collaborators at Purdue and tthree Italian research institutes/Universities.

This multidisciplinary research group was formed in 1992 to provide linkages and multidisciplinary approaches for application of non-conventional methods for cowpea improvement and germplasm management. A mode of operation for the research group has been established. The main emphasis of the research group is to increase plant resistance to flowering and post-flowering pests of cowpeas and to provide better knowledge about germplasm diversity and inter-relationships among *Vigna* species. The approaches for solving the problem of post-flowering insect pests of cowpea include the identification and characterization of resistance genes and incorporation of these genes into cowpea cultivars. This could be accomplished through conventional and wide-crosses or biotechnological innovations such as genetic transformation of cowpea through *Agrobacterium*, gene gun or electroporation as well as protoplast fusion. Collaborative activities are underway in the various laboratories within IITA and with various advanced laboratories, notably the University of Napoli, Italy; the Germplasm Institute, Bari, Italy and Purdue University, USA.

L.2.2 Outcrossing mechanism in wild cowpea

N.Q Ng and J. Apeji

Spatial separation of the anther from the stigma in wild cowpea proves to be a major cause of outcrossing. This character is genetically controlled. A systematic survey will be made to ascertain what proportion of the germplasm accessions of the various *Vigna* species including cultivated land races have wide spatial separation of the anther from the stigma. This information is important for germplasm management and utilization.

L.2.3 Cowpea interspecific hybrids for insect resistance

N.Q. Ng, T. Mesfin, L. Jackai, H. Rossel and D. A. Florini

An interspecific cowpea hybrid from crosses made between an improved cowpea and a hairy-pod wild cowpea were advance to F6 generation. Some of the agronomically promising lines were selected and tested against bruchid and aphid during 1992. Several lines were shown to be more resistant to bruchid than the resistant control (TVu 2027). These selected lines will be advanced and multiplied and they will be tested against other pests and diseases.

L.2.4 Interspecific hybridization in *Vigna*

S.R. Schnapp and N.Q. Ng

Attempts to achieve interspecific hybridization are continuing. Efforts are focused on obtaining hybrids between cowpea and wild species *V. vexillata* and *V. luteola* . Embryo rescue techniques are now available and the emphasis will be on incorporating more diversity into the crossing programme.

L.2.5 Embryo rescue of *Vigna* hybrids

S. R. Schnapp

A system has been developed for obtaining plants from self-pollinated fruits that are excised from the plant as early as one day after pollination using a combination of ovary culture and embryo culture. These methods will be applied to obtain interspecific hybrids between *V. vexillata* and *V. unguiculata* as well as *V. luteola* and *V. unguiculata*. We are also attempting to understand the physiological factors affecting embryo development in order to further optimize the system.

L.2.6 Interspecific hybridization of *Vigna* t etraploids (combines L.2.7, 2.8, 2.9)

S. R. Schnapp, F. Saccardo (Univ. of Naples), and N.Q. Ng

An alternative approach for achieving interspecific hybridization involves performing crosses at the tetraploid level. Colchicine treatment has been used to obtain putative polyploids of *V. unguiculata*, *V. oblongifolia* and *V. luteola*. Colchicine treatments from 0.1 to 0.5% applied to axillary buds resulted in a number of phenotypically abnormal plants. Seed has been collected from plants suspected of being polyploid on the basis of stomatal size and number, and a cytological examination will be performed to confirm polyploidy. Second generation plants are being evaluated to determine the stability of polyploidy. Confirmed polyploids will be used in interspecific hybridization efforts.

L.2.7 Cytogenetics of putative tetraploids

R. E. Ugborogho, F. Saccardo (Italian Institutes)
N. Q. Ng, G. Thottappilly and S. R. Schnapp

Cytogenetic confirmation of chromosome numbers will be carried out on putative polyploids. It may also be possible to identify aneuploids that may be useful in genetic studies. Further, a survey of the somatic chromosome number, size and morphology of selected accessions will be undertaken to facilitate interspecific hybridization. Karyotype differences responsible for incompatibility and embryo abortion may be detected.

L.2.8 Resistance bioassay for pod-sucking bugs

L.E.N. Jackai, R.E. Shade (Purdue Univ.) and S. R. Schnapp

A callus bioassay system has been developed for *Maruca testulalis* and efforts are underway to develop the system for application to pod sucking bugs and thrips. This system will be used to rapidly screen callus material after transformation with putative insect resistance genes.

L.3 SOYBEAN IMPROVEMENT

Project rationale

From 1986 through 1991 there has been a rapid expansion of soybean production in Nigeria and Zambia and other countries in Africa have initiated research and extension projects on soybean. As might be expected, the increase in soybean production has also brought about an increase in soybean disease. Increased insect problems are also expected. Soybean breeding for higher yield and resistance to these pests will continue in Nigeria and a new breeding effort will be initiated in southern Africa. Emphasis will be placed on (i) utilizing germplasm from strong national programs such as Brazil, Thailand, India, etc. and IITA, and (ii) further improvement of elite germplasm for resistance to pod shattering, lodging, pod bugs, frogeye leaf spot (*Cercospora*) and red leaf blotch.

Activities

L.3.1 Soybean improvement for the savanna

K. Dashiell, L. Jackai and C. Akem

Crosses are made using parents from the IITA breeding program, Brazil and Zimbabwe and germplasm lines. The populations are advanced through F_6 using the pedigree method. Screening methods are used for identifying lines with resistance to *Cercospora* leaf spot, bacterial pustule, pod shattering, pod sucking bugs and *Spodeoptera littoralis*.

L.3.2 Multilocational evaluation of soybean

K. Dashiell, L. Jackai, C. Akem, and L. Bello (University of Agric, Makurdi)

Trials are conducted at Zaria, Mokwa, Zonkwa, and Makurdi. The trials are organized into three maturity groups: early (90-105 days), medium (105-120 days) and late (120-135 days). They are fertilized with 50 kg of 15N;15P,15K and 100 kg of 0N,18P,0K per hectare; no pesticides are used. Data collected includes grain yield, disease ratings, pod sucking bug damage, nodulation, shattering, lodging, days to maturity, seed color, seed size and seed longevity.

L.3.3 Genetics of resistance to soybean insect pests

G. Semakula-Nankinga, K. Dashiell, I. Fawole (Univ. of Ibadan) and L.E.N. Jackai

The objectives of this study are: (i) to explore the possibility of existence of both pod and seed resistance to the southern green stink bug *N. viridula* in some soybean genotypes; (ii) to investigate the mode of inheritance of pod and/or seed resistance to *N. viridula* in (i) above and to determine any linkage relationships; (iii) to identify some leaf, shoot and pod characteristics that are associated with resistance to the southern green stink bug and to the defoliator *S. littolaris*; and (iv) to identify any transgressive segregants with better resistance to the two pests in crosses of resistant x resistant genotypes.

L.3.4 Breeding for resistance to frog-eye leaf spot (*Cercospora sojina*) in soybean (Glycine max (L.) Merrill).

Ebot, M.E. and K.E. Dashiell

Objectives are to investigate the response of the hybrids to the various isolates or races and to determine the mode of inheritance of the resistance gene.

L.3.5 Changes in seed quality of soybeans during seed production and storage.

D.K. Ojo and K.E. Dashiell

Objectives are to determine the potential seed longevity among contrasting soybean genotypes under tropical field conditions. In addition to determine the heritability of seed storability in a cross between 2 contrasting soybean genotypes from the point of view of laboratory germination and field emergence and to test the hypothesis that soybean seeds attain maximum quality at harvest maturity under field conditions.

L.3.6 Inheritance of promiscuous nodulation and resistance to bacterial pustule disease in soybean

B.M. Yeti and K.E. Dashiell

Objectives are to develop screenhouse techniques to simultaneously screen for nodulation and reaction to bacterial pustule disease on the same soybean plant. Investigate the possibility of a linkage between gene(s) for promiscuity and those for susceptibility to bacterial pustule.

L.3.7 Screening against the root-knot nematode

I.C. Charles, K. Dashiell, G. Atiri and C. Akem

Objectives are to screen for resistance to root-knot nematodes and SMV and to evaluate the interaction of these two pathogens on varieties that are susceptible to both.

L.3.8 Soybean breeding in southern Africa

K. Dashiell, C. Akem, and NARS scientists in Zambia

(E. Munsanje and F. Javaheri), Zimbabwe, Tanzania and Mozambique

The objectives are to incorporate promiscuous nodulation, improved seed longevity and resistance/tolerance to red leaf blotch (RLB) into lines adapted to the high latitudes and elevations in southern Africa. Discussions with scientists in the region and a literature review are needed to determine the most efficient strategies to be used in this work. Zambia has already requested collaboration in the following areas: exchange of germplasm, screening for RLB resistance, RLB etiology and epidemiology research, exchange visits of scientists, information exchange and joint publications.

L.4 NODULATION IN IITA SOYBEAN LINES

Project rationale

Identification and utilisation of promiscuous nodulation in soybeans is viewed as one of the important contributions made by the IITA soybean program. It has contributed to the expansion of soybean production and the relative high yields under low inputs farming as commonly practised in Africa. However, available evidence suggests that the yield potential may be improved by further exploiting and improving the existing symbiotic relationship between these soybean genotypes and the indigenous rhizobia populations. Understanding the host requirements and population dynamics of the compatible rhizobia therefore becomes relevant to the breeding program since it will offer strategies for effective evaluation and improvement of the existing germplasm. To this end, an IITA-NifTAL collaboration has been initiated to carry out activities that may offer the needed inputs and strategies for improvement in the sustainable nitrogen resource utilization by these soybean genotypes.

Activities

L.4.1 Evaluation of promiscuous soybeans in diverse soils

R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NifTAL)

Soils would be collected from a wide range of farmers' fields and brought to IITA for Most Probable Number (MPN) evaluation and testing in pots under greenhouse conditions. Tests would include a uniform set of soybean lines and nitrogen source treatments viz (i) dependence on indigenous rhizobia populations, (ii) inoculation with nodule isolates from promiscuous genotypes (to simulate enrichment), (iii) inoculation with *Bradyrhizobium japonicum* and (iv) mineral N addition.

L.4.2 Field trials: Evaluation of promiscuous soybeans in farmers' fields.

R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NifTAL)

Information from activity L.4.1 would be used to follow up the performance of promiscuous soybean varieties under farmer practices using several small plots. Treatments would be the same as for the greenhouse evaluation.

L.4.3 Nitrogen response and nitrogen use efficiency of promiscuous soybeans

R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NifTAL)

Selected promiscuous and non-promiscuous soybean lines would be tested in potted soils/sand in the greenhouse for relative N response over a wide range of available soil nitrogen. Nitrogen Use Efficiency would be examined using N-15 labelled fertilizer.

L.4.4 Survival and persistence of introduced *Bradyrhizobia*.

R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NifTAL)

Selected sites (3) from the 1992 field trials would be evaluated. Soil samples would be obtained from previous uninoculated, plus nitrogen, *B. japonicum* inoculated, *Bradyrhizobia*. spp. inoculated and maize plots. Sampling would be done every other month up to planting time of the season. Mini-plots from these would be planted to soybeans to evaluate the possible rhizosphere effect in the initial rapid proliferation of the rhizobia prior to root infection and nodulation.

L.4.5 Characterization of *Bradyrhizobia* isolates

R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NifTAL)

Isolates of *Bradyrhizobia* which were obtained from various locations using cowpea, promiscuous and non-promiscuous soybeans will continue to be evaluated for their symbiotic efficiencies, carbohydrate utilization, growth performance on defined media, pH reaction, and sensitivity to low levels of antibiotics.

L.5 SOYBEAN UTILISATION

Project rationale

Soybeans are an inexpensive protein source that can balance the diets of people too poor to afford animal protein. The first phase of this IDRC supported project (1987-1990), carried out at IITA and IAR&T in Oyo State, Nigeria, showed that soybean can augment traditional legumes and oil seeds and fortify high carbohydrate foods. In the second phase (1990-1994) the objective is to develop and encourage small-scale household processing and utilization in Nigeria and Ghana. We are collaborating with four institutions in Nigeria (IAR&T, NCRI, UNN, NAERLS) and one in Ghana.

Completed studies

Soybeans in Ngierian foods. (A booklet of 72 recipies), IITA, Ibadan, Nigeria.
(Osho, S.M., H.O. Ogundipe and V.A. Obatolu, 1990).

Nigerian foods were formulated from whole soybeans, dehulled soybeans, raw soyflour, processed soyflour, soybean paste, soybean grits, soybean milk, extruded soyflour, defatted soyflour and soybean milk residue.

Development and introduction of improved soybean utilization technology for use in households and small scale processing enterprises in rural Nigeria. Final report of the IDRC/IITA/IAR&T Soybean Utilization Project Phase I.
(Ogundipe, H.O. and S.M. Osho, 1990).

This project had five main objectives: (a) document the status of soybean utilization in Oyo State, Nigeria; (b) develop household technology for preparing soy-based foods; (c) develop small scale processing technology for soy-based foods; (d) introduce and assess the impact of household food technologies in rural areas; and (e) disseminate the results of the study. A baseline survey was conducted in 1987 to document the status of soybean production and utilization in three main villages: Ikoyi, Igangan and Ijaiye. Soybean cultivation was found to be relatively unimportant in the farming systems of farmers at the time of the baseline survey. About 17% of the farmers cultivated soybean in Ijaiye and 40% in Ikoyi, while no farmer cultivated soybeans in Igangan. According to the farmers, factors that would motivate them to grow more soybeans are a ready market (26%), multipurpose use (26%) profitability (24%), nutritional value (20%)

and knowledge of its cultivation (4%). Processing effectively eliminated trypsin inhibitor activity (TIA) and reduced phytic acid and tannin to acceptable levels. Soy-fortified dishes generally had higher protein and mineral content than the non-fortified versions. Product development research fortified traditional Nigerian foods with soybean to produce soy-egusi soup, soyvita (soy + maize), soygari and soylafun (soy + cassava), soymilk, soybread, sorghum/soy biscuit, soyamusa (a plantain based baby food), etc. Studies found that dry extrusion and screwpress technologies can be used to produce inexpensive and high quality soyoil, snacks, baby foods, and breakfast foods having a high caloric density, and protein quality at least similar to the conventional brands, with a good shelf life when packaged in simple materials, and much cheaper than the same products processed by conventional methods.

Soybean Utilization in Nigeria, a first year technical report for IDRC/IITA Soybean Utilization Project. Osho, S.M., Ogundipe, H.O. and Dashiell, K.E., 1991.

The status of soybean production, processing and utilization in Nigeria (edited). (Selina Adjebeng-Asem and Osho, S.M., 1991).
The report of the baseline surveys conducted by the National Institutions (IAR&T, NCRI, NAERLS and UNN) in their chosen project sites. These surveys were conducted in Oyo, Niger, Kaduna and Enugu States of Nigeria. The aim of the baseline survey was to document the status of soybean production, processing and utilization in the project sites.

Soybean Utilization in Nigeria, a second year technical report for IDRC/IITA Soybean Utilization Project. Osho, S.M. and Dashiell, K.E. 1992.
A compilation of all activities comprising soybean production and utilization at IITA and National Programs under the IDRC Soybean Project.

Consumer acceptability and quality of soy-cheese (tofu) and local cheese (warankashi) in Nigeria. S.M. Osho.
In order to access the acceptability of soybean used in Nigeria, detailed sensory evaluation and chemical composition of both the soybean curd and the local cheese (warankashi) were compared. The chemical analysis of the two samples indicated that the protein content and moisture content of soybean curd were higher than local wara while the fat content (12.5%) was much higher in the local cheese. The amino acid profiles in both samples were similar with glutamic acid at levels of 25.8% in the local cheese and 24.5% in the soybean curd. The results of the sensory evaluation showed that there were significant differences in the texture and colour of the soybean local cheese. The results indicate that local cheese and soybean curd are nutritious. (Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992 Pg 126).

Prospect of substituting skimmed milk powder (SMP) with soybean meal in the industrial processing of chocolate in Nigeria. Osho, S.M., Aruye, G.L., Aina, A.A. and Afolabi, R.
Chocolate is a confectionery that is rich in fat, essential mineral and vitamin, but low in protein content (11%). When soybean in a form of defatted extrudate was substituted for skimmed milk powder, the protein content increased from 11% to about 15%. When sensory evaluation was conducted, there was no difference both in the taste and in all parameters when compared to the plain chocolate. The fat content also increased and was found to separate from that of cocoa butter after 30 days of storage.
(Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992. Pg 175.

The devlepment of various formulae for soybean ice cream and the comparison with dairy ice cream. Osho, S.M. and Oyelola, B.
Dairy ice cream is a very popular dessert in Nigeria, as it is consumed by the old and the young. Surveys on dairy industries in Nigeria has shown that the price of dairy ice cream is out of reach to the low income group. This project's objective is to develop acceptable ice creams using soybean milk that people can afford. The soy-ice cream developed contained 75% soybean milk and 25% dairy ice cream mix and was accepted.

Chemical analysis of the soy-ice cream, when compared with the dairy ice-cream contained 4% protein and 3.7% protein respectively. Sensory evaluation showed soy-ice cream to be equally compared with dairy ice cream in all the parameters and showed that soy-ice cream was accepted. The product had greater

protein content than the dairy ice cream, because of the soybean used, which is a high vegetable protein. (Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992. pg 103).

The production of yoghurt from soybean milk in the presence and absence of starter culture. Osho, S.M. and Afolabi, R.
Yoghurt is a dairy product from cow's milk. The high cost and unreliable availability of yoghurt from cow's milk called for the use of milk extracted from soymilk to produce yoghurt in the presence of a starter culture. Two different types of methods were adopted in which soy-yoghurt was produced with starter culture and without starter culture. When the sensory evaluation of the product was evaluated and compared with the commercial dairy yoghurt, it was accepted. There was little or no difference detected between the non-starter cultured and starter cultured soy-yoghurt. The storage life of soy-yoghurt without starter culture is a few days. The chemical analysis of the three products showed that soybean yoghurt in method 1 contains 5.2% protein, method 2 contains 3.5% protein compared with the dairy yoghurt sold commercially that contain 3.4% protein. Method 1 contains more protein than the other products. There was no increase in protein, fat, moisture, carbohydrate and fibre contents of frozen yoghurt samples. Significant changes occured in flavour, color, sourness, smoothness, sweetness and overall acceptability of stored yoghurt samples after 30 days.
(Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992. Pg 114).

The utilization of soybean in the production of a beverage (coffee). Osho, S.M. and Oyelola, O.A.
Several foods have been developed from soybean which have been found acceptable in Nigeria. Research has led to the development of various substitutes that utilize soybean as their basic ingredient such as soybean beverage. The study was therefore conducted to determine the feasibility of processing a soybean beverage which is nutritious and acceptable. Chemical analysis showed a protein in the five products developed content ranging from 32.4%-39.4%, fat content ranging from 14.1%-20.5%, moisture content ranging from 3.6%-5.6% and ash content ranging from 3.6%-5.0%. The sensory evaluation results show that there are no significant differences among the formulations of beverage developed in terms of flavour, taste, and overall acceptability, however, there were significant differences in these attributes when compared to a 100% roasted soybean beverage. Sedimentation is also a problem in the 100% roasted soybean beverage.
(Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992. Pg 93.

Production and nutritional evaluation of soybean fortified malted sorghum meal extrudate. Osho, S.M. and Adenekan, I.G.
Malting sorghum with soybean grains increased the percentage of protein in the resultant blends. There was positive correlation between the soybean fortification level and protein level in the blend. The most appealing aroma/flavour and taste was obtained from a blend of malted sorghum-raw soybean as a 70:30 meal extrudate. This also had optimal nutrient density. The rigours and cost involved in getting malted soybean that is free of non-viable and deteriorating seed supersede the additional nutrient benefit derivable from the preparation exercise. Rather raw soybean flour could be used industrially to fortify the sorghum. Use of the extrusion cooking process yielded cereal/legume product of low and permissible trypsin inhibitor level.
(Published in the second year technical report for IDRC/IITA Soybean Utilization Project 1992. Pg 155.

Activities

L.5.1 Comparative analysis of acceptability and nutrient content of milk substitutes processed from two cultivars of cowpea and two cultivars of soybean
S.M. Osho
The studies is to extract milk from cowpea and soybean and determine the nutrient content, trypsin inhibitor activity and compare acceptability of the products.

L.5.2 The nutritive evaluation and the preparation of some Nigeria based food from soybean shaft.
S.M. Osho
Soybean shaft is a by-product of soybean seed which usually is used as animal feed in Nigeria. It is a comparatively easy and inexpensive product and it could be used in different ways in Nigeria. Therefore the range and nutritive value of food products processed from soybean shaft will be assessed.

L.5.3 The processing of canned soybean milk.

S.M. Osho

Soybean milk has been found to be highly acceptable in Nigeria. However, spoilage in soybean milk is still a major problem. Canning is needed to increase the shelf life of soybean milk. Chemical composition and sensory evaluation and shelf life of canned soybean milk will be assessed as well.

L.5.4 The development of powdered soybean milk in Nigeria.

S.M. Osho

Soybean milk is a widely accepted soybean product in Nigeria. However, the processing time for soybean milk has been considered to be too long. Though powdered milk is readily available in the market it is expensive. Hence there is a need to process soybean milk into powdered form. The nutritional and consumer acceptability of the soybean powdered milk will be equally accessed.

L.5.5 Nutritional and quality assessment of cowpea leaves as human food.

S.M. Osho

Cowpea leaves are part of the staple diet in Botswana and contribute to the protein need of the populace. In Nigeria, cowpea leaves are usually seen as animal feeds. Nutritional assessment and consumer acceptability and potentials of cowpea leaves as human food in Nigeria will be examined.

L.5.6 Formulation, evaluation and optimization of tortilla containing different forms of cowpea, maize and soybean flours.

S.M. Osho and J. Kling

Tortillas are eaten as staple food in Mexico and Central America, where they contribute to the nutrition of the populace. Maize is a cereal crop in Nigeria and is presently processed into different food products but not tortilla. Preliminary investigation at IITA shows that tortillas are acceptable. Tortilla fortifed with soybean will be developed thereby making an appropriate product available to the populace. Nutritional aspects with be evaluated.

L.5.7 Production and nutritional assessment of soybean fortified puffed-corn using extrusion-cooking process.

S.M. Osho

The extrusion cooking process is one of the fastest growing technologies. A cereal-based breakfast food will be developed by extruding maize which will be fortified with soybean protein to improve the amino acid profile.

L.5.8 Characterization of crude and refined soybean oil including studies of quality variation relative to shelf life.

S.M. Osho

Domestic edible oil consumption is increasing steadily in Nigeria. This study will extract and refine crude soybean oil and determine the quality characteristics of the oil.

L.5.9 Soy-fufu

S.M. Osho and M. Bokanga

One of the most popular cassava foods in Nigeria is fufu. Various methods of incorporating soybean into fufu will be tried. The parameters that will be evaluated include chemical analysis, shelf life studies, and sensory evaluation of soy-fufu.

L.5.10 Maize/soy tortilla

S.M. Osho and J. Kling

Maize tortillas serve the function of bread in Mexico and Central America, but they are low in protein. The potential of a soybean-fortified tortilla will be investigated.

L.5.11 Malted maize/sorghum/soy breakfast food

S.M. Osho and J. Kling

Malted maize/sorghum will be extruded with soybean to produce a breakfast food. Data to be collected include chemical analysis, shelf life and water absorption isotherms. Acceptability and sensory evaluation will be conducted.

Plantain and Banana Improvement Program

Plantain and banana (*Musa* spp.) are important food crops in the humid forest and mid-altitude agroecologies of sub-Saharan Africa, providing more than 25% of the carbohydrates for approximately 70 million people in the region. Plantains are high-yielding and form an integral component of the farming systems in these ecologies. In addition to being a staple food for rural and urban consumers, plantain and banana provide an important source of revenue for smallholders, who produce them in compound or home gardens as well as in large fields. The gross value for the annual production exceeds that of many other major food crops, such as maize, rice, cassava, and sweet potato.

The bulk of cultivated *Musa* are triploids ($2n = 3x = 33$). Being almost completely sterile, they develop fruit by parthenocarpy. The most important cultivars vary in their genomic constitution and are generally categorised as follows: dessert bananas (AAA), East African highland bananas (AAA), plantains (AAB), and cooking bananas (ABB). The genome for the cultivated types is derived from the diploid wild species *M. acuminata* and *M. balbisiana*, which contributed the A and B genomes, respectively.

The world production of plantain and banana is estimated at 68 million tons of which 35% is grown in sub-Saharan Africa. The different cultivated triploid *Musa* species are cultivated in distinct ecoregions. Thus, the AAB plantains are predominant in the humid lowlands of West and Central Africa, while AAA cooking and beer bananas prevail in the East African highlands. The former region harbours the world's greatest variability of plantains and is thus considered as a secondary center of plantain diversification. A similar situation is found for the specific East African bananas of the *Musa* AAA group, reflecting a long history of plantain and banana cultivation in Africa.

Pest and disease pressure on the crop has increased during the past 15 years. Also, rising population pressure has led to altered farming practices, among which shortening fallow periods are most conspicuous. At present the major production constraints of plantain and banana in sub-Saharan Africa are diseases (black sigatoka, *Fusarium* wilt), pests (weevil, nematodes), and the phenomenon of yield decline (mainly in plantains). Declining soil fertility also accounts for decreased yields.

Research on plantain at IITA began in 1973. In 1979, the center of plantain research was transferred to the Onne station, in the more suitable ecological niche of the humid forest zone. IITA included plantain and banana among its mandate crops in 1987 and the Plantain and Banana Improvement Program (PBIP) was created in 1991 as a full part of the Crop Improvement Division (CID) of IITA. PBIP scientists work closely with pathologists and entomologists of the Plant Health Management Division (PHMD) and agronomists/soil scientists of the Resource and Crop Management Division (RCMD).

During 1992, PBIP has further strengthened its collaborative linkages with several international, regional and national programs:

- the International Network for the Improvement of Banana and Plantain (INIBAP) in the areas of germplasm exchange and evaluation, and training,
- the Centre Régional Bananiers et Plantains (CRBP, Cameroon) for evaluation of breeding materials,
- the Inter-Ministerial Committee on the Control of Black Sigatoka Disease of Plantain in Nigeria, the National Horticultural Research Institute (NIHORT), the National Root Crops Reseach Institute (NRCRI), and other Nigerian NARS for evaluation of PBIP breeding materials in Nigeria,
- the Nigerian Plant Quarantine Service for movement of germplasm,
- the Crop Research Institute (CRI, Ghana) for evaluation of breeding materials,
- the Katholieke Universiteit Leuven (KUL, Belgium) in the areas of biotechnology,
- the Regional Plant Introduction Station of the USDA/ARS (Griffin, GA, USA) for molecular genetics work (RFLPs and RAPDs).

In 1993 we expect to increase our cooperation with NARS in East Africa and Latin America which have requested our breeding materials for testing in Advanced Musa Yield Trials (AMYT). Likewise we hope to strengthen our relationship with the Fundacion Hondureña de Investigacion Agricola (FHIA) for joint testing of plantain and banana hybrids in the AMYT.

PBIP was concerned with six research projects during the year:
- Gaining insight into the *Musa* genome
- Developing the *Musa* breeding capability
- Breeding for durable black sigatoka resistance

- Biotechnology for *Musa* breeding
- Post-harvest quality of plantains
- Genotype-by-cropping system interaction

In 1993, banana improvement for the mid- to high-altitude will be initiated and a banana breeder will be located in Uganda for this purpose.

General Publications

PBIP. 1992. Annual Report 1991. IITA, Ibadan, Nigeria. 30 pp.
This publication is an archival report of research results and achievements by PBIP scientists in 1991 in the areas of: (i) developing the plantain/banana breeding capability at IITA, (ii) breeding for durable host plant resistance to sigatoka leaf spot diseases, (iii) in vitro culture techniques and biotechnology for the enhancement of *Musa* breeding, and (iv) developing improved systems for sustainable and perennial plantain production.

Vuylsteke, D., R. Ortiz and R. Swennen. 1992. Plantains and Bananas. In IITA, Sustainable Food Production in Sub-Saharan Africa, Chapter 3, Crop Improvement. International Institute of Tropical Agriculture, Ibadan, Nigeria. pp 86-91.
This paper describes almost 20 years of plantain and banana research at IITA, with as most important achievements the results in: (i) morphotaxonomic studies to determine plantain diversity, (ii) agronomic and physiological investigations leading to identification of the crucial role of organic matter in perennial productivity of plantains, (iii) development of tissue culture techniques for *Musa* germplasm exchange and enhancement, and (iv) the production of high-yielding black sigatoka-resistant tetraploid hybrids of plantain.

Vuylsteke, D., R. Ortiz, C. Pasberg-Gauhl, F. Gauhl, C. Gold, S. Ferris and P. Speijer. (1993). Plantain and banana research at the International Institute of Tropical Agriculture. HortScience, in press.
Current work and prospects of plantain and banana research at IITA are presented. Projects in: (i) breeding plantain/banana for durable host plant resistance to sigatoka diseases, (ii) *Musa* germplasm enhancement using in vitro culture techniques and biotechnology, (iii) ecosystem analysis of sigatoka diseases in Africa, (iv) diagnostic survey of banana weevil and nematodes in the highland banana system of East Africa, and (v) post-harvest research are discussed.

IITA seminar series

Vuylsteke, D. 1992. Breeding plantains for resistance to sigatoka leaf spot diseases: an overview of the first 5 years. 5 May 1992.

Ortiz, R. 1992. Genetic analysis to develop new breeding schemes in *Musa* spp.: The "recreation" of plantains with sustained productivity based on disease and pest resistance. 25 May 1992.

Invited seminars

Vuylsteke, D. 1992. Production of black sigatoka-resistant plantains at IITA. Rivers Institute of Agricultural Research and Training, Rivers State, Nigeria. June 1992.

Ortiz, R. 1992. Genetic improvement of plantain and banana at IITA. Journal Club of Plant Breeding & Plant Genetics. University of Wisconsin, Madison, USA. November, 1992.

P. 1 GAINING INSIGHT INTO THE *MUSA* GENOME

Project rationale
Genetic information is required to develop scientific breeding strategies. However, few genetic studies have been undertaken in *Musa* spp., despite the importance of the crop. Moreover, several authors have claimed that "formal genetic studies of nearly or quite sterile triploids are impossible", illustrating the absence of

inheritance studies in plantain and banana during the past 40 years. As a consequence very few genetic markers are available in *Musa*. Several characteristics of the crop make genetic analysis of *Musa* spp. difficult. The low rate of hybrid progenies recovered after interploidy/interspecific crosses and resulting small sample sizes are the major obstacles to genetic analysis. Nevertheless, the production of test-cross segregating populations, obtained from triploid (heterozygous parents) x diploid (homozygous recessive parent) crosses, and diploid plantain/banana hybrids have made genetic analysis in *Musa* possible. Therefore, the plantain and banana genomes, which were inaccessible until recently, can now be investigated.

Completed studies

Ortiz, R. and D. Vuylsteke. 1992. Inheritance of black sigatoka resistance and fruit parthenocarpy in triploid AAB plantain. In Agronomy Abstracts. Amer. Soc. Agronomy, Madison, WI. p. 109.
One-hundred and one euploids (tetraploids, triploids and diploids) were obtained by crossing two susceptible triploid plantains with the resistant but wild diploid banana 'Calcutta 4' (C4). They were evaluated along with a susceptible plantain cultivar during two consecutive years in the humid forest zone of Nigeria. The segregating progenies were grouped in three classes based on their reaction to the disease: moderately resistant, less susceptible and susceptible. A dosage effect was observed in favour of the tetraploid progenies. The segregation ratio at the diploid level fitted a genetic model consisting of one major recessive allele (*bs1*) and another two independent resistance alleles with additive effects (*bsr2* and *bsr3*). Fruit parthenocarpy was also evaluated. C4 lacks *P1*, one of the three genes for fruit parthenocarpic development. All tetraploids had parthenocarpic fruits. This trait segregated in the diploid population. The segregation fitted a test-cross trisomic segregation ratio for one gene, which indicated that the plantain parents have heterozygous duplex genotypes for the *P1* locus. *P1* and *bs1* are linked (34 cM) in repulsion phase in the plantain genome.

Ortiz, R. and D. Vuylsteke. 1992. The genetics of black sigatoka resistance, growth and yield parameters in 4*x* and 2*x* plantain-banana hybrids. Abstracts of the International Symposium on Genetic Improvement of Bananas for resistance to diseases and pests. CIRAD, Montpellier, France, September 7-9, 1992.
The production of euploid segregating populations from crosses between triploid plantains and a diploid wild banana provided the means for genetic analysis in this crop. The male diploid parent was a true breeding line, therefore the derived progenies should be considered genetically equivalent to test crosses. The segregation observed in the tetraploid hybrids was due to the occurrence of a second division restitution mechanism for the production of 2n eggs in plantains. The inheritance of black sigatoka resistance was found to be due to the gene interaction (epistasis) between three independent alleles: one major recessive gene and two minor modifiers with additive effects. A similar genetic system operates at the tetraploid level but with a significant dosage effect. This results in the enhancement of black sigatoka resistance at the tetraploid level. Growth and yield parameters were compared between ploidy levels and within each family. The tetraploids had significantly higher bunch weight, average fruit weight, and fruit length and circumference than their respective diploid full-sibs. Some black sigatoka resistant tetraploids outyielded their fungicide-treated plantain parents. This suggests that the higher yield of the tetraploid hybrids was not only due to its black sigatoka resistance, but also to heterosis. A black sigatoka susceptible tetraploid (TMPx 597-4) had a poor bunch weight which was not different from that of its non-fungicide treated plantain parent. Another black sigatoka resistant hybrid (TMPx 548-4) had equal bunch weight as compared with the fungicide-treated plantain parent. Therefore, tetraploidy *per se* did not increase bunch weight. Furthermore, test-cross ploidy mean analysis revealed that epistasis was the most important type of gene action for bunch weigth and its components at the tetraploid level.

Ortiz, R. and D. Vuylsteke. (submitted). The occurrence and inheritance of albinism in banana and plantain (*Musa* spp.) HortScience.
There is great need for genetic studies in banana and plantain, since breeding endeavors have gained impetus due to increasing pest pressure. Very few genetic markers are available in *Musa* spp. as a result of a lack of inheritance studies. A diploid ($2n = 2x = 22$) plantain-banana hybrid from Africa was crossed with an improved diploid banana from Central America. Twenty-seven seedlings were produced, two of which were albinos (complete lack of chlorophyll in any plant tissue). The segregation ratio for albinism fit a 15:1 ratio ($c^2 = 0.06$, ns), indicating that albinism in *Musa* spp. is under the genetic control of at least two independent recessive alleles. This finding also demonstrates that deleterious recessive alleles are present

in both the cultivated AAB plantain gene pool and in advanced AA banana breeding populations. The latter suggests that population improvement through phenotypic recurrent selection for agronomic traits might be based on the elimination of deleterious recessive genes.

Ortiz, R., D. Vuylsteke and N.M. Ogburia (in prep.). Inheritance of waxiness in the pseudostem of banana and plantain. J. of Heredity.
Studies on resistance mechanisms have revealed that the presence of epicuticular wax on leaves appears to be involved in host plant resistance to the fungus *Mycosphaerella fijiensis* , the causal agent of black sigatoka leaf spot disease. The objective of this research was to study the inheritance of waxiness in the pseudostem, which is composed of leaf sheaths. Segregating populations were obtained by crossing triploid cultivated *Musa* and diploid improved bananas with diploid cultivated and wild bananas. Tetraploids, triploids and diploids were recovered from the $3x$ x $2x$ crosses, in which the diploid wild banana 'Calcutta 4' (C4) was the male parent . C4 bred true for pseudostem waxiness. A diploid F_1 hybrid was then selfed to produce an F_2. The F_2 population segregated into 29 individuals with non-waxy and 6 with waxy pseudostem. This suggests that pseudostem waxiness was due to a recessive allele, *wx*. The results from other crosses between C4 and other recessive waxy parents, however, indicated that alleles with mainly additive effects were responsible for modifying the action of the dominant allele *Wx* . Moreover, a clear dosage effect, i.e., an increase in expression of the trait in tetraploids, was observed. The mid-parent regression values were 0.67 ($r^2 = 0.48$) and 1.28 ($r^2 = 0.99$) for the diploid and tetraploid levels, respectively. Phenotypic recurrent selection should therefore result in an increased level of pseudostem waxiness.

Ortiz, R. and D. Vuylsteke (in prep.). Genetic analysis of apical dominance and improvement of suckering behaviour in plantain. J. Amer. Soc. Hortic. Sci.
Apical dominance (the correlative inhibition of lateral bud growth due to growth substances released by the terminal bud) has been considered as a limiting factor in the perennial production of AAB plantains. Inheritance of apical dominance in plantain-banana hybrids was elucidated by studying F1 progenies segregating for suckering behaviour, as measured by the height of the tallest sucker at flowering and at harvest. Apical dominance is controlled by one single recessive gene, *ad*. The dominant allele *Ad*, which is probably fixed in AA bananas, improved the suckering behaviour of the plantain-banana hybrids. At harvest of the plant crop, the diploid and tetraploid hybrids had already completed 75-100% of the vegetative development of the ratoon plant, whereas the plantain parents, due to their strong apical dominance, had only 50% of total pseudostem growth for the ratoon. The rate of sucker growth is a result of gibberellic acid (GA3) levels within the plant and it is thought that the *Ad* gene regulates GA3 production. However, the *Ad* gene has incomplete penetrance, genetic specificity and variable expressivity. Increased frequency of the *Ad* gene and a commensurate improvement in the suckering behaviour of the diploid population may be achieved by phenotypic recurrent selection.

Ortiz, R. and D. Vuylsteke (in prep.). Inheritance of dwarfism in AAB plantains. Plant Breeding.
Strong winds occur periodically in areas where plantains are grown, causing heavy losses. Therefore, the development of dwarf cultivars with short false internodes (< 9 cm) is desirable. The objective of this research was to elucidate the inheritance of dwarfism for the genetic improvement of dwarf plantains. A heterozygous normal plantain cultivar ($2n = 3x$) with long false internodes (19.4 ± 0.9) was crossed with a homozygous wild banana ($2n = 2x$), which has short false internodes (6.9 ± 0.4) to develop a test-cross segregating population. A total of 74 euploids ($2x$, $3x$ and $4x$) were produced. Forty-one normal and 24 dwarf diploids were obtained, which fits a 2:1 trisomic test-cross segregation ratio for one locus. Dwarfism is controlled by a single recessive gene, *dw*. At the tetraploid level, 2 normal and 6 dwarf hybrids were produced. This suggests that the *dw* locus should be close to the centromere, i.e., chromosome segregation, and there is a dosage effect of the *dw* allele at the tetraploid level. The substitution of the dominant *Dw* by its recessive allele reduced the height of the plant by 36 cm ($r^2 = 16\%$; $p = 0.005$).

Ortiz, R. 1993. Ploidy manipulations as a tool for genetic analysis and improvement of plantains and bananas (*Musa* spp.). In Peloquin, S.J. and E.T. Bingham, eds. Chromosome manipulations in plants. University of Wisconsin - Madison. pp. xxx-xxx.
This paper discusses the utilization of ploidy manipulations (scaling up and down the number of chromosomes) in the elucidation of the inheritance of 22 morphological descriptors, and several growth and yield parameters, as well as its contribution for the genetic improvements of *Musa* spp. Several conclusions

were drawn from the results: (i.) the triploid female plantain genome is not fixed, hence much more variability was recovered from $3x$ x $2x$ crosses than earlier anticipated, (ii) the success of tetraploid hybrids did not depend totally on the qualities transmitted by the pollen as was iterated before by banana breeders, (iii) 'Calcutta-4' (C4), the wild non-edible diploid banana, is not the sole source of black sigatoka resistance alleles because they are present, but not expressed phenotypically, in the plantain due to its additive/recessive genetic nature. Moreover, C4 did not transmit its inferior bunch characteristics to its progenies, i.e., fruit parthenocarpy and bunch weight of the tetraploid hybrids was not inferior to that of their French plantain parents. (iv), the idea that formal inheritance studies involving triploid *Musa* cultivars are imposible and that nothing definite can be said about the genetic nature of disease and pest resistance in this crop should be reviewed. For example it was demonstrated that black sigatoka resistance is due to an oligogenic system comprising one major recessive locus and two minor modifier loci with additive effects, (v) most of the traits investigated in *Musa* spp. are under the control of epistatic genetic systems. This was not surprising because such systems are expected in vegetatively propagated species, (vi) the production of diploid plantain-banana hybrids by $3x$ x $2x$ crosses proved to be very advantageous not only for genetic analysis but also provided the opportunity for plantain germplasm enhancement at the diploid level. This facilitates and speeds up the breeding work, (vii) the genetic knowledge accumulated through this research allows PBIP breeders to develop a breeding scheme which is currently implemented at IITA. The diploid (TMP$2x$) plantain-banana hybrids are used in crosses with AAB plantains to produce black sigatoka resistant primary tetraploids or secondary triploids.

Ortiz, R. (submitted). *Musa* genetics. In Gowen S., ed. Bananas and Plantains. Chapman and Hall.
This bookchapter reviews the current status of genetic research in *Musa* spp. Despite the triploid nature of most plantains and bananas, which could have represented a challenge and an incentive for geneticists, few genetic and cytological investigations were done after the 1950's. Early cytological research was done in the Caribbean by Cheesman, Dodds, Larter, Pittendrigh, Shepherd, Simmonds and Wilson. Currently, few researchers at IITA and CIRAD/IRFA (France) continue with basic genetic research in this crop. IITA has focused on: (i) elucidating the basis for the production of diploid progenies after triploid x diploid crosses, (ii) 2n gametes and their genetic consequences, (iii) ploidy effects in phenotypic appearance, (iv) inheritance of morphological and reproductive traits (albinism, apical dominance, dwarfism, sterility, fruit parthenocarpy, pseudostem waxiness), (v) genome differentiation between *M. acuminata* and *M. balbisiana* genomes, (vi) genetics of disease and pest resistance (especially black sigatoka), (vii) development of linkage maps, and (viii) genetic analysis of quantitative traits using: (a) test-cross ploidy mean analysis, (b) heritability estimates, (c) determination of genotype-by-environment interaction and (d) intraclass correlations.

Ortiz, R. and D. Vuylsteke. (in prep.). Trisomic segregation ratios and genome differentiation in AAB plantains. Banana Newsletter.
Plantains (*Musa* spp. AAB group) are triploid perennial herbs derived from interspecific crosses between the diploid species *M. acuminata* and *M. balbisiana*. These diploids have the AA and BB genomes. Subsequently, the AAB genome designation was given to plantain due to its interspecific origin and based on a putative differentiation between the A and B genomes. The objective of this research was to investigate whether these genomes were different with the aid of genetic marker segregation in diploid populations, derived from $3x$ (plantain) x $2x$ (wild banana) crosses. The markers analysed were *P1* (fruit parthenocarpy), *bs1* (major gene for black sigatoka resistance) and *dw* (dwarfism due to short internodes). The diploid populations were considered as test-crosses because the diploid banana was a homozygous line and the triploid plantains were found to be dominant duplex for the P_1 locus, and dominant simplex for the bs_1 and *dw* loci. The 1:1 (disomic) and 2:1 (trisomic) critical ratios were tested to determine the pattern of inheritance of the morphological markers. Segregation at the P_1 and *dw* loci did not fit a disomic but a trisomic ratio whereas segregation at the bs_1 locus fit both ratios. However, co-segregation of bs_1 and P_1 loci,which are linked repulsion phase linked in plantain deviated significantly from the expected disomic inheritance. In conclusion, plantains have a trisomic pattern of inheritance which occurs because each linkage group occurs three times instead of twice. Furthermore, there was no preferential pairing between the homologous chromosomes of the A genome but random distribution of the paired chromosomes to the cell poles during anaphase I of the first meiotic division. This implies that there is no genome differentiation between *M. acuminata* and *M. balbisiana* and therefore the AAB genomic designation for plantain should be discontinued or replaced with a more genetically specific characterization when necessary.

Vandenhout, H., R. Ortiz, K.V. Bai, R. Swennen and D. Vuylsteke. (in prep.). Ploidy effects on stomata size and density and pollen size in plantain-banana hybrids.
Euploid seedlings are normally sorted out by ploidy level through chromosome counting using root tips or pollen mother cells or by determining stomata size and density. A comparison of size and density of stomata in a polyploid series of mature plants demonstrated that stomata become larger and less numerous with increasing ploidy. This relation seems to be linear, but is not perfect because genetic background (differences between clones of the same ploidy within and between populations) and environment (differences between samples of the same clone) affect both stomata size and density. Pollen size, however, does not seem to be reliable to determine ploidy levels in plantain-banana hybrids because significant differences were not found between ploidy levels in plantain-banana euploid hybrids.

Activities

P.1.1 Inheritance studies using *Musa* germplasm
R. Ortiz and D. Vuylsteke
The inheritance of qualitative morphological descriptors will be elucidated and/or confirmed using diploid test-cross and F_2 segregating populations. Test-cross ploidy mean analysis as well as components of variance will be used for the interpretation of results about the genetic control of quantitative traits in triploid-diploid populations.

P.1.2 Linkage groups in *Musa* using conventional genetic markers
R. Ortiz
Departure from strict independent assortment is an indication of possible linkage between two loci. Contingency tables (cross-tabulation) are used to identifiy genetic markers which are linked. Chi-square tests are used to determine if each pairwise combination segregates independently. For example, the P_1 (fruit parthenocarpy) and bs_1 (black sigatoka resistance) genes cosegregate in a diploid test-cross population derived from triploid-diploid crosses. Based on this result it was established that both loci are linked (34 cM) in repulsion phase linkage in the plantain genome. Also, by means of half-tetrad analysis using data from the segregating tetraploid progenies, the bs_1 gene-centromere map distance was estimated as 0. Preliminary results also suggest that one of the two genes controlling the persistence of male bracts and neutral flowers (NFP_i) in the male axis belongs to this linkage group. This (centromere----bs_1 ----P_1 ---- NFP_i) represents the first linkage group in *Musa*, a triploid crop. Similar analyses will be continued to construct linkage groups with more morphological qualitative descriptors using the populations indicated in P.1.1.

P.1.3. Genotype-by-environment (year, location) interaction and broad sense heritability for growth and yield parameters in *Musa* hybrids
R. Ortiz and D. Vuylsteke
Preliminary results indicate that year variation significantly affected plant height, bunch weight, fruit circumference and youngest leaf spotted by black sigatoka. More important was the effect of the genotype by year interaction at Onne which only affected plant height, height of tallest sucker at harvest and fruit filling period, but not bunch weight and components of black sigatoka resistance parameters. This indicates that multilocational trials should be carried out to assess yield stability and durability of black sigatoka resistance of the plantain-banana hybrids across environments. Broad-sense heritabilities (or the ratio between the total genetic variance and the phenotypic variance) were grouped as high (plant height, bunch weight, fruit weight, length and circumference), medium (height of tallest sucker and number of standing leaves at flowering, youngest leaf spotted, and total black sigatoka index score), low (height of tallest sucker at harvest, number of hands and fingers) and very low (fruit filling period). Low broad-sense heritability values are the result of either lack of genetic variability or strong genotype-by-environment interaction for the traits studied in the reference population. Conversely, high heritability indicates that the trait is not influenced by the environment or shows a high degree of genetic variability, e.g., plant height. More comprehensive information (especially for the location effects) will be obtained from the multilocational trials (P.2.14 & P.3.4).

P.1.4 Molecular linkage map of *Musa*

R.L. Jarret (USDA/ARS), R. Ortiz, D. Vuylsteke and S. Schnapp (BRU)

The development and utilization of molecular markers of *Musa* could help to obtain basic genetic knowledge and to apply it to plantain and banana breeding. For example, they could help to elucidate the genetic nature and origin of the diploids obtained from 3*x* x 2*x* crosses involving plantains or the genetic mechanism involved in the modified megasporogenesis leading to the formation of 2n eggs in plantain. We are developing the molecular linkage map of *Musa* using restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs) and hypervariable sequences to detect variable number tandem repeats (VNTRs). RFLPs are the differences in the size of DNA fragments generated upon digestion of genomic DNA of different genotypes with restriction endonucleases. RAPDs result from differential annealing and extension of primer DNA to template (genomic) DNA in the presence of *Taq* polymerase. Tandem repeats are dispersed throughout the plant genome of most organisms and, when minisatellite repeats are used as probes, they detect individual-specific hypervariable sequences in humans, animals and plants. Various random genomic probes, random primers and cloned minisatellites, including the M13 bacteriophage repeat, are being evaluated for their ability to detect polymorphisms in segregating *Musa* populations. The most informative probe enzyme combinations, arbitrary primers or tandem repeats, for detecting molecular polymorphisms and hypervariable sequences in *Musa* segregating populations will be identified. This information will be combined with that obtained from field evaluation to identify useful molecular markers for plantain and banana breeding (see P.3.7 & P.5.7).

P.1.5 The effect of major genes in plant growth and fruit development

R. Ortiz

The effect of major genes for dwarfism (*dw*), apical dominance (*ad*) and black sigatoka resistance (*bs1*) on plant height, suckering behaviour and response to sigatoka diseases, respectively, have been documented (see completed studies). This demonstrates the effect of a single locus on quantitative trait variation in *Musa* spp. Currently, we are interested to establish the effect of the fruit parthenocarpy (*P1*) gene on fruit development as well as of other major genes on plant growth. The identification of such genes will help to speed up progress through indirect or direct selection.

P.1.6 Genetics of fruit quality

R. Ortiz and S. Ferris

There are many fruit quality attributes related to fruit palatability and durability that have to be satisfied before any improved varieties are adopted by farmers. The genetic improvement of these traits relies on the elucidation of their inheritance to determine scientific breeding strategies. Genetic analysis in two test-cross segregating populations derived from triploid x diploid crosses and data from multilocational trials will be used for this project.

P.2 DEVELOPING *MUSA* BREEDING CAPABILITY AND STRATEGY

Project rationale

Plantain and banana breeding has become the most important activity in PBIP during 1992. This resulted from significant gains in 1991 which opened up many possibilities for breeding across ploidy, species and subspecies of *Musa*. Based on those exciting results, IITA has decided to intensify and decentralize the breeding work into different agro-ecologies and gene pools by hiring a breeder for East Africa. However, there is still a need at this stage to consider breeding strategies and determine the most useful methodologies for improving this little-researched crop for African conditions and constraints. Genetic research, discussed in P.1., and breeding activities indicated below will help to achieve this goal. Recently, we defined the breeding philosophy of PBIP as follows: to develop not only disease- and pest-resistant, but also better cultivars for the smallholders who produce plantain and banana in compound or home gardens as well as in larger fields.

Completed Studies

Swennen, R., D. Vuylsteke and K. De Smet. 1991. Season dependent seed set in plantain. Banana Newsletter 14:35-36.
Average seed set in crosses of plantain cultivars with diploid bananas ranged from 0.3 to 21.7 seeds per bunch depending on the cultivar. However, variation in seed set is very wide. For example, the cv. 'Bobby Tannap', which produces on average 21.7 seeds per bunch, has once produced up to 219 seeds in a bunch. Conversely, some pollinated bunches produce no seed. This large fluctuation in seed number seems to be influenced by the season. Indeed, seed set is very high in the early part of the year, then declines to a very low level and reaches a second but smaller peak later in the year. This pattern is repeated yearly with only slight alterations. Because of this repetitive pattern of strong variation in seed set and the occurrence of similar patterns for crosses of other plantain cultivars, it is hypothesized that one or more climatic factors influence seed set.

Vuylsteke, D., R. Swennen and R. Ortiz. 1993. Development and performance of black sigatoka-resistant tetraploid hybrids of plantain (*Musa* spp., AAB group). Euphytica, 65: 33-42.
A strategy to control the black sigatoka disease (*Mycosphaerella fijiensis* Morelet) of plantain (*Musa* spp, AAB group) in Africa, targeting the incorporation of durable host plant resistance, was initiated at the International Institute of Tropical Agriculture (IITA). The commonly accepted intractability of plantain to genetic improvement has been challenged by the identification of 37 different, seed-fertile plantain cultivars and by the production of 250 hybrids in four years of breeding work. Twenty tetraploid hybrids have been selected for their increased black sigatoka resistance, high yields, large parthenocarpic fruits and improved ratooning. 'Calcutta 4' (*Musa acuminata* ssp. *burmannicoides*) was the diploid male parent of 17 of the selected hybrids, which indicates that the inferior bunch characteristics of this wild banana were generally not transmitted to its tetraploid progenies. Conversely, the 4*x* progeny of plantain readily expressed black sigatoka resistance when crossed with 'Calcutta 4'. Progenies of the triploid plantain cvs. 'Obino l' Ewai' and 'Bobby Tannap' differed in their black sigatoka breeding values, the former producing larger numbers of promising hybrids. Tetraploids obtained from crosses of plantain cultivars with the homozygous 'Calcutta 4' displayed variation in black sigatoka reaction, qualitative morphological traits and growth and yield parameters, suggesting the occurrence of segregation and recombination during the modified megasporogenesis leading to the formation of 2n eggs in the triploid female plantain parents. The triploid genome thus seems not be fixed, hence much more variability should be recovered from triploid x diploid crosses than earlier anticipated. This concept is now the cornerstone in PBIP's strategy for plantain improvement. The variation in black sigatoka reaction among the tetraploid progenies of plantain suggests that resistance could be regulated by recessive, additive genes.

Vuylsteke, D., R. Ortiz and R. Swennen. 1992. Genetic Improvement of plantains at IITA. Abstracts of the International Symposium on Genetic Improvement of Bananas for Resistance to Diseases and Pests. CIRAD, Montpellier, France, September 7-9, 1992. (Also in press in the conference proceedings).
Increasing pest/disease pressure on Musa and the unsoundness of agrochemical approaches have focussed attention on ecologically sustainable pest management interventions. For black sigatoka (BS) disease, which causes 33% yield loss in plantains at Onne, this generally entails host plant resistance, a strategy that IITA is pursuing actively. In spite of the commonly accepted intractability of plantain in terms of genetic improvement, 37 different French and False Horn cultivars have been identified with seed set rates ranging from 0.5 to 20 per bunch. Aggressive triploid x diploid pollination schemes have resulted in the selection of 20 tetraploid hybrids that combine moderate BS resistance, high yield and improved ratooning. The best performing tetraploids produce up to 100% higher yield than their plantain parent. The wild banana 'Calcutta-4' (C4) was the major pollen source, yet its inferior bunch characteristics were generally not transmitted to the tetraploid progenies. Because C4 is homozygous, the variation in BS response, qualitative traits and growth & yield parameters displayed among the full-sib tetraploids suggests the occurrence of segregation and recombination during the formation of 2n eggs in the triploid plantains. This inference challenges the fixed genome theory. Diploid plantain-derived hybrids have also been recovered from the triploid x diploid crosses. These prove to be very useful in further breeding schemes and in elucidating the inheritance of important traits.

Vuylsteke, D., R. Ortiz and R. Swennen. 1993. Genetic improvement of plantain and banana at IITA. INFOMUSA, in press.
The paper describes and shares information about the current work and findings of PBIP, especially in the utilization of aggressive pollination schemes, interspecific hybridization using ploidy manipulations (2n gametes), embryo culture, rapid *in vitro* multiplication, and field testing and selection. This resulted in the identification of improved *Musa* (plantain) germplasm at IITA. Promising breeding materials are currently undergoing multilocational evaluation in order to assess their stability of yield and black sigatoka resistance. Also new genetic information has been obtained along with the practical breeding work (see completed studies in P.1). The paper reports that a black sigatoka resistant hybrid of the AAB cv. 'Bluggoe' TMBx 612-74 has already been selected due to its high bunch weight (> 17 kg) and big fruits (>200 g) at Onne.

Ortiz, R., S. Ferris and D. Vuylsteke. (submitted). Plantain and banana breeding. In Gowen S., ed. Bananas and Plantains. Chapman and Hall.
This book chapter discusses the past work and current state of art in *Musa* breeding. A critical review of the Caribbean, Honduran, French, Brazilian, Indian, Australian and IITA programs is presented. Factors affecting the genetic improvement of banana and plantain are reported. Current and alternative breeding strategies are compared. Practical aspects of *Musa* breeding (screening for seed-fertile triploid parents, pollination, seed set, in-vitro methods, embryo rescue, propagation, evaluation, selection and registration of improved germplasm) are described. Finally, the paper explores the potential of biotechnology (mutation breeding, somaclonal variation, somatic embryogenesis/cell suspensions, genetic engineering/recombinant DNA technology, and biochemical and molecular markers) as a component of the breeding program.

Swennen, R., D. Vuylsteke and R. Ortiz. (in prep.). Phenotypic diversity and patterns of variation in West African plantains (*Musa* spp. AAB group). Economic Botany.
There are at least 116 plantain cultivars that have been grouped according to bunch type and plant size. The objective of this research was to examine the association between growth and yield parameters in the West African plantain germplasm, and to find out if the pattern of quantitative variation of bunch and vegetative traits agrees with the taxonomic grouping based on bunch type and plant size. A group of 24 plantain cultivars representing the major variability in West Africa were evaluated for nine quantitative characters. Phenotypic correlations between these traits were calculated. Giant cultivars are taller, thicker and flower much later than medium ones and they produce more foliage, resulting in heavier bunches with more hands and fingers. Principal component analysis (PCA) grouping agrees with conventional taxonomic grouping of plantains. PCA was based mainly on time to flowering, pseudostem height and number of fingers. The last two traits should be used in combination as discriminant function for grouping plantain cultivars based on the phenotypic correlations and the eigenvectors (weights in the PCA). The results also suggest that Nigeria has been a transit centre of plantain germplasm movement in West Africa.

Ortiz, R. and D. Vuylsteke. (in prep.). Plot technique studies on yield trials of plantains propagated by *in vitro* methods. Banana Newsletter.
In vitro techniques are used for conservation, distribution and rapid multiplication of selected genotypes of plantains. Phenotypic or somaclonal variation among micropropagated plants of *Musa* is a common phenomenon.The determination of yield potential of plantains should therefore consider this as well as proper field plot techniques. The aim of this research was to determine the optimum plot size and adequate number of replications for yield trials of differerent plantains which underwent rapid in-vitro multiplication before field establishement.The cultivars used represented the major plantain taxonomic groups: 'Ntanga 2' (Giant French), 'Bobby Tannap' and 'Obino l' Ewai' (Medium French), 'Big Ebanga' (Giant False Horn), 'Agbagba' (Medium 'False Horn') and 'Ubok Iba' (True Horn). A total of 120 true-type plants were sampled and their bunches weighed for each cultivar. No data transformations were required because var and sd were independent from the mean (lack of correlation) and the variance of the six plantain cultivars were homogeneous according to Box's rule. The bunch weight intra-clonal variance was not affected by the reported somaclonal variation in each cultivar . However, a negative trend was observed between both which suggest that selection against somaclonal variants was effective to reduce within clonal variation for bunch weight. Thus, soil heterogeneity (SH) was measured by the variation of bunch weight within each cultivar. SH was smaller in French plantain (0.25) plots than in Giant or Horn plantain plots (0.9-1.5). Optimum plot size (OPS) was determined by the method of maximum curvature by

plotting the coefficient of variation (Y axis) versus the number of competitive plants per plot (X axis). OPS (without including border plants) consisted of 8-12 (48-72 m^2), 15 (90 m^2) and 20 (120 m^2) competitive plants for French, False Horn and Horn plantains, respectively. The adequate number of replications (r) was determined using the following equation: $r = t^2 \times MSE \times 2 / D^2$, where t is the critical value of Student's t distribution for a significant level of a =0.05, MSE is the mean square error for bunch weight of a yield trial carried out in a randomized complete block design using 15 plants per plot and D is the desired significant mean difference expressed in kg. The number of replications decreased concomitantly with an increase in the magnitude of D, e.g. 60 reps of 15 plants each are required to detect a significant bunch weight mean difference of 0.5 kg whereas 2 reps of 15 plants each are enough to detect a significant mean bunch weight difference of 2.5 kg between 2 plantain cultivars. The detection of significant mean differences between plantain bunch weights larger than 3 kg requires only 1 rep of 15 competitive plants.

Ortiz, R. and D. Vuylsteke. (in prep.) Recommended experimental designs for selection of plantain and banana hybrids. Banana Newsletter.

Musa breeding consists of two stages: the initial production of desired genotypes by interspecific interploidy hybridization followed by several years of testing selected genotypes before a cultivar can be released. One of the limiting factors for testing the yield potential of plantain and banana is the recommended planting density (6 m^2) which requires extensive areas of land. The objective of this research was to determine the number of plants that need to be sampled per clone and the most efficient field layouts for testing bunch weight at different stages of the breeding program. Data from a yield trial of hybrids along with their plantain parent (fungicide and non-fungicide treated) were used to determine the optimum plot size and number of replications per location required for a yield trial. The method of maximum curvature was used to determine sample size. Type I (a) and Type II (b) error probabilities and the availability of breeding materials were considered in calculating the number of reps and experimental designs required for a statistically accurate evaluation of bunch weight. In the early stage of the breeding process plantain or banana parents are used as the comparison criteria for the performance of selected hybrids. In more advanced stages of the breeding process, the relative performance between hybrids can be used as the selection criteria for the best genotypes.In preliminary stages of the program (Early Evaluation Trials -EET, or Preliminary Yield Trials -PYT) breeders evaluate a large number of clones (100 or more) to determine which will be selected for further testing. However, planting material is limited (normally1-5 plants/clone) and only available for unreplicated plots or limited replications in EET or PYT, respectively. A probability level of 10% (cultivar mean - 1 standard error, SE) and 5% (cultivar mean - 2 SE) is recommended to select those hybrids with equal/higher (EET), or significantly higher (PYT) bunch weight than the parental cultivar. This approach provides a means to control Type II error (or false acceptance of null hypothesis). In the more advanced stages of the program (e.g. Multilocational testing or Advanced Trials), breeders are recommended to differentiate elite hybrids with a significance level of 1% (highest hybrid mean - 2 or 3 SE) or 0.1% (highest hybrid mean - 3 or 4 SE) which requires an increase in the number of replications to protect against Type II error. Currently we use non-replicated plots of 5 plants each for EET in the breeding station, randomized complete block design (RCBD) with 2 reps of 4 plants each for PYT in one location, and RCBD with 2 reps of 5 plants for multilocational testing in at least 10 different locations of West and Central Africa. PBIP breeders recommend that national programs use a RCBD with 4 reps of 5 plants for advanced testing of elite hybrids for at least 2 years (plant crop and ratoon) and at 10 different sites in the target ecoregion.

Vandenhout, H., R. Ortiz. D. Vuylsteke and R. Swennen (in prep.) Discrimination of ploidy levels using morphological traits in plantain-banana hybrids.

Eleven growth and yield parameters were used to group euploid (diploid, tetraploid and triploid) hybrids of three plantain-banana populations derived from triploid x diploid crosses using principal component analysis. Preliminary results indicated that the principal component 1 (x axis) was unevenly loaded, with average fruit weight as main contributor, while principal component 2 was mainly loaded on fruit filling time (days between flowering and bunch harvest) and number of fingers per bunch. The ploidy levels were grouped irrespective of their population background along the PRIN1 axis, which accounted for 74.56% of the total variation, while the populations were preferentially grouped along the PRIN 2 axis. This could suggest that ploidy has an important effect on average fruit weight in plantain-banana hybrids, while days to fruit filling and fingers per bunch are characteristics influenced by the plantain parent because the populations shared the same diploid wild banana as male progenitor.

R. Ortiz, R. Swennen and D. Vuylsteke (in prep.). A taxonomic approach to plantain breeding. Monographs of the Crop Improvement Division, IITA.

Despite the success in the direct utilization of a wild non-related diploid banana in the production of black sigatoka resistant plantain hybrids, diploid breeding is still considered as an important component for the genetic improvement of *Musa* spp. In this regard, the genome composition of triploid banana and plantain cultivars has been considered as crucial to develop breeding strategies by CIRAD/IRFA and IITA (1987-1990). This sort of information was used, by breeders in these institutes, as criteria for choosing diploid materials to transfer desired characteristics to cultivars. However, this approach does not seem to be genetically correct because ignores the occurrence of segregation and recombination during meiosis. In other words, genes and not genotypes are carried to the next generation by the gametes of the diploid parents. Moreover, important genotypic combinations are broken due to the independent assortment of genes or the occurrence of crossing overs between loci. Furthermore, selection gains in a breeding program rely on change on gene frequency, which results in the production of improved genotypes. Nevertheless, interesting diploid materials, which have been obtained at IITA using this approach, will be incorporated in the regular breeding program of PBIP to test their breeding value.

Activities

P.2.1 *Musa* germplasm working collection

D. Vuylsteke, R. Ortiz, R. Swennen (KUL) and Q. Ng (GRU)

The main goal of this activity is to collect, conserve and characterize *Musa* species and cultivars with (i) black sigatoka resistance (BSR), (ii) large, pendulous bunches, (iii) dwarf height, and/or (iv) nematode and weevil resistance, and evaluate them in the African humid forest environment represented at Onne. Conserved germplasm (>400 accessions) includes diploid species and cultivars (*M. acuminata* -AA, and *M. balbisiana* -BB), dessert bananas (AAA, AAB), plantains (AAB), and cooking and beer bananas (AAA and ABB). Over 30 sources of BSR are available in the collection. All accessions are kept in field genebanks and partly duplicated in *in vitro* tissue culture. Characterization of the germplasm will be finished this year. This year we expect to organize the field collections according to their genomic designation: Collection 1 for *M. acuminata* (accessions ordered by subspecies) and AAA banana types, Collection 2 for *M. balbisiana*, ABB cooking bananas, and other AAB cultivars, Collection 3 for AAB plantains and Collection 4 for AAA East African bananas. This field array will help to identify potential duplicates in the collections. The development of an East African banana gene bank in Uganda should be considered in the 1994 workplan. A database file including the most important descriptors is currently being developed. Duplicate in vitro samples and documentation are being kept at the Genetic Resources Unit (GRU).

P.2.2 *Musa* germplasm characterization

R. Ortiz, D. Vuylsteke, R. Swennen (KUL) and Q. Ng (GRU)

The objective of this research is to use a descriptor list to evaluate *Musa* natural germplasm (diploids, triploids and few tetraploids). Multivariate analyses such as principal components (PCA) and cluster will be used for the statistical grouping of the germplasm (see completed studies in P2). The development of a discriminant function will be required to assign clones to each taxonomic group. Preliminary principal component analyses on the Asian (collection from the Philippines) banana cultivars using quantitative growth & yield parameters (pseudostem height and girth, leaf length and girth, bunch weight, number of hands and fingers in the second hand, finger length and diameter, weight of peel and number of suckers at harvest) did not result in any clustering for *M. acuminata* accesions, which had a wide dispersion in the principal component diagram. Nevertheless, AA and AAA bananas did not overlap. Conversely, most of the AAA bananas overlapped with the other cultivars in the AAB and ABB taxa. This could indicate that PCA based on bunch and vegetative quantitative traits should only be considered to discriminate between ploidy levels in the same taxon. The first principal component, which explained 87% of total variation, was unevenly loaded on plant height and leaf length while the second principal component (7% total variation) was loaded mainly on weight of peel. Therefore, the right upper quadrant included tall cultivars with heavy bunches of large fingers and high weight of peel, e.g. AAB Horn plantains and ABBB natural hybrid. The ABB cooking bananas, which were initially considered as BBB (Cardaba, Saba, etc.), were grouped in the right lower quadrant, which was characterized for containing cultivars with large leaves. This demonstrates that some quantitative traits along with PCA could be used to discriminate between taxonomic groups in *Musa*. The documentation of the collection and of the hybrids will be carried out in collaboration with GRU and included in a database for worldwide distribution with the cooperation of INIBAP.

P.2.3 Environmental effects on male fertility and seed set in plantain

R. Ortiz

Research is underway to link changes in climatic factors with fluctuation in seed set but also to determine how these affect male fertility. Elucidation of such a relationship could assist breeders to keep seed set at high levels throughout the year or to decide when pollinations should be carried out. Consequently, more progeny should be produced and the chances to obtain desired genotypic combinations would be increased. Currently pollen samples are collected daily from all the male parents and observed under the microscope for stainability with acetocarmine glycerol jelly (acgj). The observed seed set will be correlated with the daily pollen stainability scores to determine any potential relationship. Likewise, pollen stainability and seed set will be correlated with meteorological data (air temperature, rainfall, solar radiation, relative humidity, etc.) to establish the effect of them in male and female fertility, respectively.

P.2.4 Male fertility in *Musa* germplasm

R. Ortiz

Triploidy *per se* has been considered as one of the potential causes of sterility in plantain and banana. However, bananas such as 'Yangambi Km 5' and 'Valery' have been found to produce functional pollen grains. Moreover 37 seed-fertile plantains have been reported to produce viable 2n and n eggs. Therefore, triploidy itself should not be the only cause of sterility in triploid cultivars. Chromosome aberrations such as translocations (interchanges, sometimes referred to as structural hybridity) and inversions also cause female and male sterility in diploid bananas. However, sterility in diploid bananas does not result only from structural hybridity but seems to be also under genetic control. Preliminary results indicate that male sterility in diploid plantain-banana hybrids could be due to the interaction of a plantain sensitive cytoplasm with at least three recessive nuclear genes from banana. The cytoplasmic effect cannot be demonstrated by reciprocal crosses due to high male sterility in plantains. The use of diploid plantain-banana hybrids in crosses with 'Calcutta 4' will produce test-cross segregating populations to confirm this cytoplasmic-genetic model for male sterility. Typical test cross ratios (male fertile:male sterile) are expected when the plantain-banana hybrids are used as females, while no segregation (all male fertile) should be the outcome when 'Calcutta 4' is the female parent. The objectives of this activity are also to a) determine pollen viability through microscopic studies, and b) identify the period of maximum pollen viability. Pollen stainability, using acetocarmine glycerol jelly (acgj), will be used for a preliminary screening of the diploid collection. Controlled crosses (diploid x diploid and triploid x diploid) will be performed using pollen samples collected during different stages of male flower development. Pollen samples will be stained with acgj before their utilization in crosses. Seed set will then be correlated with pollen stainability scores.

P.2.5 Ploidy levels of *Musa* hybrids

H. Vandenhout, R. Ortiz, D. Vuylsteke, R. Swennen (KUL) and S.K. Hahn (BRU)

The verification of the ploidy levels in the progenies from triploid x diploid crosses is required due to the production of diploid, triploid and tetraploid hybrids. Ploidies are estimated by phenotypic appearance and confirmed either by root-tip chromosome counting or stomata size and density. Protocols for DNA flow cytometry analysis and ploidy determination using *in vitro* plantlets are currently in development.

P.2.6 Diploid *Musa* breeding

R. Ortiz, D. Vuylsteke and R. Swennen (KUL)

Plantain and banana breeding has long been considered essentially as diploid breeding due to the triploid nature of the cultivated types. In this regard, an important step in the breeding program is the production of improved diploid material to be used as male parents in both triploid x diploid crosses (to produce primary tetraploids) and tetraploid x diploid crosses (to produce secondary triploids). The selection of diploid material is based on both female and male fertility, BSR and good bunch characteristics. In 1992, eight improved diploid banana clones (TMB2x 9719-7, TMB2x 8848, TMB2x 9869, TMB2x 8075-7, TMB2x 9128-3, TMB2x 9076, TMB2x 8532-1 and TMB2x 5265-1) combining BSR, a pendant high-yielding bunch with long parthenocarpic fingers, were identified. For example TMB2x 9128-3 (a progeny from the cross 'Tjau-Lagada' x 'Pisang Lilin') had a bunch of 12.2 kg with 19 hands and 316 fingers. It had higher bunch weight but smaller fingers than SH-3362 (improved diploid clone from FHIA) at Onne. In 1993, the improved diploids will be multiplied along with selections of previous years(TMB2x 366-8, and TMB2x 384-30) for their use on a large scale in the crossing blocks. In this way their breeding values will be assessed.

P.2.7 Plantain germplasm enhancement at the diploid level

D. Vuylsteke, R. Ortiz and S. Ferris

The plantain-derived diploids recovered from the triploid x diploid crosses prove to be very advantageous to plantain breeding, because they provide opportunity for germplasm enhancement at the diploid level and simplify genetic analyses due to disomic inheritance. This facilitates and speeds up the breeding work. They have been used in the extension of the initial breeding approach to include the production of secondary triploids by further crossing the primary tetraploid hybrids with these diploids. TMP2x 1605-1, TMP2x 1549-7, TMP2x 1199-6, TMP2x 1448-1, TMP2x 1586-2, TMP2x 1549-5, TMP2x 4400-8, TMP2x 4281-2, TMP2x 4600-15, TMP2x 1489-3, TMP2x 2625-20, TMP2x 1518-4, TMP2x 2348-6, TMP2348-7 and TMP2x 1297-3 have been selected and multiplied for further inclusion in 1993/1994 crossing blocks. The plantain-derived diploids should be evaluated for fruit quality traits prior to their utilization as regular progenitors in the breeding program. (see also P.1.6.)

P.2.8 Outcrossing rates in wild diploid *Musa* spp.

R. Ortiz, R.L. Jarret (USDA/ARS), D. Vuylsteke and M. Mutsaerts

'Calcutta 4' (C4), a homozygous, wild diploid seeded clone was used to determine the outcrossing rate in diploid germplasm. Seeds were extracted from selfeed, open pollinated (OP) and non-pollinated, bagged bunches of C4. A comparison between hand pollination (C4 selfed) vs OP was done. Early results (start of rainy season) indicated that OP yielded more seeds (a total 1400 seeds per bunch; 330 with well formed embryos) than selfing (a total of 150 seeds; 14 with well formed embryos). No seeds were obtained from bagged, non- pollinated inflorescences. However, an increase in seed set was observed in later hand pollinations which were done at the end of the rainy season. Furthermore, seeds were also obtained in the non-pollinated bunches (which were bagged to avoid pollination by bees). The results suggest that apomictic seed development could be possible in the female flowers of the inflorescence of C4. Molecular markers (see P1.4) will be used for the analysis of C4 OP seeds. This will allow us to determine the rate of outcrossing (mainly done by bees) in this clone.

P.2.9 Combining ability in plantain germplasm

R. Ortiz and D. Vuylsteke

Progeny testing of the plantain cultivars should be carried out to assess their combining ability for bunch weight and black sigatoka resistance before using them as elite progenitors in a breeding program. In this regard, plantain cv. 'Obino l'Ewai' had greater breeding value than cv. 'Bobby Tannap', not only because the former has a higher black sigatoka resistance inheritance rate, but also because its progenies have significantly better average bunch weight. Both traits, black sigatoka-resistance and yield, are currently the most important parameters used in the early evaluation of breeding materials, again emphasizing that 'Obino l'Ewai' seems to be a more valuable maternal source for plantain improvement as compared with 'Bobby Tannap'. The cv. 'Bungaoisan' was the best parent in the production of diploid-derived plantain hybrids with high bunch weight and adequate black sigatoka resistance.

P.2.10 Production of secondary triploids and tetraploids

R. Ortiz, D. Vuylsteke and R. Swennen (KUL)

Secondary triploids and tetraploids have been produced by tetraploid x diploid and tetraploid x tetraploid crosses, respectively. The parents were tetraploid hybrids (TMPx), diploid natural germplasm, an improved diploid from FHIA (SH-3362) and plantain-derived diploids (TMP2x). High seed set has been obtained in both types of crosses, which challenges the common idea that tetraploid hybrids have low male fertility. The first secondary triploids and tetraploids have been field-established in early evaluation plots in 1992 and preliminary yield trials will be carried out in 1994 to identify the best type of breeding methodology for further improvement of *Musa* germplasm. The combining ability of the parents will also be assessed to identify progenitors for the breeding program.

P.2.11 Breeding the False Horn plantain gene pool using somaclonal variation

D. Vuylsteke and R. Ortiz

Occurrence of French reversion variants among in vitro propagated populations of False Horn plantains provides opportunity to breed this highly sterile gene pool. Indeed, the French variant is more fertile that the original 'Agbagba' cultivar. Moreover, the off-type did not only show a different inflorescence type, but also shorter stature and fruit filling period, lower fruit weight, more hands and fingers than the true-type. The French reversion variant was also significantly different from the naturally occurring French cultivars for all growth and yield parameters. This indicates that somaclonal variation has only partially

affected the characteristics of the 'Agbagba' cultivar. Therefore, the utilization of the French reversion variant as parent in triploid x diploid crosses could be considered as a way for the genetic improvement of the highly sterile False Horn plantains. TMPx 1112-1 and TMPx 1297-3 are a triploid and diploid, respectively, black sigatoka-resistant hybrid derived from crosses between the 'Agbagba' French reversion and 'Calcutta 4'. A potential procedure to improve the False Horn gene pool will be to cross TMPx 1112-1 and TMPx 1297-3 and select for larger and fewer fingers, i.e., the False Horn ideotype, in the segregating population. Directional selection for reduced number of fingers could result in larger fruit size and concomitant bunch weight increase. This scheme has been put in practice and currently euploid hybrids (diploids, triploids and tetraploids) from the cross TMPx 1112-1 x TMPx 1297-3 are being evaluated in the field.

P.2.12 Breeding for dwarfism in plantains

D. Vuylsteke and R. Ortiz

A dwarf False Horn and French plantain cultivar have been established in pollination blocks at Onne for crosses with diploid parents. Also, dwarf diploid and tetraploid progenies have been selected from the cross 'Bobby Tannap' x 'Calcutta 4' (see completed studies in P.1.). These will be used in the development of a plantain population with short stature to solve the problem of winddamage in the crop.

P.2.13 Physiological research to enhance *Musa* breeding

I. Ekanayake (TRIP), R. Ortiz and S. Ferris

There are several physiological traits that can be used as indirect criteria for selection. Preliminary results indicate that high values for stomatal resistance to water vapour (sec cm^{-1}) in the afternoon could be a good indicator of potential drought tolerance. Low resistance values in the afternoon means that their stomata remain open and have increased water loss. The AAB cooking banana cultivars 'Fougamou' and 'Bluggoe' were identified as promising cultivars for dry areas while the AAB plantain 'Bobby Tannap' and the AAA desert banana 'Valery' could be very susceptible to drought according to this evaluation. Stomatal resistance in the morning has been suggested as an indirect measurement of photosynthesis. High resistance values (stomata closing) in the morning could indicate less productivity. However, preliminary results suggested that the utilization of this parameter as a predictor of high economic yield (i.e. bunch weight) could be unreliable for black sigatoka resistant plantain hybrids. Research will continue in this area to identify the best time and leaf age and surface for sampling as well as the location effect on this measurements.

P.2.14 Multi-site evaluation trials of selected *Musa* hybrids

R. Ortiz, D. Vuylsteke, PBIP and TRIP breeders in Nigeria, Cameroon and Uganda, PHMD scientists and NARS cooperators

The objective of this series of trials is to investigate the genotype-by-environment interaction for important traits and to determine the stability of yield and black sigatoka resistance (BSR) of promising hybrids across different environments in Africa. These trials are an important step in the testing of hybrid plantain/banana varieties before their release through NARS. The performance of twelve tetraploid BSR hybrids (TMPx 548-4, TMPx 548-9, TMPx 582-4, TMPx 1621-1, TMPx 1658-4, TMPx 2481, TMPx 2637-49, TMPx 2796-5, TMPx 4698-1, TMPx 5511-2, TMPx 6930-1 and TMBx 612-74) along with their three female triploid parents ('Obino l'Ewai', 'Bobby Tannap' and 'Bluggoe'), two male diploid parents ('Calcutta 4' and 'Pisang lilin') and three reference cultivars ('Agbagba', 'Cardaba' and 'Valery') are being evaluated in IITA stations and with the cooperation of NARS in Nigeria (10 sites), Cameroon (2 sites), Uganda (1 site) and Ghana (1 site), where a MET was planted in 1992 (except Uganda: 1993). MET-2 sets are also being sent to NARS in other continents (Cuba, Dominican Republic and Australia) in 1993 which have requested to test IITA improved *Musa* germplasm in their ecologies.

P.2.15 Advanced *Musa* Yield Trials

D. Vuylsteke, R. Ortiz, PBIP and TRIP breeders in Cameroon and Uganda, and NARS cooperators

The objective of the AMYT is to identify elite genotypes for potential release as new cultivars by the NARS according to the specific regulations in each country. In 1993, selected materials from the multilocational trials (PITA-1= TMPx 548-4, PITA-2 = TMPx 548-9, PITA-3 = TMPx 5511-2, PITA-4 = TMPx 582-4, PITA-5 = TMPx 2796-5, PITA-6 = TMPx 4698-1, PITA-8 = TMPx 7002-1, and BITA-1 =612-74) along with local cultivars (plantains in West & Central Africa and highland cooking bananas in East Africa) used as checks, will be included in an Advanced *Musa* Yield Trial (AMYT). In consultation with FHIA, we are also

including an improved cooking banana hybrid (FHIA-3) from this Honduran breeding program in this first AMYT. The AMYTs will be undertaken simultaneously in several local evaluation sites of various countries (Uganda, Burundi, Kenya & Zanzibar in East Africa, Côte d'Ivoire, Congo, Nigeria and Ghana in West & Central Africa) and in which the NARS would be the executing agencies. The results of such trials would thus be of regional relevance. AMYT will evaluate the previously selected promising hybrids over a period of at least two years in a RCB design. Criteria for selection will not only include disease resistance and productivity, but also local preferences as they affect consumer acceptability.

P.2.16 Defining an ideotype for plantain breeding

R. Ortiz, R. Swennen (KUL), D. Vuylsteke and S. Ferris

It has been reported that both pseudostem height and bunch weight are correlated with the leaf area index. This suggests that tall plantains with big leaves have high yielding bunches because they have more leaf area for photosynthesis. Therefore, the identification of correlated characters will be useful to define an ideotype for plantain breeding. Data from six plantain cultivars (giant and medium, Horn, False Horn and French) will be analyzed to determine cause-effects relationships by means of path analysis (see P.7.1.). Also post-harvest attributes will be considered for the definition of a plantain ideotype.

P.2.17 A selection index for multitrait selection in *Musa* hybrids

R. Ortiz

The breeding product, an improved cultivar with sustainable production, is a combination of several traits, e.g. a high and stable yielding plantain with black sigatoka resistance and fruit quality. This clearly reflects that the economic value of the final product depends on more than one trait. Therefore, simultaneous selection for several traits should be considered. Several methods are available for multitrait selection: (i) tandem selection in which one trait is used as selection criteria at any one time, but the chosen trait is varied from cycle to cycle of selection, (ii) independent culling or when a separate threshold is set for each trait, and the selected individuals are those who are simultaneously above the threshold for each trait, and (iii) simultaneous selection based on an index developed taking into consideration the variance/covariance structure, genetic and phenotypic correlations and economic weights that the breeder gives to the different traits according to their economic importance. A selection index will be developed for diploid and tetraploid populations based on early evaluation trials of plantain-banana hybrids over the plant crop and the ratoon.

P.2.18. The effect of inbreeding and heterosis on growth and yield parameters

R. Ortiz

This experiment aims to establish the importance of heterozygosity and inbreeding in bunch weight and other agronomic traits. The experimental materials are currently being produced. The experiment will consist of 2-way, 3-way and 4-way tetraploid x diploid, triploid x tetraploid and triploid x diploid crosses between unrelated (Q=0) and related parents (Q>0). An early evaluation trial in 1994 and a preliminary yield trial in 1995 of the hybrid progenies along with their parents will be carried out to determine what could be the best crossing scheme to continue improving *Musa* germplasm.

P.2.19 Computerization of PBIP breeding program

R. Ortiz

A database using DBASEIII+ has been developed with the pollinations records (1987-1992) to analyze the results of six years of crosses. This will help to determine which are the parents with high levels of fertility and seasonal effects. The database includes the cross number, parentage (female and male parents), pollination dates, date of seed extraction, and number of seeds per bunch. We hope that this database will be updated daily with the pollinations of the current day in 1993. The organization of the fields is crucial for the breeding program. Currently EXCEL has been used extensively for field maps. However, we feel that a better field arrangement could be achieved using a computerized database of the breeding materials at different testing stages (early observations, preliminary trials or multilocational evaluation). This will help to find the materials and pedigrees and make decisions for further testing or utilization as parents in the crossing block. Moreover, materials with common origin (parentage and/or year of release) can be grouped together to compare them in the same micro-environment. This computerized database will include the full description (based on morphological/molecular markers) of the improved TMP(B)x germplasm which will be registered by IITA.

A new data sheet has been developed for the record keeping in early evaluation trials (EET), preliminary yield trials (PYT), multilocational evaluational trials (MET) and advanced *Musa* yield trials

(AMYT). Plant height, height of tallest sucker at harvest, days to flowering, days to harvest, number of standing leaves at flowering, youngest leaf spotted by black sigatoka, bunch weight, number of hands and fingers, fruit length (representative finger of 2nd hand), and fruit circumference (representative finger of 2nd hand) are the traits that will be recorded in the breeding books because these are the traits taken into consideration by breeders to make their selections.

P.3 BREEDING FOR DURABLE BLACK SIGATOKA RESISTANCE

Project rationale

The black sigatoka leaf spot disease (*Mycosphaerella fijiensis*) is considered the most important constraint to plantain and banana production worldwide. The fungus, introduced to Africa about two decades ago, spread rapidly through all production zones. The pathogen causes severe leaf necrosis and reduces yield by 30-50%. All plantain germplasm at Onne (115 cultivars), collected from West and Central Africa, tropical America and the Philippines, is equally susceptible to BS. Chemical control strategies exist, but are socio-economically inappropriate in the framework of the resource-poor smallholdings which grow the crop in Africa. In addition, fungicide applications are hazardous to health in the village homesteads. Resistance breeding is generally considered as the most appropriate component intervention to control sigatoka leaf spot. Therefore, a genetic improvement program was started by IITA in 1987, targeting the incorporation of durable host plant resistance to black sigatoka in plantain and banana.

Completed studies

Swennen, R. and D. Vuylsteke. 1993. Breeding black sigatoka resistant plantains with a wild banana. Trop. Agric. (Trinidad) 70: 74-78

Plantain (*Musa* spp., AAB group), an important staple food crop in the lowland humid tropics of Africa, Latin America and the Caribbean, is threatened by a fungal leaf spot disease called black sigatoka. Two African plantain cultivars of the medium French category were crossed with 'Calcutta 4' (*Musa acuminata* ssp. *burmannicoides*), a wild diploid banana highly resistant to black sigatoka. Four tetraploid hybrids, selected from the progenies of the crosses, showed high levels of resistance to black sigatoka and produced bunches up to 125% heavier than their fungicide-treated plantain parents. The inferior fruit size of the wild banana was not reflected in these hybrids. The wild diploid 'Calcutta 4' can thus be used in a plantain breeding program.

Ortiz, R. and D. Vuylsteke (submitted). Inheritance of black sigatoka disease resistance in plantain-banana (*Musa* spp.) hybrids. Theoretical and Applied Genetics.

Black sigatoka (*Mycosphaerella fijiensis* Morelet), an airborne fungal leaf spot disease, is a major constraint to plantain and banana production worldwide. Gaining further knowledge of the genetics of host-plant resistance will enhance the development of resistant cultivars, which is considered the most appropriate means to achieve stable production. Genetic analysis was conducted on one-hundred and one euploid ($2x$, $3x$ and $4x$) progenies, obtained from crossing two susceptible triploid plantain cultivars with the resistant wild diploid banana 'Calcutta 4'. Segregating progenies, and a susceptible reference plantain cultivar, were evaluated over two consecutive years. Three distinct levels of host response to black sigatoka were defined as follows: susceptible (< 8 leaves without spots), less susceptible (8 - 10) and partially resistant (> 10). Segregation ratios for resistance at the $2x$ level fitted a genetic model having one major recessive resistance allele (*bs1*) and two independent alleles with additive effects (*bsr2* and *bsr3*). A similar model explains the results at the $4x$ level assuming that the favourable resistance alleles have a dosage effect when 4 copies of them are present in their respective loci (bsi^4). The proposed model was further validated by segregation data of S1 progenies. Mechanisms of black sigatoka resistance are discussed in relation to the genetic model.

Vuylsteke, D., R. Swennen and R. Ortiz. 1993. Registration of 14 improved Tropical *Musa* Plantain Hybrids with black sigatoka resistance. HortScience, in press.

Improved genetic material of *Musa*, developed by IITA, is 'released' to national programs whenever it proves useful and with 'no strings attached'. Registration of the improved germplasm developed by IITA breeders is being done in order to place the material in the public domain, i.e., prevent the germplasm from being protected by others. Moreover, official registration provides an accurate description of the material

and establishes a benchmark of advancement. The tetraploid hybids TMPx 548-4, TMPx 548-9, TMPx 582-4, TMPx 1621-1, TMPx 1658-4, TMPx 2637-49, TMPx 2796-5, TMPx 4479-1, TMPx 4698-1, TMPx 4744-1, TMPx 5511-2, TMPx 5706-1, TMPx 6930-1 and TMPx 7002-1 were included in this first registration of IITA improved *Musa* germplasm with black sigatoka resistance. A limited amount of *in vitro* plantlets are available upon request. Recipients are asked to give appropriate recognition of the source of germplasm if it is used in the development of a new germplasm, parental line or cultivar.

Gauhl, F., D. Vuylsteke, K. Mobambo, R. Ortiz, C. Pasberg-Gauhl and R. Swennen. 1993. Yield loss in plantain from black sigatoka leaf spot and field performance of resistant hybrids. Field Crops Research, in press. (A preliminary report was published as abstract in Phytomedizin 22(4): 55, 1992).
BS resistance and yield performance of three tetraploid hybrids were evaluated and compared with the maternal triploid plantain cultivar 'Obino l'Ewai' with and without fungicide treatment. Plantain yield loss from black sigatoka was 33%, as calculated from the difference in yield between the fungicide-treated and non-treated plantain (23.5 and 15.7 $mt.ha^{-1}$, resp.). Yield loss ensued from a reduction in the number of fruits per bunch and lower fruit weight. Black sigatoka also caused delayed flowering and harvest and premature fruit ripening. Sigatoka evaluation at flowering indicated significantly less leaf spot damage in the three hybrids (TMPx 548-4, 548-9 and 597-4) as compared with the susceptible plantain. At harvest, however, hybrid TMPx 597-4 displayed a susceptible response, whereas hybrids TMPx 548-4 and 548-9 still showed field resistance. These two hybrids also had improved agronomic traits, such as high yield, shorter stature, earlier maturity and better suckering. Yield of TMPx 548-9 (33.5 $mt.ha^{-1}$) was 43% higher than that of the fungicide-treated and twice that of the non-treated plantain, suggesting that its higher yield was due not only to black sigatoka resistance, but also to heterosis. TMPx 597-4 had poor yield performance (12.7 $mt.ha^{-1}$), indicating that tetraploidy *per se* does not increase yield.

Pasberg-Gauhl, C., F. Gauhl, D. Vuylsteke and R. Ortiz. 1992. Sigatoka research at IITA. Phytomedizin 22(4): 54-55. (Abstract).
In 1986, the black sigatoka (BS) disease, caused by the fungus *Mycosphaerella fijiensis*, was identified for the first time in Nigeria. No resistance or tolerance to BS has been found among the 115 plantain (*Musa* AAB) cultivars in IITA's *Musa* collection, which is maintained at the Onne High Rainfall Station, southeastern Nigeria. In 1987, a program aimed a breeding for durable host plant resistance to BS in plantain was initiated. Several promising tetraploid hybrids have been produced and evaluated along with their triploid plantain parent and other reference clones. The hybrids showed reduced BS severity and high yields when compared with the female plantain parent. The genotype x environment interaction is currenty being tested in multilocational evaluation trials in collaboration with national research programs at different locations in Nigeria, Cameroon and Ghana. Additional research will broaden the understanding of the epidemiology and biology of *M. fijiensis*. This includes studies of symptom development and monitoring of airborne inoculum. A volumetric spore trap with an automatic data logging weaher station was set up in January 1992 for detailed ecosystem analysis in the humid forest zone of West Africa.

Ortiz, R., D. Vuylsteke, H. Vandenhout and I. Ekanayake (unpubl.). Mechanisms of black sigatoka resistance.
Different mechanisms of host plant resistance have been postulated by biochemical and morphological comparison of black sigatoka susceptible and resistant cultivars. Low stomata density and an increase in epicuticular wax in the leaves of the resistant cultivars have been considered as mechanisms which may protect the plant against the sigatoka disease. Indeed, black sigatoka resistant cultivars such as AAB cooking bananas or the diploid dessert banana 'Pisang Lilin' show greater accumulation of epicuticular wax on the leaves. This reduces the accumulation of moisture and, thus, retards the establishment and germination of spores, especially in emerging leaves. However, the highly resistant (almost immune) wild banana 'Calcutta 4' lacks this characteristic. Moreover, preliminary evaluation of susceptible and resistant clones in the TMPx germplasm indicates that leaf waxiness should not be considered as a major mechanism for black sigatoka resistance. Others have indicated that high stomata density could be a characteristic of susceptible cultivars because this facilitates the fungal penetration in leaf tissue. Nevertheless, evaluation for stomata density in the TMPx germplasm (see completed studies in P.1.) suggested that this trait should not be considered as a resistance mechanism to black sigatoka; e.g. the moderately resistant clone TMPx 548-4 had a high stomata density, while the susceptible clone TMPx 597-4 had a low stomata density. Furthermore, the opening/closing of the stomata (see P.2.13) does not correlate with the expected

resistance to black sigatoka. Therefore, both morphological traits (low stomata density and leaf waxiness) are certainly not the sole and major mechanisms of black sigatoka resistance in the TMPx germplasm. Probably, black sigatoka resistance in this germplasm may be based on either resistance of the host to phytotoxins produced by the pathogen or on the fungitoxic activity of polyphenols in healthy tissue of resistant genotypes. A graduate student of PHMD/KUL will continue with this investigation as part of her PhD research.

Activities

P.3.1 Development of durable black sigatoka-resistant hybrids by combining different sources of resistance

D. Vuylsteke and R. Ortiz

Most *Musa* breeding programs have used 'Calcutta 4' as primary source of resistance to black sigatoka. 'Pisang Lilin' has also been used in the development of the TMPx germplasm at IITA. Recently, pathologists in New Zealand reported that BS pathotypes from Papua New Guinea and the Pacific can attack these sources of resistance. The search for other sources of resistance could be one way to address this problem. An alternative way to achieve durable host plant resistance may be to incorporate different sources of resistance (based on their response to different isolates of black sigatoka) in the same genotype. To this end, several secondary triploids and tetraploids have already been produced and field-established in early evaluation trials at Onne. Moreover, plantain-derived diploid hybrids (see P.2.7.) have been crossed with different BS resistant diploid bananas. In this way we are developing populations with a broad genetic base for sigatoka resistance which may result in durable resistance.

P.3.2 Clonal evaluation of BSR 4*x* hybrids - the ratoon cycle

K. Mobambo, D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasberg-Gauhl and R. Swennen (KUL)

This experiment is carried out to evaluate black sigatoka resistance and yield performance of tetraploid hybrids as compared with the maternal (plantain) cultivar under fungicide and non-fungicide conditions at Onne. Results of the plant crop are reported in completed studies in P.3.

P.3.3 Multi-location evaluation trial of BSR 4x hybrids at IITA stations

D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasberg-Gauhl and TRIP breeders in Nigeria and Cameroon

The objective of this set of replicated trials is to test the performance of the improved TMPx under different agroecological conditions. A first set of tetraploid hybrids (TMPx 548-4, TMPx 548-9, TMPx 597-4, TMPx 582-4, TMPx 1112-1, TMPx 1658-4, TMPx 2796-5 and TMBx 612-74) are currently undergoing multilocational evaluation trials (MET) in Nigeria (Ibadan and Onne) and Cameroon (M'balmayo). Four black sigatoka resistant ABB cooking banana cultivars ('Bluggoe', 'Cardaba', 'Fougamou' and 'Pelipita') and six reference clones (the susceptible plantain parents 'Bobby Tannap' and 'Obino l' Ewai', the diploid resistant banana parents Pisang Lilin' and 'Calcutta 4', the susceptible plantain 'Agbagba' and susceptible banana 'Valery') have also been included in this first MET, which was planted in 1991. This experiment also provides to PHMD pathologists the opportunity to study the symptomatology and pathogenic variation of the sigatoka disease in different West and Central African ecosystems.

P.3.4. Multi-site evaluation trials of selected *Musa* hybrids

R. Ortiz, D. Vuylsteke, PBIP and TRIP breeders in Nigeria, Cameroon and Uganda, PHMD scientists and NARS cooperators (see P.2.14.)

P.3.5 Advanced *Musa* Yield Trials

D. Vuylsteke, R. Ortiz, PBIP and TRIP breeders in Cameroon and Uganda, and NARS cooperators. (See P.2.15.)

P.3.6 International *Musa* Testing Program

D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasberg-Gauhl, INIBAP and FHIA scientists

The IMTP, set up by INIBAP, aims at the identification of germplasm (natural or improved) with resistance/tolerance to a specific disease or pest through testing on a global scale. The first IMTP concentrates on black sigatoka resistance and involves 6 selection sites in two continents (Africa and tropical America). IITA participates as an equal partner in the IMTP being carried out in Africa. The current IMTP-1 trial centers on the evaluation of seven hybrids of banana and plantain produced by FHIA (Fundacion Hondureña de Investigacion Agricola, La Lima, Honduras), along with 11 BS standard hosts and reference

cultivars in observation (unreplicated) plots. This research is part of IITA's collaboration with INIBAP and FHIA. Preliminary results showed that FHIA-1 (a 'Prata' banana hybrid), FHIA-2 (a 'Cavendish' banana hybrid) and FHIA-3 (a 'Cardaba' derivative) have partial resistance to black sigatoka leaf spot disease while the remaining plantain hybrids (derived from either Maqueño or AVP-67) were susceptible or less susceptible to BS. The black sigatoka-resistant plantain hybrids PITA-2, PITA-3, PITA-4, PITA-5 and PITA-6 (see P.2.15.) have been suggested by PBIP to enter the IMTP-2 along with breeding materials from FHIA, EMBRAPA (Brazil), CIRAD/IRFA (Guadaloupe) and INIVIT (Cuba).

P.3.7 Molecular mapping of black sigatoka resistance genes

R. Ortiz, R.L. Jarret (USDA/ARS), D. Vuylsteke and S. Schnapp (BRU)

The objective of this research is to identify molecular marker loci linked to black sigatoka resistance (BSR) loci. As such, the quantitative trait variation (QTV) for BSR can be dissected into its components and the suggested main types of gene action controlling BSR phenotypic expression (see completed studies in P.1. and P.3.) can be confirmed. Furthermore, the markers linked to BSR could be useful for marker assisted selection (MAS) in the development of diploid, triploid and tetraploid breeding materials during the early stages of plant development (see P.5.7.). Alternatively the identification of functional gene sequences and/or protein-derived products conferring resistance to this disease could be useful for the production of diagnostic kits for the *in vitro* selection and rapid multiplication of black sigatoka resistant genotypes. Two test-cross populations derived from triploid-diploid crosses and a F_2 generated by selfing a susceptible TMP2x have been evaluated for the disease and are being scored using molecular markers described in P.1.4.

P.4 BANANA IMPROVEMENT FOR MID TO HIGH ALTITUDES

Project rationale

The world's highest per capita consumption of bananas is in the East African highlands. Pest pressure is higher in this region as compared to West and Central Africa, because of the additional presence of *Fusarium* wilt disease and banana bunchy-top virus. Genetic improvement of the highland cooking and beer banana cultivars (AAA and ABB) that are adapted to this agroecology seems possible because seeds have been produced in a number of these cultivars. Moreover, methods developed by PBIP (see P.2.) for the genetic improvement of plantains can be extended to AAA and ABB bananas. IITA has decided to hire a breeder to be posted in its station at Namulonge (Uganda) in 1993 as an outposted member of the Onne-based team.

Activities

P.4.1 Screening for female fertility in East African *Musa* germplasm

D. Vuylsteke and R. Ortiz

The two most common cultivars in that region ('Igitsiri' and 'Igisahira gisanzwe') were multiplied *in vitro* and later field-established in a larger pollination block at Onne station. Hybrid seeds will be produced during 1993 by crossing these black sigatoka susceptible bananas with diploid resistant accessions. Also a more systematic screening for seed-fertility in this gene pool will be done in 1993 using the cultivars planted in the East African banana collection at Onne (see P.2.1.)

P.4.2 Mid-altitude cooking/beer banana breeding

PBIP breeder in Uganda, D. Vuylsteke and R. Ortiz

It is envisaged to send hybrid materials, produced at Onne, in seed form to the IITA regional station in Namulonge for clonal production, evaluation and selection for improved yield, plant type and resistance to sigatoka diseases, fusarium, weevil and nematode pests in this suitable highland environment. The PBIP breeder will also collaborate closely with Ugandan and other national scientists in the East African region and assist them in breeding and evaluation methodology.

P.4.3 Advanced *Musa* Yield Trials

D. Vuylsteke, R. Ortiz, PBIP and TRIP breeders in Cameroon and Uganda, and NARS cooperators. See P.2.15.

P.4.4 Banana seed health

D. Florini, H. Rossel, F.&C. Gauhl and D. Vuylsteke

True seed produced at the Onne station will be examined to determine which microorganisms are present and, if required, how health status can be improved, before seed is sent to Uganda (see P.4.2).

P.5 BIOTECHNOLOGY FOR *MUSA* BREEDING

Project rationale

Recent concern over rising pest and disease pressure on banana and plantain has spurred genetic improvement programmes. Hence attention has focused on the collection, movement and conservation of *Musa* germplasm. However, efforts to propagate, conserve and breed cultivated *Musa* are fraught with many obstacles (slow propagation, low reproductive fertility, lack of genetic variability) specific to the biology of this vegetatively propagated crop. A wide array of plant tissue culture and molecular genetic techniques is increasingly being used as an enabling and enhancing technology for the handling and improvement of *Musa* germplasm.

Completed studies

Dheda, D., B.J. Panis, R. Swennen and D. Vuylsteke. 1992. The applicability of embryogenic cell suspension cultures from vegetative tissue to different banana varieties. Banana Newsletter 15: 43-44.

A cell suspension technique was established using explants excised from the upper part of the meristem shoot-tips proliferating on semi-solid medium with 1mM 2,4-D and 1 mM zeatin. Successful plant regeneration through somatic embryogenesis was achieved following four steps without 2,4-D. Plantlets from somatic embryos grew normally in the greenhouse. Then, genetic stability was evaluated in a field comprising 140 flowering plants at Onne. Only 0.7% off-types were recorded. The protocol has been succesfully tested in ABB cooking bananas cv. 'Bluggoe', 'Saba', and 'Cardaba', the AAB plantain 'Three Hand Planty' and the wild species *M. balbisiana*. Results indicated that the production of embryogenic cells is related to a high production of meristematic globules in liquid medium. The production of embryogenic suspensions of five varieties belonging to two ploidy levels and three genome combinations, holds promise that the established protocol will be applicable to a very wide range of *Musa* varieties. Since the explant consists of vegetative tissue, this protocol is applicable to seed sterile bananas and plantains thereby paving the way for genetic manipulation of the cultivated *Musa* genome.

Swennen, R., D. Vuylsteke and S.K. Hahn. 1992. The use of simple biotechnological tools to facilitate plantain breeding. In G. Thottappilly, L. Monti, D.R. Mohan Raj & A.W. Moore (eds). Biotechnology: Enhancing research on tropical crops in Africa. CTA/IITA, Ibadan, Nigeria. pp. 69-74.

Plantains are a staple food crop in sub-Saharan Africa. Black sigatoka, a leaf spot disease, is the principal cause of current yield losses. Only 3 years after its inception, the plantain breeding programme at the International Institute of Tropical Agriculture (IITA) has produced four black sigatoka-resistant plantain hybrids by crossing the triploid plantains with a wild diploid. A few simple biotechnologial rools, such as introduction of germplasm through in vitro meristem culture, in vitro micropropagation, and embryo rescue culture, were pivotal in this achievement.

Vuylsteke, D. and R. Swennen. 1992. Biotechnological approaches to plantain and banana improvement at IITA. In G. Thottappilly, L. Monti, D.R. Mohan Raj & A. W. Moore (eds). Biotechnology: Enhancing research on tropical crops in Africa. CTA/IITA, Ibadan, Nigeria. pp. 143-150.

Banana/plantain genetic improvement programmes have been spurred by the rapid spread of black sigatoka disease, but the genus *Musa* is intractable in terms of conventional breeding strategies. The potential of biotechnology in banana and plantain improvement may therefore be considerable. Simple tissue culture techniques, such as shoot-tip culture and embryo culture, can overcome some of the obstacles impeding breeding progress. These techniques have contributed significantly to IITA's success in the production of black sigatoka-resistant plantains. Assistance in the development of tissue culture laboratories has been provided to Nigerian national programmes, thereby strengthening their capability in biotechnology. Cell culture and RFLP mapping, both essential to the future implementation of molecular genetic techniques in *Musa* improvement, are being researched in collaboration with advanced laboratories.

Vuylsteke, D. and R. Swennen. 1993. Genetic improvement of plantains: the potential of conventional approaches and the interface with in vitro culture and biotechnology. In Biotechnology for the improvement of bananas and plantains, Proceedings of an INIBAP workshop, San José, Costa Rica, January 1992. In press.
The current achievements of plantain improvement at IITA and the utilization of *in vitro* culture and biotechnology in support of plantain and banana breeding are described. Applications of shoot-tip culture, embryo culture/rescue, cell suspensions and somatic embryogenesis, and somaclonal variation in the genetic improvement of plantain are discussed. It is concluded that despite the success of conventional breeding at IITA, recombinant DNA technology may be of great benefit to the improvement of *Musa* cultivars that are difficult to breed (e.g. 'Cavendish') and to incorporate genes coding for characters that are not available in the *Musa* gene pool, such as banana bunchy top virus. It is, however, advocated that biotechnology projects be fully integrated into conventional breeding programs in order to take full advantage of biotechnological developments and products.

Jarret, R.L., D. Vuylsteke and L.J. Dunbar (1993). Detecting genetic diversity in diploid bananas using PCR and primers from a highly repetitive and dispersed DNA sequence. Plant Mol. Biol. In Press.
The polymerase chain reaction (PCR) was used to detect polymorphisms among 29 diploid clones of *Musa acuminata* Colla from Papua New Guinea. Primer sequences were derived from a 520bp highly repetitive and dispersed sequence isolated from *M. acuminata* ssp. *malaccensis*. Primers, used individually, detected a total of 48 polymorphisms that were scored as unit characters and used to generate a Jaccard's similarity index. The relative abundance of variability within the PNG diploid bananas reflects the extreme variability within the *M. acuminata* gene pool. Principal coordinate analysis (PCO) was used to cluster clones and the unweighted paired-group method of analysis (UPGMA) was used to compute genetic distance among the materials examined. PCR with primers from a highly repetitive sequence is a rapid and reliable means of detecting genetic diversity in *M. acuminata*.

Activities

P.5.1 *Musa* germplasm exchange in vitro
D. Vuylsteke and S. Ng (TRIP)
Aseptic shoot-tip culture, in combination with third-country quarantine, is used as a vehicle for the safe exchange of banana/plantain germplasm. Shoot-tip cultures confer considerable advantages for the international transfer of germplasm because (1) the mass of plant material involved in the movement is greatly reduced, (2) the plant material is contained, (3) they overcome nearly all of the problems associated with non-obscure pests and pathogens, and (4) they are amenable to rapid multiplication. In 1992, no introductions were made but materials were distributed to NARS for the multilocational trials (see P.2.14). In 1993, diploid *M. acuminata* materials from Papua New Guinea will be introduced with the cooperation of the Nigerian Plant Quarantine Service and the INIBAP Transit Center at KUL. Also, genetic materials will be distributed to NARS carrying out advanced yield trials of improved *Musa* germplasm (see P.2.15). Over 500 germplasm accessions, among which 110 plantain cultivars and about 300 plantain hybrids, are maintained *in vitro* for germplasm conservation and as duplicates of the field genebank (see P.2.1.). The *in vitro* collection is partly duplicated at the Ibadan tissue culture laboratory for safety reasons, where it is maintained under minimal growth conditions.

P.5.2 In vitro propagation of selected genotypes
D. Vuylsteke and NARS collaborators
Shoot-tip culture is a well-established, adequate and relatively simple *in vitro* method for the rapid propagation of selected *Musa* materials and the production of clean planting material. Multiplication rates are several orders of magnitude higher than those obtained with conventional methods, which is of great value for the multiplication of newly bred genotypes or to speed up the testing of selections by plant breeders. Micropropagation continued to be an important tool in the rapid deployment of new breeding schemes and trials by supplying large numbers of plants of female and male parents for the crossing blocks and of BSR hybrids for the evaluation trials. Micropropagated plants of BSR cooking bananas were distributed to Nigerian national programs (see P.8.4). In 1992, 2000 plants of the cv. 'Cardaba' were distributed in this framework. Technical support on *in vitro* propagation of *Musa* was provided to tissue culture laboratories of Nigerian NARS.

P.5.3 Embryo rescue in *Musa*

G.I. Harry, D. Vuylsteke and U.U. Ebong (RSUST)

Hybrid plant production in the most common triploid *Musa* clones is hampered by low seed and by low seed germination rates. Seeds of plantain crosses germinate in soil at a rate of only 1%. Aseptic embryo culture techniques are routinely applied to increase seed germination rates by a factor of 3 to 10. An average of about 1000 plantain hybrid seeds are handled *in vitro* on a monthly basis, in support of the breeding program. A research fellow is investigating factors that influence immature embryo germination to further increase germination rates.

P.5.4 Somaclonal variation studies in *Musa*

D. Vuylsteke and KUL cooperators

The increased genetic variation among plants regenerated from *in vitro* culture has been termed as somaclonal variation, a ubiquitous phenomenon. The frequent use of *in vitro* culture techniques for the handling of *Musa* germplasm warrants investigations into the occurrence of somaclonal variation in this genus. In collaboration with the KUL the genetic stability of cooking banana plants (*Musa* spp, ABB group, cv. 'Bluggoe') regenerated from cell suspension cultures by somatic embryogenesis is investigated (see completed studies in P.5.). In a continued joint effort with this university, plants obtained from cryopreserved cell suspensions are also screened for somaclonal variation at Onne station. A first batch of plants recovered from cryotreated suspensions proved to be all off-types with a "diploid"-like appearance (slender, erect leaves), which was already clearly apparent at the nursery stage. These off-type plants did not survive in the field. This experiment was repeated by regenerating plants from 5 different batches of suspension culture (5 replicates), which were subjected to 3 treatments: 1) control, 2) cryoprotection with DMSO but no freezing, and 3) cryoprotection and freezing. Observations at the nursery stage of the first 2 replicates showed only off-type plants, indicating that something is wrong with the stock culture from which the culture batches were produced. The origin of these problem cultures is now being traced backwards in order to find out where the initial genetic instability occurred (maybe already in the shoot-tip cultures from which the suspensions were derived). New suspension cultures, also from other *Musa* cvs., among which plantains, are in the process of initiation and regeneration in order to examine the effect of culture process and regeneration mode on somaclonal variation.This activity is of significance to the long term conservation of genetic resources of *Musa* and other crops, for which cryopreservation of *in vitro* cultures has been advocated.

P.5.5 Molecular linkage map of *Musa*

R.L. Jarret (USDA/ARS), R. Ortiz, D. Vuylsteke and S. Schnapp (BRU)

(see P.1.4)

P.5.6 Molecular mapping of black sigatoka resistance genes

R. Ortiz, R.L. Jarret (USDA/ARS), D. Vuylsteke and S. Schnapp (BRU)

(see P.3.7)

P.5.7. Molecular methods in *Musa* breeding: marker assisted selection (*)

R. Ortiz, R. L. Jarret (USDA/ARS), D. Vuylsteke, F. Rosales (FHIA), R. Rowe (FHIA) and S. Schnapp (BRU)

A project has been proposed to FAO to initiate this research in collaboration with FHIA and USDA/ARS. Molecular markers (see P.1.4.) along with field evaluation of IITA and FHIA breeding materials and conventional genetic analysis will be used to elucidate the genetic basis for black sigatoka (see P.3.7.) and burrowing nematode resistance in plantain and banana. The most informative markers (probe enzyme combinations, arbitrary primers or tandem repeats) are those linked to loci coding for a specific resistance. They will be used as 'landmarks' for marker assisted selection (MAS) in early stages of plant development and for introgression of exotic germplasm into the cultivated *Musa* gene pool. The efficiency of MAS will depend of several factors such as (i) linkage disequilibria, (ii) number of marker loci, (iii) population size, (iv) heritability of the trait of interest, and (v) the percentage of additive variance explained by the marker. An improvement of such technology for plantain and banana breeding should be achieved by developing an optimum selection index which combines information on molecular with phenotypic measurements. Therefore, both molecular and field work are required to achieve this goal.

P.5.8 Development of a secure and rational management system for in vitro propagated and conserved germplasm of *Musa*

D. Vuylsteke, L. Withers (IPGRI), R. Swennen (KUL), R. Ortiz and possibly other cooperators

In vitro culture is a valuable component of current propagation, conservation and improvement strategies for *Musa*. However, the full exploitation of *in vitro* culture is hindered by the occurrence of somaclonal variation. Many somaclonal variants are not apparent until in the field, often only at fruiting. This has serious practical and economic consequences for banana/plantain production at the farm level, threatens the security of *in vitro* conservation, and hampers the efficient application of biotechnological approaches to genetic improvement. Research over recent years has advanced towards characterizing somaclonal variants and understanding some of the underlying factors. However, there is a need to develop a strategy for the rational management of *in vitro* collections for *Musa* germplasm conservation. A project under FAO's Associate Professional Officer (APO) scheme has been proposed to address this issue in collaboration with IPGRI, KUL and others. The project mainly aims at investigating factors that influence the nature and extent of somaclonal variation in plantains and bananas in order to develop improved *in vitro* culture management procedures for the control of instability in culture. The project will also explore methods for the early detection and characterization of somaclonal variants with a focus on biochemical and molecular markers. This endeavour may eventually result in a genetic map of somaclonal variation in *Musa*, which could elucidate the underlying causes and origins of this phenomenon.

P.5.9 Transmission of chloroplasts in *Musa*

R.L. Jarret (USDA/ARS), R. Ortiz and D. Vuylsteke

The determination of the mode of inheritance (uniparental vs biparental) of chloroplasts in *Musa* spp. will be carried out by using cpDNA probes and intra- and inter-specific crosses. 'Calcutta 4' (*M. acuminata* ssp. *burmannica*) has been crossed as male and female with *M. a.* ssp. *banksii* acc. No. 237. Also *M. balbisiana* acc. No. I-63 was crossed as female with C4. Several thousand seeds were obtained from this cross. In 1993 we will do the reciprocal cross to complete the set of materials for this experiment. Seeds or *in vitro* plantlets will be sent to USDA/ARS for DNA extraction and fingerprinting.

P.5.10 Application of recombinant DNA technology for the development of disease-resistant plantains and bananas using antifungal proteins

B. Cammue (KUL), R. Swennen (KUL), D. Vuylsteke, R. Ortiz and BRU

The two principal aims of the project are the direct production of transgenic cultivars and the incorporation of antifungal protein in IITA's *Musa* breeding populations for further manipulations by conventional methods. Recent research at KUL has led to the discovery of plant-seed antifungal proteins that inhibit *Mycosphaerella fijiensis* and *Fusarium* spp. A protocol for the production of transgenic *Musa* plants has also been developed at KUL. It consists of shooting tungsten particles coated with plasmids containing the GUS marker gene and an antibiotic resistance gene into embryogenic cell suspension cultures. Many cells with transient expression were recovered. After a culture of about three months in a liquid medium containing an antibiotic, few cells were selected. These cells were transferred onto a semi-solid medium where they multiplied. All cells coloured blue within 2 hours when treated with X-gluc. This is a confirmation of the first stable transformation in *Musa* and that it was possible to select those cells among non-transformed cells. Based on this we propose the introduction of genes encoding antifungal proteins into the genome of banana and plantain using recombinant DNA techniques as described above. The utilization of different resistance genes also provides a means to increase the durability of the resistant genotypes. Moreover, the proposed recombinant technology seems to be the only means for the genetic improvement of certain groups of *Musa* such as the Horn plantains and the 'Cavendish' bananas. This project combines the comparative advantages in *Musa* genetics and improvement of the institutions involved. The hybrid *Musa* germplasm (TMPx or TMBx) developed by PBIP/IITA as well as its expertise in field testing and selection are the contributions of IITA to this project.

P.5.11 Molecular and biochemical studies of *Musa* fruit ripening

G. Tucker, Vendrell (Univ. Nottingham), S.Ferris, R. Ortiz and M. Bokanga

See P.6.10.

P.6 POST-HARVEST QUALITY OF PLANTAINS

Project rationale

Promising black sigatoka-resistant hybrids arose from crosses with wild and cultivated relatives, which have very different fruit characteristics. Hence, hybrids are likely to have altered fruit quality which will probably affect consumer acceptability. Research is needed to determine the criteria associated with plantain fruit quality and to give breeders methods for rapid, efficient screening to identify superior clones. IITA decided to intensify this work by hiring a full-time postdoctoral research fellow for postharvest research in 1992. This postdoctoral project investigates fruit palatability (flavour, texture, response to cooking procedures) and durability (shelf life, ripening, handling and storage aspects) with the goal of defining the important quality parameters and identifying rapid screening techniques for breeders' use. Quality assessment of hybrids is also required to ensure successful introduction of new varieties. The methodologies involved are sensory evaluation (taste panels), surveys and physicochemical measurements.

Completed studies

Smith-Kayode, O., P.O. Ogazi, Ore-luma Taylor, D. Vuylsteke and N.M. Ogburia. 1992. Potentials of the informal sector food processing: evidence from fried plantain products. In Abstracts of the International Food Technology Exposition and Conference. The Hague, Netherlands, 15-18 November, 1992.

Plantain (*Musa* spp. AAB group), an emerging and versatile processing material, was investigated in the context of informal sector food processing. Technical data from a study carried in Nigeria using selected cultivars namely 'Agbagba', 'Ubok Iba', 'Big Ebanga', 'Obino l'Ewai' and 'Ntanga-2' processed at 200^{0}C-210^{0}C into "lpekere" and "dodo" (fined unripe and ripe pulp snacks, respectively) gave yields of 24-31% and 20-22% respectively. Both products are popular in the snack market and highly acceptable to school age children. Consumer acceptance observation showed that they are prefered on the basis of unit price and perceived higher food value relative to other snack food products. Investement in fried plantain processing is convenient because of low technology required, initial fixed costs and working capita. Raw materials supply status and conditions necessary for enhanced growth of this informal business in lowland humid tropics are discussed.

Eggleston, G., Swennen, R, and Akoni S. 1992. Physicochemical studies on starch isolated from plantain cultivars, plantain hybrids and cooking bananas. Starch/Stärke 44: 121-128.

Starches from mature, unripe fruit pulp of plantain cultivars (*Musa* spp., AAB group) representing the wide variability in Africa, tetraploid and diploid plantain hybrids and starchy cooking bananas (*Musa* spp., ABB group) were isolated and characterised. In general, studies revealed very compact irregularly shaped and sized granules, with low amylose content (9.11-17.16%), highly resistant to bacterial a-amylase attack. Brabender amylograms showed very restricted swelling type patterns with great stability and negligible retrogradation. Results indicate that differences in physico-chemical properties exist amongst the three *Musa* fruit group starches. Plantains represent a chemical/molecular homogeneous group, but heterogeneous for granule structure. Ploidy level affected hybrid properties. ABB cooking bananas exhibited highly pronounced restricted swelling and high gelatinisation and pasting temperatures, indicating a more ordered, very strongly bonded granule structure; chemical and physical properties varied considerably within the ABB genotype.

Eggleston, G., Swennen, R, and Akoni S. 1992. Differences in composition and texture among plantains, plantain hybrids and a cooking banana. In Westby, A. and P.J.A. Reilly (eds), Traditional African Foods - Quality and Nutrition. Proceedings of a workshop, 25-29 Nov 1991. pp. 179-185.

A comparative and systematic study of post-harvest fruit characteristics, relevant to consumption and processing, of three plantain cultivars which represent the major variability of plantain subgroups in Africa was undertaken and compared with a black sigatoka resistant cooking banana and two resistant tetraploid plantain hybrids, in order to assess their alternative use. Differences in the morphological characteristics of the fruits are described. Pulp-to-peel ratios (wet basis), pulp moisture contents and carbohydrate compositions of all the unripe (green) and ripe (yellow) fruit pulp were measured. Pulp-to-peel ratios (wet basis) and pulp moisture contents of all the fruits increased on ripening; in the unripe form, no significant differences were detected among the plantains and they were lower than the values for the cooking banana and hybrids. The cooking banana demonstrated the highest degree of starch hydrolysis on ripening, which was similar to the behaviour previously reported for dessert bananas (*Musa* spp., AAA

group). Starch hydrolysis was lowest in the plantains and the plantain hybrids showed intermediary behaviour. Significant differences in texture were detected among the unripe and ripe plantains, a plantain hybrid and a cooking banana; the cooking banana was significantly softer than the plantains. The effect of traditional African plantain processing methods on fruit texture are discussed.

Thompson, A.K., Al Zaemey, A.B.S. and Ferris, R.S.B. 1992. Aspects of handling bananas and plantains. Tropical Agriculture Association Newsletter, Vol 12 (3): 15-17.
Damage to plantain fruit caused early ripening which leads to postharvest loss. Studies in the controlled environment showed that impact damage on unripe fruit had no effect on bruising and fruit ripening. Abrasion damage only reduced the ripening period of fruit stored at humidities below 100 % RH. In was considered that abrasion may cause early ripening by increasing the rate of ethylene production and consequent increase in respiration. Some of the detrimental effects caused by damage could be moderated by applying a sucrose ester based fruit coating to the peel after they had been damaged.

Ferris, R.S.B., Hotsonyame, G.K., Wainwright, H., and Thompson, A.K. (in press). The effects of genotype, damage, maturity, and environmental conditions on the postharvest life of plantain. Tropical Agriculture.
Mechanical damage is a major factor contributing towards the postharvest losses of plantain fruit. This study investigated the effects of both maturity, genotype, and damage on the ripening period of three genotypes of plantain (*Musa* spp, AAB group), in both ambient and tropical (Ghana), and controlled environmental conditions (U.K.). It was found that there were no differences between the genotypes studied. The most mature fruit at harvest ripened the most rapidly, but maturity had no effect on % moisture loss of fruit. At an ambient tropical humidity (RH) of between 70 - 96 %, only abrasion significantly increased the rate of ripening. Abrasion significantly increased fruit moisture loss and reduced the ripening period of the least mature fruit by up to 39 % compared with the control. In the controlled environment, at 100 % RH, impact and abrasion had no effect on the ripening period of plantain fruit; however, at between 55-65 % RH, abrasion significantly increased the rate of moisture loss and caused fruit to ripen significantly more rapidly than impacted and control fruit. It was concluded that abrasion accelerated the rate of ripening of plantain fruit at humidities below 100 % RH, by increasing the peel permeability to water vapour. This exposed the fruit to an increased water stress which initiated early ripening.

Ferris, R.S.B., Wainwright, H. and Thompson, A.K. (submitted). The effects of morphology, maturity and genotype on the ripening and susceptibility of plantains (AAB) to mechanical damage. Fruits.
Plantain (*Musa* AAB) is an important staple food crop grown in the lowland humid tropics. Retail surveys have indicated high levels of plantain loss during marketing and mechanical damage is considered to be a major factor contributing towards early ripening and postharvest loss. This study investigated the response of three plantain genotypes, True horn (Ubok Iba), False horn (Agbagba), and French plantain (Obino L'ewai), at two levels of maturity, to impaction, abrasion, and incision damage. The smaller Obino L'ewai fruit ripened more rapidly than the larger Ubok Iba and Agbagba plantain due to having a higher surface to volume ration and thinner peel. Abrasion significantly reduced the ripening periods of Agbagba and Obino L'ewai, but abrasion had no significant effect on the ripening of Ubok Iba. The ripening period of Ubok Iba was significantly reduced by incision. The genotypic response to damage indicated different mechanisms of climacteric initiation, related to morphological and physiological traits.

Activities

P.6.1 Fruit quality evaluation of *Musa* hybrids
S. Ferris, M. Bokanga, R. Ortiz and D. Vuylsteke
Rapid success in the plantain breeding program has produced many promising black sigatoka-resistant plantains. These are currently being field tested for resistance-durability and agronomic performance. Postharvest research will focus on fruit ripening characteristics of the hybrids compared with parents and plantain from the favoured False Horn gene pool. Fruit material will be obtained from the MET experiments I and II, IMTP plot and hybrid collections, (i.e., most recent promising selections). Key criteria to be assessed include: fruit ripening period, fruit weight loss during ripening, initial sugar, sugar:starch ratio's, starch hydrolysis during ripening, pulp: peel ratios, rate of tissue softening, dry matter content, and mineral composition. These data will be used to obtain a ripening profile specifically for the hybrids but also to provide data of ripening characteristics across the genepool, for further use by the breeding team.

Studies on dry matter content will also be used to determine effect of dessication period on the % germination of seed. This will only apply to pollinated bunches.

P.6.2 Sensory evaluation of *Musa* hybrids

S. Ferris and NARS cooperators

Objective physiological measurements (see P.6.1.) will be complemented with subjective studies of fruit palatability. This data will provide information on important fruit quality characteristics for consumers and indicate potential for consumer acceptability. Taste panels will be used for sensory evaluation of boiled unripe and fried ripe fruit Criteria for testing include taste, colour, sweetness and texture. Initial studies are being conducted at Onne station, but when fruit are available at multilocation sites, taste panel testing will be set up at NARS sites for further evaluation. Postharvest training was given to trainees during the plantain technology training course and this information will be followed by further training at key sites before hybrids are harvested.

P.6.3 Fruit damage susceptibility evaluation

S. Ferris

Postharvest losses of perishable tropical fruit has been estimated at levels from 25 % to > than 80 %. Losses are a product of the perishable nature of the crop, high ambient temperatures and handling systems which cause mechanical damage to fruit. Damage susceptibility is therefore an important factor contributing towards hybrid acceptance in the retail / marketing sector. Fruits which are more resistant to damage have the advantage of a longer potential marketing period. Damage studies will be used to determine effect of damage on tissue injury and ripening period of hybrids and traditional plantain.

P.6.4 Effects of black sigatoka disease on post harvest qualities

S. Ferris and NARS cooperators

It has been established that black sigatoka has a significant effect on fruit maturation (the period between flowering and harvest), sigatoka-infected plants having a shorter fruit filling period than fungicide-treated fruit. Effects of sigatoka on fruit ripening remain unclear and this study aims to determine whether sigatoka also causes a reduction in ripening period and how sigatoka affects fruit quality characteristics and shelf life.

P.6.5 On-farm assessment of yield and quality loss from black sigatoka

S. Ferris, F. Gauhl, D. Vuylsteke, R. Ortiz, C. Pasberg-Gauhl and NARS

(see P.8.3.)

P.6.6 Physiology of fruit maturation

S. Ferris and I. Ekanayake (TRIP)

Yield data of plantain and bananas is calulated by harvesting fruit at a specified date, determined by the number of days after flowering. This method relies on a previous knowledge of clonal maturation time at a given site. Information of previous optimal harvest date is not available for hybrids and guidelines for intersite comparisons are unclear. Furthermore operators using standard duration periods for bunch maturation are liable to harvesting underfilled bunches, making yield data prone to underestimation. Studies are therefore required to determine or evaluate optimum time of harvest. Methods for testing maturation period include monitoring days from flowering - relative to female parents, simple climactic relation, i.e., heat units, and monitoring phenotypic signs which indicate full maturity and observing how farmers judge when to harvest.

P.6.7 Fruit storage

S. Ferris and NARS cooperators

Plantains are highly perishable and lose quality rapidly after harvest. As such, postharvest losses are a major constraint to banana/plantain production in Africa. Methods to prolong shelf life include simple storage techniques to determine the effects of storage unit, sunlight, temperature, humidity, plastic film and ethylene scrubbers on ripening period. It is envisaged that experimentation will be conducted on site, on farm and at market locations.

P.6.8 Plantain utilisation

S. Ferris and NARS cooperators

Hybrid plantains closely resemble but are not identical to parent plantains. Hence there is a need to compare the utilisation characteristics of hybrids with parents as differences between hybrids and parents may confer advantage e.g. in terms of cooking time or sugar content. Initial studies will investigate preparation times for hybrids in traditional dishes. Later studies will investigate simple processing techniques such as vinegar yields from high sugar content hybrids, for storage purposes.

P.6.9 Genetics of fruit quality

R. Ortiz and S. Ferris

(see P.1.6.)

P.6.10 Molecular and biochemical studies of *Musa* fruit ripening

G. Tucker, Vendrell (Univ. Nottingham), S.Ferris, R. Ortiz and M. Bokanga

Work being conducted by Drs. Tucker and Vendrell is concerned with the effect of polygalacturonase on cell wall degradation which causes the fruit tissue softening associated with fruit ripening. Drs. Tucker and Vendrell are looking at polygalacturonase in banana and have started to screen for mRNAs for PG message. Identification of mRNAs and cDNAs for the key cell wall degrading enzymes would provide good RFLP probes to assist with breeding work. It is intended to use the IITA *Musa* collection as the base from which to isolate a range of ripening response material for testing following primary screening at Onne station. Advance of this project is subject to external funding.

P.6.11 On-farm cassava processing technology

Y.W. Jeon, L. Halos, S.Ferris

This collaborative project will support the cassava technology and processing equipment project being developed and promoted by Dr. Jeon. PBIP will supervise this project at one of PBIP's on-farm sites near Port Harcourt, Rivers State. It is hoped to develop this key project site to provide an active demonstration for the new cassava processing technology along with IITA cropping systems and new hybrids.

P.7 GENOTYPE BY CROPPING SYSTEMS INTERACTION

Project rationale

Careful management of organic matter is essential to achieve sustained perennial productivity of plantain under large scale field production conditions. Agroforestry systems such as alley cropping and the management of regrowth of natural bush fallow species in plantain fields are being investigated for their capacity to maintain productivity over long periods of cultivation without degradation of the resource base. Also, the performance of improved genotypes relative to cropping systems should be assessed. On-site cultural practices used in breeding trials may differ considerably from typical farmer practice and could influence genotype selection. Research on this project is carried out in cooperation with agronomists/soil scientists of RCMD.

Completed studies

Witters, S., D. Vuylsteke and R. Swennen (in prep.). Determination of the perennial yield potential of plantains.

The objective of this activity was to determine the long-term yield potential of six cultivars representative for the cultivar variability in the plantain subgroup. Such information may be useful to identify a plantain category that has greater potential for perennial production and to define a plantain ideotype for breeding (see P.2.16). Results from the plant crop indicated large differences in bunch weight, i.e., virtual yield, among the different plantain cultivars. Within the same inflorescence-type category, the giant plantains showed better yield performance than their medium-sized counterparts. However, giant plantains had a longer production cycle as they required significantly more time from planting to harvest. Hence, the yield potential adjusted per unit time ($mt.ha^{-1}.yr^{-1}$) did not differ among the giant and medium cultivars within the False Horn plantain category. Surprisingly, the differences in yield potential between the French plantains were still significant with the giant cultivar outyielding all other cultivars. Bunch weights were generally not very different between plant crop and 1st ratoon, but due to the signifcantly shorter cycles of

the ratoon, the potential bunch and pulp yields of the ratoon were higher than those of the plant crop for all cultivars, except for 'Ntanga 2' where they were equal. This suggests that the yield decline had not yet begun in the 1st ratoon, indicating good management of the field (healthy *in vitro* planting material, long fallow, mulching, fertilization and black sigatoka disease control). Results from the next ratoons should demonstrate if there are differences between cultivars in sustained perennial productivity (see P.7.1).

Ruhigwa, B., R. Swennen and M. Gichuru (in prep.). Plantain production in an alley cropping system on an Ultisol in southeastern Nigeria.
The results of the plant crop indicated that plantains grown in *Acioa barteri* alley cropping plots and in elephant grass mulch plots performed better than in plots with the other hedgerow species. Growth of the plantain crop as indicated by pseudostem height at 6 months after planting showed that the plantain plants in the control and the *Acioa barteri* plots grew faster than in the other treatments. Early vegetative vigour is positively correlated with yield, thus the *Acioa* and control treatments resulted in the highest yields. The plantain yields were slightly lower in the *Acioa* alleys than in the control *Pennisetum* mulch plots (15.1 and 17.8 $mt.ha^{-1}$, resp.); however, the *Pennisetum* mulch must be obtained from external fields, i.e., it is not an alley cropping system, which requires higher land and labour input. Therefore, alley cropping with *Acioa* seems to be a more promising system for field production of plantains. The superior performance of plantain in *Acioa* alleys and *Pennisetum* mulch plots may be the result of improved soil properties and good weed suppression in those treatments. *Acioa* leaves decompose slowly which ensures a permanent mulch cover, unlike the litter and prunings of *Gmelina, Alchornea* and *Cassia*. *Acioa* also produces a greater biomass in the initial pruning. An additional advantage of *Acioa* is that it requires only two prunings per year against 4-6 prunings for the other hedgerow species. This research was carried by a research fellow.

Daramola, B., M. Gichuru, D. Ladipo (ICRAF) and D. Vuylsteke (in prep.). Survey of changes in floristic composition within natural multispecies hedgerows under plantain cropping at Onne, S.E. Nigeria.
A botanical survey was conducted to determine the number of plant species and the changes occurring in the floristic composition of natural hedgerows established from fallow species that were allowed to regrow in order to form a spontaneous multispecies hedgerow under plantain cropping. The study was conducted in a 4 ha, 5-year old plantain field which was planted in a manually cleared 10-year old bush fallow (see P.7.1.). About 120 different plant species were identified in these natural hedgerows, which coppiced regularly to provide mulch to the plantain. An increase in the number of plants per unit area was observed with increasing plot age, i.e., the longer the plot was cultivated with plantain, the higher the number of plants in the hedgerows. The most frequent species were *Anthonata macrophylla, Milletia aborensis* and the aggressive shrubby weed *Chromolaena odorata*. The presence of highly nodulating tree species and the high biomass produced by plants such as *Chromolaena odorata*, which provide a source of mulch, could explain the good perennial performance of plantain in this field. Research by RCMD and ICRAF will continue on the management of the multispecies hedgerows and to determine the rate and pattern of increase in the population of shrubs, trees and herbaceous annual species found in this field. The effects of the multispecies hedgerows on the nutrient status of this site will also be monitored.

Activities

P.7.1 The perennial yield potential of plantains
D. Vuylsteke, R. Swennen (KUL) and R. Ortiz
This research aims to determine the long-term yield potential of six plantain cultivars representing different taxonomic groups. The experiment, planted in 1987 with *in vitro* produced plants, will continue for at least 5 years. The trial was established in a 10-year old bush fallow which was cleared manually and not burnt. In the 3m wide alleys between the rows of plantain, the natural shrub and tree species of the bush fallow were allowed to regrow in order to form a spontaneous, multispecies alley cropping system. Through regular coppicing, the prunings of the multispecies hedgerows served as mulch for the plantain crop, as such providing the organic matter required for sustained plantain productivity. In 1991, a research scholar from Belgium performed the data analysis of the first 2 production cycles, the plant crop and the 1st ratoon (see completed studies in P.7.). In 1993 the experiment will be continued and the analysis for the five year period (1987-1992) will be done considering the effects of years (Y), plantain cultivars (P), and the YxP interaction in the following traits: pseudostem height, total number of leaves, number of leaves at

flowering, length/width ratio of the seventh leaf, height of tallest sucker at flowering and at harvest, days to harvest, bunch weight, number of hands and fingers, and length of production cycle.

P.7.2. Crop management practices for sustainable and perennial plantain production
M. Gichuru (RCMD), D. Vuylsteke, R. Ortiz and PHMD scientists

Plantains, with their shallow root systems, perform extremely well in household gardens, benefiting from regular input of household organic matter, but not in open fields , especially in the highly weathered soils of West and Central Africa. Even liberal application of inorganic fertilizers fails to halt the rapid decline in vigor and yield. This has compelled farmers to regularly move to new fields in cleared forest or bush fallow for plantain cultivation. Such practice is, however, not sustainable in view of the increasing population pressure on land and enhanced environmental awareness. Previous agronomy research has clearly demonstrated that proper management of organic matter is essential for maintaining plantain productivity over the long term without causing environmental degradation. Recently, we have observed that plantains perform very well in an agroforestry system with spontaneous multispecies hedgerows, in which the regrowth of more than 120 shrub and tree species of the natural fallow is managed by regular coppicing. This system seems promising in terms of maintaining productivity over extended cultivation periods as well as conserving the plant diversity for bush fallow periods.

In a new experiment, we will investigate the response of a local plantain cultivar and a BSR hybrid (TMPx 548-4) to such multispecies alley cropping with low, medium and high fertilizer input, and in comparison to the farmers' practice. The effect of long-term plantain cultivation on soil, environment and pest buildup will be studied. This research fits in the framework of management of natural resources for development of sustainable and stable plantain production systems, yet also emphasizing plantain productivity through crop improvement. The interrelationship between resource management, cropping systems and crop improvement will be investigated in order to achieve maximum interaction between these components.

P.8 NARS COLLABORATION AND TRAINING

Project rationale

Because of the recent expansion of IITA's plantain/banana improvement work, NARS collaboration is less developed than for other IITA crops. PBIP aims to strengthen NARS through (i) cooperative research, (ii) consultancy support, (iii) individual training: scientific visits and PhD students, (iv) group training, (v) transfer of improved *Musa* genetic materials and production packages, and (vi) publication of production manuals, research guides and other training materials.

Completed studies

IITA. 1992. An introduction to the IITA High Rainfall Station, Onne, Rivers State, Nigeria. IITA. 8 pp.

This brochure reports the achievements of IITA researchers working in this station in (i) plantain and banana breeding, (ii) soil management, (iii) cropping systems, and (iv) agroforestry research. It also explains how this station, which was established primarily to conduct research in a major agroecological zone, has considerable potential to foster active collaboration with NARS. This station, the home of PBIP, acts as a satellite of IITA, and collaborates with national research institutes, agricultural development projects, as well as universities located in an area with the highest population density in West & Central Africa.

Activities

P.8.1 Multilocational trials
R. Ortiz, D. Vuylsteke and cooperators at IITA and NARS
(see P.2.14)

P.8.2 Advanced *Musa* yield trials
D. Vuylsteke, R. Ortiz, IITA and NARS cooperators
(see P.2.15)

P.8.3 On-farm assessment of yield and quality loss from black sigatoka

S. Ferris, F. Gauhl, D. Vuylsteke, R. Ortiz, C. Pasberg-Gauhl and NARS

Plantain yield and fruit quality loss from black sigatoka leaf spot have been assessed at IITA station sites. These plots are subject to high inputs of fertilizer and management practises and may not be fully representative of real farming situations. Further assessment of the host-pathogen interaction requires information from farmers plots to determine effects of sigatoka disease on yield and fruit quality. Experimental sites will be located on NARS stations and on-farm, offering excellent opportunities for collaborative studies and feed-back on the performance of TMPx hybrids under diverse management situations.

P.8.4 Cooking bananas for Nigeria

D. Vuylsteke, R. Ortiz, S. Ferris and NARS

PBIP cooperation was requested by the Commissioner of Agriculture of Rivers State, Nigeria, to continue providing planting materials (suckers and *in vitro* plantlets) of the cooking banana cv. 'Cardaba', which has been released as a new cultivar for this area. In 1993, PBIP will continue providing support to the local goverment in the distribution of this cooking banana to farmers. Evaluation of acceptability will be initiated in collaboration with NARS. (See also P.5.2.).

P.8.5 Black sigatoka-resistant plantains for Nigeria

R. Ortiz, D. Vuylsteke, C. Anojulu (FACU) and other IITA and Nigerian NARS cooperators

The Nigerian Interministerial Committee for the Control of Black Sigatoka Disease requested the concourse of PBIP for the development of new breeding materials and technology in this area. In 1992, both programs started a joint effort for the testing of TMPx germplasm in 11 Nigerian sites to identify promising genotypes for future cultivar release (see also P.2.14). Group training for Nigerian cooperators (see P.8.7.) has also been provided in 1992.

P.8.6 Graduate training

D. Vuylsteke and R. Ortiz (supervisors)

1. In vitro embryo rescue for the enhancement of hybrid plantain production - G.I. Harry (see P.5.3).
2. Determination of ploidy levels and ploidy effects on growth and yield parameters in plantain-banana hybrids. H. Vandenhout (see completed studies in P.1 & P.2. and P.2.5 & P.3.5)

P.8.7 Plantain Research and Technology Transfer training course

M.T. Ajayi (Training Program), R. Ortiz (technical coordinator) and other IITA and NARS scientists

This annual group training course is offered for three weeks in November to 20 young African scientists and technicians. NARS scientists participate as resource persons on specific topics.

P.8.8 Major constraints to *Musa* production in Africa (*)

NARS, D. Vuylsteke and R. Ortiz

The plantain group training course (see P.8.7.) provides a forum to discuss the most important limiting factors for the production of plantain and banana in Sub-Saharan Africa. In 1992, course participants from 10 countries (Côte d'Ivoire, Ghana, Togo, Nigeria, Cameroon, Sao Tome & Principe, Zaire, Uganda, Tanzania and Malawi) indicated that (i) the disease complex (mainly sigatoka, banana weevil and nematodes), (ii) yield decline, and (iii) post-harvest losses are the major constraints to banana/plantain production in Africa.

P.8.9 Training materials

Training Program, NARS and PBIP scientists

In cooperation with the Training Materials Unit, PBIP scientists are publishing Crop Production and Research Guides. Crop Production Guides contain information for crop production, training and research. Although the information is directed at an intermediate level, it can be adapted for communicating with farmers. Research guides are based on documents produced for training. Their objective is to provide information and guidance to researchers and technicians conducting research essential to agricultural development. The Research Guides can be used in both research and training.

The following are the guides which should be published during 1993:

Swennen, R., R. Ortiz and D. Vuylsteke. **Morphology and growth of plantain.** IITA Crop Production Guide.

Gauhl, F., C. Pasberg-Gauhl, D. Vuylsteke and R. Ortiz. **Multilocational evaluation trial of IITA *Musa* Hybrids. Protocol and guidelines for field management and data collection.** IITA Research Guide.
Vuylsteke, D. and R. Ortiz. ***Musa* taxonomy and diversity.** IITA Research Guide.
B.A. Adelaja (NIHORT) and D. Vuylsteke. **Field techniques for rapid multiplication of banana and plantain.** IITA Research Guide.
Vuylsteke, D. **Tissue culture for *Musa* multiplication.** IITA Research Guide.
Ortiz, R. **Statistical tools for plantain researchers.** IITA Research Guide.
Ortiz, R. **Genetic analysis for plant breeders.** IITA Research Guide.
Ferris, S. **Postharvest physiology and biochemistry of plantain ripening.** IITA Research Guide.
Ferris, S. and Smith-Kayode, O. **Plantain storage.** IITA Research Guide.
Ferris, S. **Plantain fruit quality: criterion and evaluation.** IITA Research Guide.
Ferris, S. and Ogazi, P.O. **Plantain processing and utilisation.** IITA Research Guide.
Akalumhe, Y.O. and Ferris, S. **Plantain marketing I - Overview.** IITA Research Guide.
Akalumhe, Y.O. and Ferris, S. **Plantain marketing II - Techniques for rapid rural surveying.** IITA Research Guide.
Ferris. S. **Plantain marketing III - Experimental methods to determine postharvest loss.** IITA Research Guide.

P.9 ONNE STATION

Activities

P.9. 1 Upgrading of physical facilities at the Onne High Rainfall Station of IITA in support of plantain and banana research and training

D. Vuylsteke and P.D. Austin

Onne station is one of four stations of the IITA in West and Central Africa. Its research farm of 100 hectares is located in southeastern Nigeria, in an area marked by extreme population pressure on the land. The station is representative for the humid rainforest ecosystem, characterized by high rainfall and highly leached, acid ultisols. IITA has recently emphasized the role of the Onne station in its research agenda. It is the base of IITA's plantain and banana improvement program, which has achieved rapid success in the production of hybrid varieties resistant to the black sigatoka disease. In addition, the Onne station is an important test site for IITA's resource and crop management work, particularly for developing sustainable agroforestry systems for the humid lowlands. The station also has considerable potential to foster active collaboration with national agricultural research systems (NARS).

The innovative research on plantain and banana improvement and agroforestry systems, as well as the collaboration with NARS in the region, have attracted increasing training activities and linkages at Onne station. Facilities at the station are rustic and there are no suitable facilities for participants in training programs, which impedes the development and transfer of technologies developed at IITA, and Onne in particular, to NARS and farmers in the region. Furthermore, the potential for successful longer term programs of plantain/banana breeding can only be achieved given adequate logistic and backup support. This project, funded by the Belgian Administration for Development Cooperation (ABOS/AGCD), aims at upgrading the physical facilities and research support services at the Onne station of IITA in view of enhancing the research and training capability in plantain/banana improvement.

Genetic Resources Unit

The Genetic Resources Unit (GRU) has the responsibility to collect, characterize, conserve and distrubte the germplasm of food legumes, rice, root and tuber crops and plantain/banana in Africa. Small collections of maize and a few other crops and many agroforestry species are also held to service particular needs of Program researchers and researchers from other IARCs and of national researchers. Plantain and banana germplasm is mainly an active working collection, kept at the Onne Station where researchers are located who manage the material. A duplicate of this plantain collection is maintained as *in vitro* culture at the Tissue Culture Laboratory in IITA's main campus in Ibadan to enhance the security of this collection. The diverse germplasm collections available at IITA are essential tools for IITA's breeding programs, as well as for researchers world-wide and for posterity.

G.1 GERMPLASM COLLECTION

Project rationale

Collection of the germplasm of African food crops is essential to counteract the genetic erosion that is occurring as a result of changes in land use. We organize collections in-country with NARS on a target basis. This germplasm serves as an invaluable resource for present and future crop improvement efforts. In addition, it ensures the conservation of the genetic diversity which is needed to maintain sustainable food production and sound enviornment.

Completed studies

Maroya, N. and N.Q. Ng. (unpubl.). Collecting cassava germplasm in the Republic of Benin

During 1989/90, we collected some cassava germplasm in a multicrop collecting mission in the Republic of Benin. However, several regions were not adequately covered. The national program of the Republic of Benin was interested to collect more local cassava germplasm for evaluation. Funded by the RRPMC project, a cassava germplasm collecting expedition was organized during 1992 to the Central and Southern regions of the country. This resulted in collecting 207 samples of cassava germplasm. These germplasm materials are being maintained in the country. Arrangements will be made to duplicate maintenance of some of this collection at IITA.

Padulosi, S. Plant Exploration in Congo, GRU Exploration Report.

Congo was successfully explored during the months of June - July 1992 by covering the areas (central and northern regions) not previously collected by the earlier expedition which IITA mounted in 1988. A total of 333 accessions were gathered, which included both cultivated and wild IITA mandated species (accessions were maize 41, wild and cultivated yam 33, cowpea 12, Bambara groundnut 16, wild legumes 19, wild *Vigna* spp. 96 and cassava 77) along with 39 samples of groundnut gathered from the national germplasm collection held at Loudima Station. A highlight of the survey of the wild *Vigna* species distribution in this part of Africa was the finding for the first time of two wild *Vigna* species, *V. longitoba* and *V. juruana* in the eastern part of the county. The mission also confirmed the presence of the newly described variety of wild species, *V. unguiculata* to subsp *dekindtiana* var congolensis Padulosi, which is endemic only in this country.

Activities

G.1.1 Collecting cassava and maize germplasm in Nigeria

N.Q. Ng, T.G. Nandang, COSCA team and NRCRI and IAR&T Scientists

During 1992, GRU had in collaboration with COSCA team visited 60 Nigerian villages surveyed previously to collect clonal cassava varieties identified by the COSCA survey. A total of 150 distinct named varieties was collected from farmers in those villages and these were brought back to IITA for multiplication, evaluation and conservation. A duplicate of 125 clonal materials of this collection was given to NRCRI for its use. Further collection will be made in another 15 COSCA villages surveyed previously.

Through RRPMC supported project, NRCRI scientists, collected 465 samples of cassava from regions different from where the above samples were collected. After initial multiplication at Umudike, distinct genotypes of this collection will be duplicated at IITA.

Our collaborators in IAR&T collected 284 samples of maize germplasm from various parts of Nigeria during 1992, with funds provided by RRPMC project. These materials will be evaluated and multiplied by the national scientists during 1993 and a duplicate of the multiplied materials will be transfered to IITA for storage and for use in breeding programs.

During a collecting expedition in Nigeria, 15 samples of *Dioscorea* species and 5 of wild *Vigna* were also collected, which were brought back to IITA for research and conservation.

G.1.2 Germplasm collecting expedition to Uganda

S. Padulosi

Cleareances which delayed commencement of the mission were finally concluded. All the necessary documents requested by the Local Authorities were successfully processed and the exploration is now expected to take place sometime in early 1993.

G.1.3 Collecting germplasm in Guinea-Conakry

El Sanoussy Bah, Sekouna Camara, N.Q. Ng, J. Kling, S.K. Kim and M.D. Winslow

Funded by RRPMC project and based on a request by the national program of Guinea (Conakry), an exploration mission for collecting cassava and maize germplasm was conducted in the Basse and Moyenne regions of the country, between 1 November and 15 December, 1992. This mission had collected 104 samples of cassava and 194 samples of maize from those two regions. Plans are made to further collect the germplasm of these two crops in the Haute and Forestiere regions of the country during 1993. Yam germplasm will also be collected duirng this time. Plans are also being made to characterize this germplasm collection in Guinea and to duplicate the germplasm for conservation and use at IITA.

G.1.4 Wild *Manihot* species collecting expedition in Brazil

S.K. Hahn, A.C. Allem and N.Q. Ng

IITA in collaboration with EMBRAPA, CIAT, and IBPGR participated in a collecting expedition in the Amazon region of Brazil to collect germplasm from the wild primary gene pool of cassava, namely *Manihot flabellifolia* and *M. peruviana*, during the period between 12 May and 12 June 1992. In addition to collecting germplasm for use in plant breeding and for conservation, the exploration trip also tried to amass further evidence on the theory that cassava was domesticated from stocks of either or both of these wild species. Forty-nine accessions from fourteen species of *Manihot* were collected. This and other collections of seeds should provide useful materials for a followup study on the diversity and evolution of cassava germplasm by morphological, cytological and DNA techniques to be carried out at IITA.

G.2 GERMPLASM CONSERVATION, DOCUMENTATION AND DISTRIBUTION

Project rationale

Germplasm conservation is a high priority worldwide, and crop improvement researchers have a special responsibility to conserve germplasm since the successful spread of improved varieties is one cause of the erosion of existing genetic diversity in crop species. Enlarging and maintaining the existing germplasm collection will provide needed genetic diversity for direct use in farming, or use in plant breeding to produce improved genotypes suitable for different agricultural systems. Good germplasm management and well documented germplasm information will lead to efficient use and improved security of the germplasm being conserved.

Activities

G.2.1 Cowpea germplasm conservation

N.Q. Ng, A.E. Adegbite and T. Odega

In 1992, more than 3,000 accessions of cowpea germplasm were multiplied/rejuvenated mostly during the second rains and dry season, in the fields on IITA campus in Ibadan. Some accessions had to be initially multiplied in pots in screenhouse. Seeds of 15,000 accessions are maintained in an active collection store (5°C, 30% R.H.). More than 7,500 accessions are also in long-term storage in sealed aluminium containers (-

20°C, 5% moisture content). Seed viability was tested for all accessions prior to long-term storage. Only those that showed a germination rate of more than 85% were processed for long term storage. Accessions that showed a germination rate of less than 85% will be rejuvenated as soon as possible. Over 1700 accessions of cowpea were sent to Georgia, USA for their evaluation as well as for back-up duplicate storage.

G.2.2 Cowpea core collection

N.Q. Ng

Data on agrobotanical traits, resistance to diseases, pests and physiological stresses as well as agroecological information are being analysed to choose a set of accessions to represent a core collection. Other characters such as DNA Polymorphism is also being investigated (see section under cowpea DNA Polymorphism).

G.2.3 Virus cleanup of cowpea germplasm

N.Q. Ng, H. Rossel, A.E. Adegbite and B.B. Singh

Some cowpea viruses are seed-born such as cowpea aphid-borne mosaic, cucumber mosaic and cowpea yellow mosaic, which are common on-site at IITA. Although attempts were made to rogue out virus-infected plants during field multiplication, it appears that seed stocks of many germplasm accessions still contain viruses. For international distribution, it is important that they be cleaned. 185 accessions suspected to contain virus were planted out in an insect-proof screenhouse in 1992 and plants exhibiting symptoms were rogued; seeds were harvested from healthy plants only. In addition, 1,600 accessions of germplasm were planted in Samaru for field evaluation for pest resistance. Any plant with primary infection of viruses was rogued as soon as symptoms became evident. There was no outbreak of aphid, and no secondary spread of virus was observed to have taken place. Thus, it was possible to harvest seeds from healthy plants of around 1,000 accessions, which should be virus free.

G.2.4 Wild *Vigna* germplasm conservation

S. Padulosi, N.Q. Ng, K. Sotonwa and J.O. Apeji

About 300 accessions were multiplied as part of the on-going germplasm rescue/multiplication activities for these species. Donations have been received from NARS and IBPGR and their material and passport data have been up-dated into the Unit's wild *Vigna* database. Many accessions need to be further multiplied to provide sufficient seeds for conservation and to meet the high demand for seeds for research world wide. New accessions will be acquired or collected.

G.2.5 Yam field genebank

N.Q. Ng, D. Ogunsola and S.Y.C. Ng

Yam germplasm conservation is an arduous task. Most germplasm accessions do not produce flowers or seeds, thus they have to be maintained vegetatively in field nurseries and/or by *in vitro* culture. Field rejuvenation/multiplication is slow because only a few tubers are produced per plant and the crop growth cycle is long (6 to 9 months). Furthermore, tubers are bulky, requiring lots of space to store; and they lose viability in less than a year, so the whole propagation process has to be repeated annually.

Since 1992, we adopted the minisett propagation technique, by growing small planting setts on ridges covered with plastic mulch to produce small 'seed yams' (tubers) for storage. Plastic mulch reduces foliar diseases and suppresses weeds as well as eliminating staking. We also found that some 'seed yams' stored at about 18°-20°C and 50% R.H. could be stored for up to a year, but still they need to be grown annually. During 1992, more than 2,500 accessions of the existing collection were propagated and maintained in the field as well as in yam store/barn. This is supplemented by *in vitro* meristem culture to conserve this valuable germplasm.

G.2.6 In vitro yam germplasm conservation

S.Y.C. Ng, N.Q. Ng and R. Asiedu

Over 800 accessions of yam germplasm were transfered from field collections to *in vitro* culture using meristem culture. Out of these, 600 accessions showed growth. Routine maintenance of over 1,700 accessions of different yam species *in vitro* was a major task. The maintenance of the existing *in vitro* collections and transfer of yam germplasm to *in vitro* culture will continue to be a major activity.

White yam single node cuttings were able to establish in culture media with a range of sucrose concentrations (1 to 10%) with or without the addition of mannitol. In most cases, mannitol reduces the

plant height and leaf size of the plantlets. Plantlets incubated at normal incubation condition have higher plant height than those incubated under lower temperature and light intensity. Survival rate after one year of culturing indicated that media containing 1 and 3% sucrose with or without mannitol had a higher survival rate and that cultures incubated at lower temperature also had higher survival rate.

G.2.7 Yam germplasm characterization

N.Q. Ng, R. Asiedu, H. Rossel, C. Akem, A.E. Adegbite and NRCRI

A total of 1,000 yams (*D. rotundata* and *D. alata*) germplasm accessions (including 64 from NRCRI and 353 from TRIP breeding lines) were assessed at IITA, Ibadan for up to 149 agrobotanical descriptors including flowering characters. In addition, the incidence of virus and fungus diseases were also evaluated. The same set of materials were also grown at Abuja where 20 agrobotanical characters including flowering and the incidence of virus and fungus diseases were scored. About 700 accessions of these germplasm were similarly assessed at Uturu for flowering characters, yields and fungus disease incidence. Data are being processed and documented. These will be collated and analyzed during 1993. Another 1,000 germplasm accessions will be planted at Ibadan and Abuja for characterization in 1993.

G.2.8 Cassava germplasm conservation and characterization

N.Q. Ng, T. Nandang, S.Y.C. Ng and H. Rossel

A total of about 1,700 clones of cassava germplasm were maintained as a field gene bank. Ten cuttings of each clone were planted at close spacing at 25cm x 50cm. The materials were re-planted in a new field every 9 months or less to avoid over growth and reduce risk of loss. A new collection of about 150 clones collected within Nigeria during 1992 were also established in the same field. About 400 accessions, planted on ridges at normal spacing which had completed one year growth cycle, were characterized for up to 69 agrobotanical descriptors. Virus disease incidence on these collections was also recorded. Additional accessions were planted for characterization. Shoot tips of African local germplasm were regularly collected from the field gene bank for *in vitro* culture for the conservation of these germplasm accessions. These activities will be continued in 1993.

G.2.9 Rice germplasm conservation

N.Q. Ng and E.S.O. Erimah and T. Odega

Although the mandate for rice improvement has been transferred to WARDA, IITA retains responsibility for the germplasm collection for the time being. The rice collection consists of 9,473 accessions of Asian domesticated rice (*O. sativa*), 2,503 of African domesticated rice (*O. glaberrima*) and 379 of wild *Oryza* species. About 90% of the collection has been characterized with data stored in a computer bank. During 1992 about 1,000 accessions were multiplied and/or rejuvenated and we continued to process seeds for long-term storage (-20°C, 5% seed moisture content). Over 1,200 accessions of *O. glaberrima* were given to WARDA and NCRI scientists for drought resistant screening during 1993.

G.2.10 Miscellaneous leguminous germplasm and Agroforestry species conservation

N.Q. Ng, A.E. Adegbite, T.G. Nandang and T. Odega

During 1992, a total of about 160 accessions of 11 food legumes and one cover crop (67 African yam bean, 33 Winged bean, 19 Lablab bean, 9 Lima bean, 9 Pigeon pea, 7 Kersting's groundnut, 7 Rice bean, 6 Mung bean, 5 Jack bean, 4 Sword bean, and 3 Mucuna) were multiplied/rejuvenated at IITA headquarters, to replenish the seed stock for use by the various programs at IITA and national programs, and also for conservation.

These germplasm multiplication plots also served the purpose for use by the IITA Research Group for Integration of Legumes into Farming Systems to select genotypes for potential use in specific ecologies or farming systems.

In collaboration with the ICRAF scientist, GRU maintained some 300 accessions of germplasm of about 100 species of multipurpose tree and shrub species in its gene bank and compiled a list of these species that are also maintained in Arboreta at IITA headquarters in Ibadan and IITA sub-station in Onne, Port Harcourt. We also collaborated with the ICRAF scientist in seed storage studies on some of those species.

G.2.11 Germplasm distribution

N.Q. Ng, J. Obarinde and T. Odega

We responded to about 200 requests for plant genetic resources that are maintained in GRU and their related information during 1992. We distributed 9171 samples of germplasm world-wide during the year.

59% of the materials were requested by IITA researchers. This is an ongoing activity that will continue to be a major service function of the unit in future.

G.2.12 Storage of yam seeds and pollens

N.Q. Ng

It is believed that true seeds of yams are probably orthodox, which could withstand dessication and be expected to survive for considerable periods at low temperatures and low seed moisture content. However, storage characteristics of seeds of various species is unclear. It is planned to conduct research to clarify how seeds of different yam species behave during storage in various standards and this would help in conserving yam seeds. Little has been studied on pollen storage of yam, as a means to conserve germplasm. Quite a big proportion of yam germplasm accessions produce male flowers in Ibadan. An investigation on the possiblity of using pollen storage to conserve yam may help the conservation of yam germplasm.

G.2.13 Health status of germplasm collections

N.Q. Ng, D. Florini, H. Rossel and other pathologists

Health status of the seed collections presently maintained in genebank or those that will be harvested from multiplication/rejuvenation fields will be checked or tested for safe transfer.

G.3 GENETICS AND PHYLOGENY OF GERMPLASM

Project rationale

Better knowledge about genetics and phylogenetic position of germplasm will enhance the usefulness of the germplasm for crop improvement. In recent years, collections from many poorly defined species have been obtained, particularly from wild *Vigna* and *Dioscorea*

Completed studies

Barone, A., A. Del Giudice and N.Q. Ng (1992). Barriers to interspecific hybridization between *Vigna unguiculata* and *Vigna vexillata* Sex Plant Reprod. 5: 195-200

Interspecific hybridization between *Vigna unguiculata* and *V. vexillata* always failed: no seed was obtained in both crossing directions. Two different barriers to crosability were found: a pre-zygotic barrier and post-zygotic one. Many abnormalities were observed in pollen-tube development, which reduced the percentage of fertilization to 18-30%. Differences in the percentage of fertilization were detected between the two accessions of *V. vexillata* involved in the interspecific crosses. The development of the interspecific embryo was analyzed and the embryo and endosperm nuclei always degenerated 5-8 days after pollination. The growth of the embryo stopped at a globular stage, which is too early for excision and *in vitro* culturing.

Marconi, E., N.Q. Ng and E. Carnovale 1992 (in press). Protease inhibitors and lectins in cowpea. Food Chemistry

Wild and cultivated accessions of cowpea were analysed for trypsin and chymotrypsin inhibitors, and lectins in order to assess: their variability; their influence in the mechanism of Bruchid Resistance (BR); their evolution during species domestication.

Wild *Vigna vexillata* showed significantly higher values ($P < 0.0001$) for trypsin and chymotrypsin inhibitors and significantly lower values ($P < 0.025$) for lectins as compared with cultivated *V. unguiculata* accessions. This can suggest that domestication may operate an indirect selection for these characters. High degree of correlation between CIU and TIU ($r=0.959$) and significant correlation ($P<0.01$) between TIU and Protein content were found.

The high resistance to Bruchid and the high TI content of *V. vexillata* suggest that even where there is no direct relationship between BR and ITU (any case, cultivated accessions with a low TIU content prove to be likewise resistant), protease inhibitors promote (or are a component of) the plant's defence mechanism.

Ng, N. Q. and S. Padulosi 1992. Constraints in the accessibility and use of germplasm collections. Page 45-50 In: Thottappilly, G., L. Monti, D.R. Mohan Raj, and A.W. Moore (eds.). Biotechnology: Enhancing Research on Tropical Crops in Africa. CTA/IITA co-publication. IITA, Ibadan.

The problems associated with the lack of precise techniques for measuring the diversity of a crop species, selecting materials for accessions, and determining the appropriate size for a core collections, as well as the

boundaries of a genepool were discussed. The most important constraints to accessibilty and use of germplasm collections were listed and areas where biotechnology techniques can offer help were indicated. Among the research areas considered important for better utilization of germplasm are taxonomy of wild species, techniques for determination of a representative collection, reproductive biology and germplasm enhancement, conservation techniques and genetic stability under storage, evaluation for specific agronomic traits (particularly for stress resistance), and non-destructive pathogen testing techniques.

Padulosi, S. and N.Q. Ng (in press). A useful and unexploited herb, *Vigna marina* and the taxonomic revision of its genetic diversity. Bull. Jard. Bot. Nat. Belg.
Potential use of *Vigna marina* were reviewed and the morphological variability of the species studied. Major phynotypic differences between varieties from East and West Africa were found and these seems to be related to their geographical distribution. Upon such findings, a new delimitation of the species into two subspecies, i.e., subsp. *marina* and subsp. *oblonga* was proposed.

Agwaranze, N.F., N.Q. Ng and T.A.O. Ladeinde 1992. Morphological variability and the inheritance of pubescence in *Vigna vexillata* Page 27: In: Joint cowpea Biotechnology workshop, Bari, (Italy) June 29 - July 1, 1992
The morphological variability in *Vigna vexillata* based on 39 characters has been studied. A cluster and principal components analyses grouped the lines into 3 distinct varieties. The grouping did not follow any definite pattern with regard to geographical area of collection.

Study on the mode of inheritance of the hair characters was conducted using two independent analyses (diallel of the F1 and generation mean of the F2 and backcross populations) in a complementary manner. Both indicated high additive gene effects and partial dominance in the inheritance of the traits. Narrow sense heritability was quite high in all the analysis.

Estimates of the combining ability effects revealed that the general combining ability effects was more important than the specific combining ability.

The additive gene action and high heritability suggest that both pedigree breeding and recurrent selection programmes may be successful in selecting hairy lines of *Vigna*.

Venora, G., S. Padulosi and K. Sotonwa. Stomatal variation in *Vigna unguiculata* In: Joint Cowpea Biotechnology Workshop, Bari, Italy 29 June - 1 July, 1992
The study has been conducted on leaves from various accessions of wild *Vigna unguiculata* selected in order to represent each of the different varieties recognised within the wild cowpea group. Three leaves per each variety were analysed and both the upper and lower surface were studied by calculating the following parameters: leaf area, no. of stomata per mm^2, stomatal length and width and transpiring area. The results pointed out interesting correlations between stomatal and leaf traits while also indicating that almost all the 13 varieties investigated were able to be distinguished on the basis of stomatal traits.

Padulosi, S., G. Venora and N.Q. Ng. Cytogenetics studies within *V. unguiculata* (unpubl.)
Analyses of methaphases chromosomes of representative accessions of wild cowpea have been carried out by means of a computerized image analyser equipment. The results indicates that no significant differences among varieties at the level of karyotype can be detected within wild *V. unguiculata*. However the study has indicated that the average length of the karyotype of wild *V. unguiculata* is generally shorter than that of the cultivated cowpea.

Padulosi, S. and W. De Leonardis. Pollen/seed anatomy of wild *Vigna unguiculata* (unpubl.)
Detailed measurement of pollen grains of wild cowpea varieties along with morphological studies of seeds of the same accessions have provided additional information on the variability to be found within wild *V. unguiculata* material. Scanning electron microscope images of pollen exine strongly suggest that material gathered from Southern African countries is definitively more primitive than that originated elsewhere in Africa, thus supporting the hypotheses that the species *V. unguiculata* might have originated in that region.

Paul, C.P., N.Q. Ng and T.A.O. Ladeinde 1992. Submitted to Journal. Diallel anaylsis of resistance to rice yellow mottle virus in African rice *Oryza glaberrima* Steud.
The genetics of resistance to rice yellow mottle virus (RYMV) in *Oryza glaberrima* Steud. was studied in a full diallel crosses, involving four resistant and two susceptible genotypes. The F1 hybrids obtained from

resistant and susceptible parents showed various degree of susceptibility to the virus. Estimates of genetic parameters following Hayman's method confirmed the significant additive effects with some degree of non-additive gene action in determining disease reaction.

The Wr, Vr graphic analysis showed that resistance to RYMV in *O. glaberrima* is largely controlled by recessive genes with partial dominance. Similarly, Griffings' method also revealed that general combining ability (GCA) was much important than specific combining ability (SCA) effects. Generation mean analysis also indicated that RYMV is controlled by additive gene effects with two to four genes.

Galasso, I. (IITA fellow in Bari), D. Pignone and P. Perrino (Plant Germplasm Institute, Bari, Italy). Cytotaxonomic studies in *Vigna*. I. General technique and *Vigna unguiculata* C-banding. Carylogia 45(2): 155-161 1992

In order to assist cytotaxonomic investigations in the genus *Vigna*, a number of different cytological techniques have been explored with inadequate results, due to the extremely small size of its chromosomes. Therefore, a critical analysis of the steps involved in the preparation of cytological slides was conducted. Modification of the prefixation, fixation, maceration and squashing steps have been explored. As a consequence, a schedule is proposed that allows to obtain a good number of well spread, well shaped metaphase plates and that applies to all the species examined. As a result a C-banding karyotype was obtaines in *Vigna unguiculata*, the most important cultivated species of the genus. The karyotype is described in a genotype considered as the genetic standard for this species.

Activities

G.3.1 Phylogeny and Biosystematics of African *Vigna* : hybrid between *V. oblongifolia* and *V. ambacensis*

N.Q. Ng, J. Apeji, A.E. Adegbite and H. Mignouna

Systematic crossing among different *Vigna* species continued in 1992 from project initiated earlier with an over all objectives: (1) determine genetic affinity between species and clarify taxonomy; (2) determine the gene pool boundary of cowpea; and (3) find 'bridging species' to help crossing cowpea with wild *Vigna*. During 1992, a putative F1 hybrid of a cross between *V. oblongifolia* and *V. ambacensis* and the two parents were studied by morphological and cytological characters as well as DNA-RFLP markers to varify the cross. Variability and crossability among various distinct botanical varieties within and between the two parental species will further be studied. Other crosses among *Vigna* species will also be investigated.

G.3.2 Wild African *Vigna* palinological analyse

De Leonardis, S. Padulosi and N.Q. Ng

Aiming at investigating the taxonomic relationships among the African *Vigna* species, representative wild germplasm accessions of this large genus have been selected for a comprehensive palinological study: their pollen dimension, shape, polarity, openings, and sculptures of the exine, will be analysed by optical images microscope and tridimensional electron scanning microscope.

G.3.3 Phenetic studies in four species of yam (*Dioscorea*)

S.I. Chukwuma, N.Q. Ng and M.O. Akoroda

Variability of morphological characteristics of the shoot and tubers of four species of yams (*D. rotundata, D. alata, D. cayenensis and D. praehensilis*) propagated by the 'seed yam' and 'minisett yam' propagules are being characterized and compared. This study will be continued in 1993.

G.4 NARS COLLABORATION AND TRAINING

Project rationale

One of the role of GRU is to collaborate with and strengthen NARS in the conservation and utilization of plant genetic resources. GRU strengthens NARS through (a) cooperative research and germplasm exploration, conservation, characterization and documentation, (b) distribution of germplasm on request, (c) individual and group training and (d) germplasm networks to facilitate cooperation in plant genetic resources conservation and use between the NARS, and between the NARS and CG centers.

Completed activities

Ng, N.Q. and R. Asiedu, CIAT and IBPGR (unpubl.). Cassava germplasm international workshop
In collaboration with CIAT and IBPGR, an international workshop on cassava genetic resources was organised in Cali, CIAT, 19-23 August, 1992. Representatives of NARS from Asia, Africa and America, CIAT, IBPGR and IITA and many individual scientists attended the workshop. The workshop reviewed the past activities on collection, utilization, conservation and documentation of cassava genetic resources and made recommendation for future activities.

Ng, N.Q., IITA Training Unit, and other IITA scientists, FAO and IBPGR (unpubl.).
IITA/FAO/IBPGR organised a training course on Plant Genetic Resources Conservation, Utilization and Management at Ibadan from 23 September to 5 October, 1992. 14 NARS scientists and senior technicians who were sponsored by FAO/IBPGR/IITA attended the course.

Ng, N.Q. and R. Asiedu (unpubl.). Evaluation of yam germplasm at Uturu by NRCRI scientists.
Technical and financial support as well as planting setts of about 1,000 accessions of yams (*D. rotundata* and *D. alata*) were provided to NRCRI Umudike, Nigeria for evaluating yam germplasm at Uturu (see G.2.7).

Ng, N.Q. amd ICP (unpubl.).
Gave advice to IAR&T scientists on drying (dehumidification) and refregeration equipment for the installation of a maize working collection seed store in Ibadan. RRPMC funded the seed storage equipment.

Ng, N.Q. (unpubl.). Collecting cassava germplasm in the Republic of Benin.
Provided technical advice and financial support for the NARS of the Republic of Benin for collecting cassava germplasm as well as many other crops.

Ng, N.Q., R. Asiedu and S.Y.C. Ng 1992. Cassava Genetic Resources Programme at the International Institute of Tropical Agriculture, Ibadan. CIAT/IITA/IBPGR International Workshop on Cassava Genetic Resources, Cali, CIAT, August 1992
Gives accounts of the past and present activities on cassava germplasm, its distribution to NARS and subsequent use in cassava breeding and conservation at IITA, and thoughts on the need for future collecting, characterization, documentation, conservation at IITA and for strengthening the collaboration with NARS in these activities.

Ng, N.Q., D.O. Ladipo, B.T. Kang and N.A. Atta-Krah 1992. Multipurpose tree and shrub germplasm evaluation and conservation at IITA. ICRAF MPT - Germplasm Resources Centre Consultation, Nairobi, June 1992
With increasing need for enviornmental stability and soil fertility maintenance in the humid and sub-humid tropics, inclusion of leguminous woody species in farming systems has become increasingly important. An overview of IITA's past and present activities in collection, conservation and use of MPTs germplasm and its future plans and collaboration with ICRAF in these activities are discussed and recommended.

Activities

G.4.1 Cassava and maize germplasm collecting and characterization in Nigeria
N.Q. Ng, A. Dixon, R. Asiedu, S.K. Kim, J.G. Kling, NRCRI and IAR&T scientist
Provided advice on cassava and maize germplasm collecting and characterization that were/will be conducted by NARS scientists. RRPMC provided the financial support (see G.1.1.).

G.4.2 Cassava Genetic Resources Network
N.Q. Ng, R. Asiedu, A. Dixon, S.Y.C. Ng, CIAT, IBPGR and NARS
The CIAT/IITA/IBPGR International Workshop on Cassava Genetic Resources held at CIAT, Cali, Colombia, August 18-23, 1992 established a Steering Committee to implement the recommendations of the workshop on: (1) Global conservation stragegy for cassava germplasm, (2) Human resources development, and (3) Cassava Genetic Resources Database and Information Systems. Two members each from NARS from Latin America, Africa and Asia; one member from IITA, IBPGR and CIAT constituted the members of the steering commiittee. The committee will monitor most urgent activities at the regional level, particularly,

APPENDIX 39

LATE WHITE SAVANNA TRIAL 2-3

EXPERIMENT : 8326

LOCATION : GUSAU

SOWING DATE : 1983

No OF REPS = 4

No OF ENTRIES = 25

HYBRID	GRAIN YIELD KG/HA	% BEST CHECK	% BOTH CHECKS	DAYS TO SILK	PLANT HEIGHT (CM)	EAR HEIGHT (CM)	ROOT LODGING (1-5)	EAR ASPECT (1-5)	% MOIS-TURE
8326-16	7606	134	155	57	177	103	2.0	1.0	20
8326-17	7244	127	148	57	176	96	1.7	1.0	21
8326-19	7187	126	147	58	177	82	1.7	2.0	22
8326-13	7110	125	145	58	200	111	2.7	1.2	21
8326-14	7054	124	144	59	197	97	2.5	1.7	19
8326-4	6981	123	142	59	201	103	1.5	1.7	21
8326-22	6859	121	140	46	172	90	1.7	1.5	22
8326-10	6798	120	139	59	196	95	2.7	1.0	21
8326-6	6727	118	137	61	195	92	3.2	1.2	23
8326-3	6677	117	136	58	193	105	2.0	2.0	21
8326-23	6525	115	133	59	172	88	1.0	2.2	22
8326-12	6450	113	132	59	155	86	2.2	2.5	21
8326-20	6289	111	128	59	192	100	2.2	1.5	21
8326-8	6092	107	124	58	165	95	2.0	1.0	21
TZB	5684	100	116	61	192	108	3.0	2.0	21
8326-2	5449	96	111	59	198	100	3.0	2.2	22
8326-21	5395	95	110	59	193	112	3.0	2.0	19
8326-15	5335	94	109	59	193	106	3.0	2.0	20
8326-11	5259	93	107	59	191	100	3.5	2.2	19
8326-1	5171	91	105	62	187	90	2.5	2.0	24
8326-9	5148	91	105	60	186	118	2.7	2.2	24
8326-18	4422	78	90	59	171	90	2.5	1.7	20
8326-5	4399	77	90	60	168	87	3.2	1.7	21
PR.7843	4125	73	84	63	203	125	3.5	2.7	22
8326-7	3676	65	75	58	197	111	4.5	2.7	19
MEAN	5987	105	122	59	186	100	2.6	1.8	22
C.V. %	14.2	14.2	14.2	9.5	9.1	15.5	28.4	23.4	11.1
LSD 5 %	1197	21.1	24.4	7.8	23.8	21.8	1.03	0.80	3.4

Biotechnology Research Unit

The roles of the Biotechnology Research Unit at IITA are to identify, introduce, adapt and develop new techniques that can strengthen ongoing research activities elsewhere at IITA, in particular helping to overcome problems that are intractable by conventional breeding and refinements of virus diagnostic methods. The Unit is presently concerned with DNA analysis, protein analysis, virus diagnostics, cytogenetics and tissue culture. Research on transformation of genes using novel biotechnological approaches into elite lines of IITA's mandated crops will also commence from this year as methodologies and materials required for it are obtained or developed.

The major breakthrough of the biotechnological research in 1992 has been the success for the first time in regeneration of plantlets at relatively high rate from the callus cultures initiated from plumules and radicles of cowpea. This has opened up a new era for possible transformation with desirable genes, particularly pest resistant genes into cowpea by use of novel biotechnological techniques. Another achievement has been construction of an RFLP linkage map containing 90 DNA markers in cowpea in collaboration with University of Minnesota and University of Ibadan. The current map establishes 10 linkage groups of 11 chromosomes.

Techniques for non-radioactive DNA detection have been optimized for RFLP analysis for *Vigna* . RFLP markers have been identified that can be used for unambiguous determination of *Vigna* species. Polymorphisms have been identified by both RFLP and more sensitive RAPD (Random Amplified Polymorphic DNA) methods that could be useful in establishing phylogenetic relationships in *Vigna* . A total of 50 selected accessions of cowpea from diverse geographical areas has been surveyed using RAPD marker technology to detect genetic diversity in cultivated cowpea. These methods can be applied routinely to facilitate the cowpea breeding, particularly in isolation and introgression of pest resistant genes into cowpea through interspecific hybridization.

A methodology to isolate genomic DNA in cultivated and wild *Dioscorea* has been developed. DNA polymorphism has been detected by the use of Polymerase Chain Reaction (PCR) technology using RAPD markers in 3 cultivated and 9 wild Dioscorea species. The data obtained so far from the experiment were used to draw a preliminary phylogenetic tree in the genus *Dioscorea* based on RAPDs.

B. 1 MOLECULAR MARKER TECHNIQUES

Completed studies

C. A. Fatokun and N. Young . 1992. Molecular taxonomy of *Vigna*.
A numerical taxonomy of the genus *Vigna* was carried out following RFLP analysis of 44 different species belonging to four genera. The distinctness of the Asiatic grams in subgenus *Ceratortopis*, cowpea in section *Catiang*, bambarra groundnut and members of the subgenus *Plectotropis* was elucidated by the study. (Accepted for publication in *Theoretical and Applied Genetics*).

C. A. Fatokun. 1992. Detection of quantitative trait loci (QTLs) for seed weight in cowpea
A genome map for cowpea was used to identify QTLs for seed weight in cowpea. Two loci on different linkage groups controlled most (about 50%) of the variation for seed weight in cowpea. The markers which bound these segments of the cowpea genome coincide with those found bounding the main QTLs for seed weight in mungbean (*V. radiata*) indicating conservation of these segments. (Published in *Genetics*, November 1992).

Activities

B.1.1 RAPD to assess the genetic diversity in cultivated cowpea
H. Mignouna, N. Q. Ng and cowpea breeders
In order to determine the feasibility of using RAPD marker technology to detect genetic diversity in cultivated cowpea, a preliminary survey of 50 selected accessions of cowpea from diverse geographical areas has been undertaken. Two commercially available random primers were evaluated for the ability of detecting polymorphism on the basis of those primers, and the degree of genomic polymorphism was estimated. The methodology involving RAPD analysis is now well developed. This work will continue

with the screening of 200 accessions of diverse geographical origin and as well as related wild species. Eighty additional primers have been obtained and will be used for screening of germplasm.

B.1.2 Molecular characterization of improved IITA elite clones of root crops

H. Mignouna S. K. Hahn and R. Asiedu

Until recently, biochemical markers for isozyme analysis and isoelectrofocusing, in combination with morphological and physiological characters, and pest and disease reactions have been established as tools to assess genetic diversity in cassava, sweet potatoes and yams. In addition to those classical markers, we have initiated studies aimed at developing molecular markers, mainly RAPDs, to characterize 15 sweet potato clones, 15 cassava clones and 6 yam clones. Results so far obtained showed a high degree of polymorphism in each crop, allowing us to distinguish clones from one another. This work will be continued to add more markers and give a good fingerprint of each clone.

B.1.3 Construction of a yam genomic library

H. Mignouna, R. Asiedu, B. Afolabi,V. Chikaleke, and Q. Ng

Initial attempts to isolate mitochondrial DNA from *Dioscorea alata* and *Dioscorea rotundata* were unsuccessful. Nevertheless, we have developed a methodology to isolate genomic DNA in cultivated and wild *Dioscorea*. DNA polymorphism has been detected by the use of Polymerase Chain Reaction (PCR) technology by Random Amplified Polymorphic DNA (RAPD) markers in 3 cultivated and 9 wild Dioscorea species. Data obtained so far allow us to draw a preliminary phylogenetic tree in the genus *Dioscorea* based on RAPDs. This research will be continued in 1993 by using mitochondrial DNA genomic clones as probes for genomic DNA to study phylogeny in the genus *Dioscorea*. Putting together the DNA polymorphism detected at nuclear and cytoplasmic levels (namely mitochondrial and chloroplast DNA) will provide a definitive understanding of the genetic diversity and phylogenetic relationship in *Dioscorea*.

B.1.4 Analysis of proteins and messenger RNAs

P. Petrilli, J. Tonukari, H. Mignouna, and B. B. Singh

The cowpea is the major host of *Striga gesnerioides* in Africa. By capitalizing on the availability of some resistant cowpea accessions to Striga, our project intends to distinguish, by comparative analysis of resistant and susceptible accessions, the gene(s) responsible for such resistance.

Furthermore, it is our intention to fully characterize, at the amino acid sequence level, both storage seed proteins and those newly synthesized proteins appearing during germination.

Electrophoretic patterns of proteins and RNAs extracted from the different available accessions of cowpea will be compared in order to detect differences at molecular level between striga resistant and susceptible accessions. Extraction will be performed on dormant and germinating seeds.

B. 2 NON-SEXUAL GENE TRANSFER TECHNIQUES

Completed studies

S.Y.C. Ng and O.J. Adeniyi(unpublished).
Somatic embryogenesis and plant regeneration in cassava.

Media and procedure for somatic embryogenesis from cassava young leaves have been developed. Cassava leaves were cultured on MS medium supplemented with 2,4-D. Globular embryos were obtained after 21 days in culture. The embryos were transfered to modified MS medium supplemented with zeatin for maturation. Plantlets were obtained after one or two transfer to the same medium. Studies were carried out to compare the response of 19 cassava clones to the media. The frequency of somatic embryogenesis varied from 7 to 70% depending on the genotype. Plantlets were obtained from eight of the cassava clones studied.

S.Y.C. Ng , A.S. Afolabi and G. Thottappilly (In preparation). Plant regeneration in cowpea(*Vigna unguiculata* L. Walp.).

Plant regeneration was achieved from callus cultures initiated from plumules and radicles of cowpea in MS medium containing NAA. Shoot bud formation was obtained two to four weeks after calli were transfered to regeneration media. Plantlets obtained were successfully established in soil. The procedure and media developed were used to regenerate plants from several cowpea varieties.

Activities

B.2.1 Plant regeneration from cowpea protoplasts

G. Thottappilly, A. S. Afolabi, S. Y. C. Ng and G. Ude

The best way to rescue genetically transformed plants is to regenerate *in vitro* a complete plant from a single cell. Unfortunately, cowpea is considered a "recalcitrant" to *in vitro* regeneration. Recent developments at IITA and Scottish Crop Research Institute (Dundee, Scotland) have shown that cowpea regeneration is possible from callus. However for developing a reliable system for gene expression analysis in cowpea, regeneration from callus will result only chimaeric plants. As a first step in establishing a reliable and easily accessible gene transfer systems, an efficient procedure for cowpea protoplast culture and fertile plant regeneration will be attempted.

Recently, at the University of Georgia, groundnut - another recalcitrant legume - has been regenerated using protoplast from immature cotyledons and nurse culture. Attempts will be made using this approach to regenerate cowpeas from protoplasts.

B.2.2 Callus induction and plant regeneration in yam

S.Y.C. Ng and S.H. Mantell (Wye college, UK)

Preliminary studies showed that embryoid structures were obtained from explants, such as embryos, tuber pieces, stem segments and leaves, of *D. rotundata*. Detail studies will be carried out to refine the culture media (callus induction and regeneration) to achieve plant regeneration.

B.2.3 Anther culture in cassava

S.Y.C. Ng and K.V. Bai

Anther/microspore culture can be used to produce haploids and di-haploids, which would be very useful for genetic studies in cassava. Anthers containing microspores at different stages of development were identified and anthers were cultured with or without cold treatment. Calli were obtained from anthers that contained immature pollen and that cold treatment promoted callus formation. Further studies will be carried out using different media formulations and at different period of flowering season.

B.2.4 A polyethylene glycol-mediated protoplast transformation system for production of fertile transgenic cowpea plants

G. Thottappilly, A. S. Afolabi and S. Y. C. Ng

This project is dependent upon the successful regeneration of cowpea protoplasts. Polyethylene glycol (PEG), poly-L-ornithine or calcium phosphate have each been used in the incubation mixture of protoplasts and plasmid DNA to increase the frequency of tranformation. A wide variety of plant species have been successfully transformed by protoplast transformation augmented by these chemicals.

B.2.5 Protoplast transformation system by electroporation

I. Ingelbrecht, G. Thottappilly, A. S. Afolabi, S. Y. C. Ng and J. Ikea

A short pulse of high voltage electricity produces transient pores in the plasmamembrane, facilitating uptake of macromolecules into protoplasts. This process requires specialized equipment by which field strength and pulse durations can be manipulated. High voltage pulses combined with PEG have been used to improve tranformation frequency.

B.2.6 Gene tranformation in cowpea and plantain by DNA particle gun

G. Thottappilly, S. Y. C. Ng, D. Vuylsteke, J. Ikea and A. S. Afolabi

Collaborators: R.A. Bressan, P. M. Hasegawa, P. Dunn, L. Monti

Once the gene gun arrives at IITA, tranformation will be tried in cowpea and plantain using various reporter genes including GUS and luciferase genes. Our collaborators at Purdue are developing a system for high frequency transformation using the gus reporter gene. Particle bombardment of immature embryos resulted in chimeric transformations. Putative non-chimeric transformants have been identified in the second generation and these are being subjected to molecular verification.

While more refined protocols for screening transformants are being developed, the construction of vectors for both microprojectile bombardment and Agrobacterium transformation is underway. *Bacillus*

thurengensis toxins that are effective against *Maruca testulalis* have been identified and cloned into expression vectors. Constructs with other insect resistance genes including wheat germ agglutinin and a-amylase inhibitors are also available.

B.2.7 *Agrobacterium fumefaciens*-mediated gene transfer in cowpea

G. Thottappilly, I. Ingelbrecht, S. Y. C. Ng and A. S. Afolabi

The best available method for gene tranformation is based upon the ability of the bacterium *A. tumefaciens* to transfer and integrate the T-DNA region of its Tiplasmid into the recipient genome. With the recent developments of cowpea regeneration at IITA, attempts will be made for a successful transformation.

B.2.9 *Agrobacterium* gene transfer in cassava

S.Y.C. Ng, O.J. Adeniyi and E. Filippone (Italy)

The procedure and media for plant regeneration via somatic embrogenesis were established in cassava. While continue working on the improvement of this system, it will be used for gene transfer experiment using *Agrobacterium* carrying Kanamycin resistant gene and GUS gene. Two selected cassava clones which have the highest somatic embryogenesis frequencies and plantlet regeneration rate will be used.

B.2.10 Use of a defective replicase gene to induce resistance to cucumber mosaic virus in transgenic banana/plantain

S.Y.C. Ng and R. Asiedu

Flowering and seed set in yam is erratic and is influenced by the environment and genotype. Previous research had established the procedure and culture medium for mature embryo culture of yams (*D. rotundata and. D. abyssinica*) using MS, NN and LS medium.

The immature embryo/ovule of the open pollinated *D. rotundata* and *D. alata* clones will be cultured *in vitro* at different stages of development. The media and procedures developed will be used for the culture of hybrid embryos/ovules between wild *Dioscorea* sp. and cultivated yam.

B. 3 NEW CYTOGENETIC TECHNIQUES

Completed studies

B.3.2 S. K. Hahn, K. V. Bai and R. Ugborogho. DNA flow cytometry in yams

Ploidy was examined using DNA flow cytometry for over 300 accessions of yams including *D. rotundata, D. cayenensis, D. burkilliana, D. liebrechtsiana, D. mangenotiana, D. minutiflora, D. praehensilis, D. smilacifolia, D. togoensis* and the segregating seedlings of open pollinated populations of *D. rotundata and D. praehensilis* as well as the living materials of the natural vegetation in a forest in Nigeria. *D. rotundata* showed a range of ploidy levels from 4x to 8x; *D. cayenensis* from 4x to 8x; the intermediate type 8x; *D. burkilliana* from 4x to 16x; *D. lierechtsiana* 6x; *D. mangenotiana* from 10x to 16x; *D. minutiflora* from 8x to 12 x; *D. praehensilis* 4x to 6x; *D. smilacifolia* from 4x to 6x; *D. togoensis* 4x. The segregating families resulting each from the open pollinated *D. rotundata* and *D. praehensilis* showed segregation into individuals with different ploidy levels ranging from 4x to 8x and from 4x to 6x respectively. It, therefore, seems that tremendous variation in ploidy occurs both in cultivated (both non-segregating and segregating) and in wild species.

Activities

B. 3.1 DNA flow cytometry in cassava

S. K Hahn, K. V. Bai and S. Y. C. Ng

Four colchicine induced tetraploids and one octaploid, and one spontaneous hexaploid were identified using DNA flow cytometry. One aneuploid was also confirmed with the method. Ploidy was identified for several clones which were not confirmed cytologically. The technique will be continuously applied for ploidy evaluation.

B. 4 MOLECULAR DIAGNOSTICS

Activities

B.4.1 Indexing of tissue culture materials of white yam using PCR, universal potyvirus monoclonals and polyclonal antiserum

G. Thottappilly, S. Y. C. Ng, A. Alonge and A. S. Afolabi

Indexing of white yam for yam mosaic virus has been done using polyclonal antiserum. Since the universal potyvirus monoclonal antibodies react with yam mosaic virus, this will be tested to see whether we can index white yam more reliably. Also, by using potyvirus group-specific primers, attempts will be made to amplify YMV thereby making virus indexing more reliable.

B.4.2 Monoclonal antibody production lab at IITA to produce monoclonal antibodies to viruses, fungi, pathogenic bacteria and symbiotic rhizobia

G. Thottappilly, A. Alonge and H. W. Rossel

The technique of cell hybridization or fusion is a direct application of recent developments in biotechnology. That monoclonal antibodies (MAbs) are epitope specific rather than pathogen specific confers several advantages. The assay can be very selective because MAbs will differentiate closely related strains. The property of epitope specificity can be further exploited by selecting MAbs which will react with epitopes common to a large number of pathogens in a group. Another major advantage of MAbs is the assured continuing supply of high quality reagent. In 1990, IDRC funded a project to produce MAbs at Vancouver Research Station and to provide reagents to the national programs. Three years later, by the end of the first phase of the project, Vancouver Research Station has transferred the technology to IITA and continue to give back-stopping support to IITA. In 1993, production of monoclonal antibodies at IITA will be initiated.

B.4.3 Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms within Nigerian populations of whitefly

G. Thottappilly and PHMD Entomologist

By using iso-enzyme electrophoresis, two biotypes within populations of the whitefly *Bemisia tabaci* from cassava, okra and other host plants were identified in Cote d'Ivore. One was found only on cassava and egg plant, the other was polyphagus, but did not infect cassava.

Using RAPD, whitefly populations within Nigeria will be investigated. If there are differences the implications of these findings in relation to the role of *B. tabaci* as a virus vector will be studied. Also, the whiteflies from forest, transition and savanna regions will be compared by RAPD, and their feeding behaviour using the monitor as well as vector transmission studies will be compared.

B.4.4 Nucleic acid detection of ACMV

G. Thottappilly, H. W. Rossel and S. Y. C. Ng

ACMV indexing is important for international exchange of cassava germplasm. The methods must be very sensitive, reliable, and also rapid and easy to use, preferably capable of detecting virus strains.

Recent developments in biotechnology permit detection of pathogens by means of highly specific assay such as serological techniques using monoclonal antibodies. Another promising technique is based on nucleic acid hybridization. Still another highly promising technique is PCR.

Molecular probes have been produced by PCR amplification of viral DNA using a primer sequence that is specific for whitefly transmission in gemini viruses. These probes can be used directly as a screening tool. Amplification of crude extracts could also serve as a screening technique, offering further enhanced sensitivity.

B.5 TRAINING/WORKSHOP/COLLABORATIVE WORK

Activities

B.5.1 Biosafety meeting, Ibadan, IITA, June 1993.

B.5.2 Plant Cell and Tissue Culture and Biotechnology workshop, Ibadan, IITA, 4-22 October,1993.

B.5.3 Workshop on "Application of monoclonal antibodies for identification of cowpea viruses", October/November, 1993.

B.5.4 Safe handling of radioactive materials - date to be fixed. This is mainly for IITA researchers. This will be done in collaboration with the Radiation Protection Agency of Nigeria.

B.5.5 Collaboration with national programs on application of monoclonal antibodies.
IDRC has agreed to provide funds for a second phase mainly to help the national programs. IITA will coordinate this activity

B.5.6 Development of biosafety guidelines
G. Thottappilly and all involved in Biotechnology research
The priority in 1993 is to develop biosafety guidelines.

LIST OF ACTIVITIES

ROOT AND TUBER IMPROVEMENT PROGRAM

T. 1 Cassava Germplasm Introduction and Evaluation

T.1.1 Broadening the germplasm base
M. Porto, N.Q. Ng and R. Asiedu

T.1.2 *In vitro* conservation and distribution

T.1.3 Measuring diversity in cassava
A. Dixon and R. Asiedu

T.1.4 Cassava germplasm evaluation
A. Dixon, R. Asiedu, M. Porto, M. Bokanga, I. Ekanayake, C. Akem and Entomologist

T.1.5 Evaluation at the Mbalmayo Station
A. Dixon, M. Bokanga and J. Ngeve

T.1.6 Field performance of virus-free cassava
A.O. Akano, R. Asiedu, S.Y.C. Ng, H. Rossel and G. Atiri (UI)

T.1.7 Cyanogenesis in cassava: genetic variation, de novo biosynthesis in roots, and DNA probes for CNP screening
M. Bokanga, S.Y. Ng, J. Mignouna, B. Halkier and B.L. Moller (RVAU, Denmark)

T.1.8 Screening for flexible harvest time
A. Dixon, P. Ntawuruhunga and M. Bokanga

T.1.9 Cassava adaptation to inland valleys
A. Dixon, I. Ekanayake and M. Wouamane

T.1.10 Relationship between seed size and ploidy in cassava
A. Boateng, S.K. Hahn and S.Y. Ng

T.1.11 Characterization of a mutant cassava clone using molecular markers
V, Boateng, S.K. Hahn and D.H. Mignouna

T.1.12 Comparison of spontaneous sexual and asexual tetraploids, artificial tetraploids induced by colchicine, and their diploids
S.K. Hahn and J. Udosen

T.1.13 Yield loss assessment and screening of cassava germplasm for resistance to the spiralling white fly
A. Dixon, I. Ekanayake and Entomologist

T.2 Enhancement of Cassava Germplasm Pools

T.2.1 Agroecology-based population improvement
A. Dixon, Pathologist and Entomologist

T.2.2 Selfing and outcrossing in cassava
A. Dixon and R. Asiedu

T.2.3 Back-up source populations for specific characters
A. Dixon, M. Porto and R. Asiedu

T.2.4 Inheritance studies in cassava

T.2.5 Interspecific hybridization in cassava
R. Asiedu, K.V. Bai, M. Bokanga and M. Fregene

T.2.6 Cytogenetics of *Manihot* species and hybrids
K.V. Bai and R. Asiedu

T.2.7 Cytological screening of progenies from cassava polyploids and 2n gamete clones
K.V. Bai, A. Dixon, R. Asiedu and S.K. Hahn

T.2.8 Induction of polyploidy in cassava
K.V. Bai and S.K. Hahn

T.2.9 Tetraploid population improvement
S.K. Hahn, A. Dixon and K.V. Bai

T.2.10 Genotype X Environment studies
A. Dixon, R. Asiedu and J. A. White

T.2.11 Mechanisms of CGM resistance
A. Dixon, I. Ekanayake, M. Porto, V.B. Mbuyongha and N.E. Nukenine

T.2.12 Field evaluations of cassava plants regenerated through somatic embryogenesis
S.Y.C. Ng and O.J. Adeniyi

T.2.13 Molecular Markers for ACMV resistance in cassava
M. Bonierbale CIAT), J. Thome (CIAT), M. Mignouna,
R. Asiedu, H. Rossel, S.Y. Ng and M. Porto CIAT)

T.3 Physiology of Adaptation in Cassava

T.3.1 Screening for drought tolerance
I.J. Ekanayake, M. Porto and M. Bokanga

T.3.2 Adaptation to water deficits
M. Porto and I. J. Ekanayake

T.3.3 Screening for cold tolerance
I.J. Ekanayake, M. Porto and M. Bokanga

T.3.4 Screening for tolerance to low light intensity
I.J. Ekanayake and A. Dixon

T.3.5 Flowering in cassava
I.J. Ekanayake, M. Simwambana, S. Jagtap and T. Ferguson

T.4 Food Quality of Root and Tuber Crops

T.4.1 Testing of a simple method for linamarase preparation
M. Bokanga, Nars scientists

T.4.2 Cassava processing and detoxification

M. Bokanga, S. Essers (WAU, Netherlands), N. Mlingi (TNFC, Tanzania) and H. Rosling (Uppsala U. Sweden)

T.4.3 Nutrient supply and cyanogenic potential
J.H. Bradbury (Australian National Univ.) and M. Bokanga

T.4.4 Bitter compounds in cassava roots
M. Bokanga and J.H. Bradbury (ANU, Australia)

T.4.5 Assessment of food quality of root and tuber crops
M. Bokanga, F. Nweke, O. Tewe (UI), A. Larbi (ILCA, Ibadan) and N. Poulter (NRI)

T.4.6 Assessment of cassava flour quality for breed making
M. Bokanga and J.A. Delcour (KUL, Belgium)

T.4.7 Food systems analysis of the competing use of wheat and cassava products in Africa
Economist, M. Bokanga

T.4.8 Postharvest biodeterioration of cassava roots
M. Bokanga and I.J. Ekanayake

T.4.9 International workshop on the safety of cassava as food and feed
M. Bokanga, Coordinator

T. 5 Yam Improvement

T.5.1 Cytogenetics of Dioscorea species and hybrids
K.V. Bai and R. Asiedu

T.5.2 Optimum pollination period in yam species
K.V. Bai and R. Asiedu

T.5.3 Gender expression in *D. rotundata*
V. Chikaleke, R. Asiedu and I. Ekanayake

T.5.4 Yam germplasm characterization
N.Q. Ng, R. Asiedu, I. Ekanayake, A. Dixon, M. Bokanga and NRCRI

T.5.5 Yam germplasm evaluation
R. Asiedu, C. Akem, G. orkwor (NRCRI), Q. Ng and D. Dashiell

T.5.6 Yam germplasm distribution
S.Y.C. Ng, R. Asiedu, G. Thottappilly and N.Q. Ng

T.5.7 Effect of plant age on yam meristem culture
S.Y.C. Ng

T.5.8 Yam *in vitro* microtuberization and mass propagation form node cuttings
S.Y.C. Ng and S.H. Mantell (Wye College, UK)

T.5.9 Field performance of virus-free yam
S.Y.C. Ng, H.W. Rossel and I. Ekanayake

T.6 Collaboration with NARS and Training

T.6.1 International testing of cassava

A. Dixon, R. Asiedu, S.Y.C. Ng, J.B.A. Whyte and NARS (West and Central Africa)

T.6.2 National cassava trials in Nigeria
A. Dixon and NRCRI staff

T.6.3 Tropical Root and Tuber Crops Bulletin
A. Dixon and H. Mutsaers

T.6.4 Training courses
M. Ajayi, I.Ekanayake, M. Bokanga, S.Y. Ng, J.Whyte, A. Dixon, J. Gulley and NARS

T.6.5 Yam monograph
I. Ekanayake and G.C. Orkwor

T.6.6 Graduate training

T.6.7 Collaboration with CTCRI, UWI and ROTREP on yam research
R. Asiedu, S.G. Nair (CTCRI), T. Ferguson (UWI) and R. Dadson (ROTREP)

T.7 ESARRN Network

T.7.1 Regional research coordination
M. Alvarez, J. Ikeorgu and NARS (East and Southern Africa)

T.7.2 Training
J. Ikeorgu, M.N. Alvarez and NARS (East and Southern Africa)

T.7.3 Characterization and diagnosis
M.N. Alvarez, F. Nweke and NARS (East and Sourthern Africa)

T.7.4 Broadening the germplasm base
M.N. Alvarez, M. Porto, R. Asiedu and NARS (Eastern and Southern Africa)

T.7.5 Evaluation and distribution of germplasm
J. Ikeorgu, M. Alvarez and NARS (East and Southern Africa)

T.7.6 Post-harvest technology
M. Alvarez And R. Sauti (Malawi)

T.7.7 Technology transfer
J. Ikeorgu, M. Alvarez and NARS (East and Southern Africa)

MAIZE IMPROVEMENT PROGRAM

M.1 Maize Improvement for the Savanna

M.1.1 Breeding for high yield in the savanna

M.1.2 Breeding for nitrogen use efficiency
J.G. Kling, H.A. Akintoye, S.K. Kim, J. Fajemisin and P. Salle

M.1.3 Root growth and nitrogen use efficiency
J.G. Kling, W. Horst, G. Weber, H. Heuberger and S. Oikeh

M.1.4 Breeding for drought tolerance
B. Badu-Apraku, J. Fajemisin, S.K. Kim, D. Hema and J.G. Kling

M.1.5 Breeding for *Striga* tolerance/resistance (STR)
S.K. Kim, J.G. Kling, J. Fajemisin, A. Diallo, B. Badu-Apraku, D. Berner, V. Adetimirin, C. Thè (IRA, Cameroon), M. Esseh-Yovo (DRI, Togo), P.Y.K. Sallah (CRI, Ghana) and L. Akanvou (IDESSA, Cote divoire)

M.1.6 Screening diverse germplasm for *Striga* resistance

M.1.7 Screening for resistance to *Striga aspera* and *S. asiatica*
S.K. Kim, V. Adetimirin, M. Esseh-Yovo, J. Iken, S. Lagoke and D. Berner

M.1.8 Genetics of resistance to *Striga* (inbreds and hybrids)
V.O. Adetimirin, S.K. Kim and M.E. Aken'Ova

M.1.9 Inheritance of *Striga* resistance in OP's
L. Akanvou (IDESSA), J.G. Kling and M.N.A.B. Fakorede

M.1.10 Effect of maturity group on *Striga* resistance
L. Akanovou (IDESSA), D. Berner, J.G. Kling, A. Diallo, J. Fajemisin and M.A.B. Fakorede

M.2 Maize Improvement for the Forest

M.2.1 Breeding for high yield in the forest
J.G. Kling, S.K. Kim, C. Thè (IRA, Cameroon) and M. N'Kishama (PNM, Zaire)

M.2.2 Resistance to downy mildew
J.G. Kling, S.K. Kim, K.F. Cardwell and M. Omidiji (IAR&T)

M.2.3 Green maize studies of hybrid vs. OP varieties
S.T. Yoon and S.K. Kim

M.2.4 Studies on yield components
S.T. Yoon and S.K. Kim

M.2.5 Fresh maize and cassava intercropping in the forest ecology
S.T. Yoon, S.K. Kim And A.G. Dixon

M.2.6 Green maize yield and weed control when intercropped with melon
S.T. Yoon, S.K. Kim, I.O. Akobundu and B.A. Gbadamosi

M.2.7 Comparison of streak screening methods
M. Esseh-Yovo, S.K. Kim and J. Kling

M.2.8 Resistance to MSV
M. Esseh-Yovo, S.K. Kim and J. Kling

M.2.9 Breeding for resistance to the pink stem borer
J.G. Kling, S.K. Kim, O. Gold and N.A. Bosque-Pérez

M.2.10 Breeding for resistance to the African sugarcane borer (*Eldana saccharina*)
J.G. Kling, S.K. Kim, O. Gold, and N.A. Bosque-Pérez

M.2.11 Stem borer resistance on-farm

M.2.12 Breeding for weevil resistance
J.G. Kling, D.K. Kossou, S.K. Kim and N.A. Bosque-Pérez

M.2.13 Screening inbreds against *Sitophilus* weevils
D.K. Kossou (Univ. of Benin), C.G. Yallou and S.K. Kim

M.2.14 Improving post-harvest properties of maize for Benin
C.G. Yallou, D.K. Kossou (Univ. of Benin), J.G. Kling and A.E. Okoruwa

M.3 Maize Improvement for the Mid to High Altitudes

M.3.1 Mid-altitude breeding in Nigeria
S.K. Kim and R. Olafare (UTC Seeds)

M.3.2 Mid to high altitude breeding in Cameroon
N.Beninati (IITA-NCRE), M. Ndioro and I. TAbi (IRA), and J. Foko (Univ. Centre Dschang)

M.3.3 Lower limit mid-altitude maize for Togo
M. Esseh-Yovo and S.K. Kim

M.4 Maize Grain Quality

M.4.1 Environmental effects on maize grain quality
A. Okoruwa and J.G. Kling

M.4.2 Rapid test for milling performance
A. Okoruwa and J.G. Kling

M.4.3 Improving traditional hand milling
B. Assa Kante (IER, Mali), A. Okoruwa and J.G. Kling

M.4.4 Oil content of whole vs. dehulled maize
A. Okoruwa and J.G. Kling

M.4.5 Inheritance of grain oil content
J.G. Kling and A. Okoruwa

M.4.6 Evaluating floury conversions
A.E. Okoruwa and J.G. Kling

M.4.7 Potential of maize tortillas in Nigeria
V.A. Obatolu (IAR&T), A.E. Okoruwa and J.G. Kling

M.4.8 Fortification of maize tortillas with soybean
S.M. Osho, R. Abiodun (soybean util.), and A.E. Okoruwa

M.4.9 Improving post-harvest properties of maize for Benin
D.K. Kossou, C.G.Yallou, J.G. Kling and A.E. Okoruwa

M.4.10 Disease resistant popcorn varieties
S.K. Kim and J.G. Kling

M.5 Collaboration with NARS and Training

M.5.1 SAFGRAD Network, terminal phase
S. Badu-Apraku

M.5.2 International germplasm trials
B. Badu-Apraku, J.G. Kling, S.K. Kim, J. Fajemisin and S. Adewunmi

M.5.3 Fitting varieties to environments
J.G. Kling, A. A. Akalumhe, S. Jagtap, J. Fajemisin, A.O. Diallo and S.K. Kim

M.5.4 Usefulness of IITA germplasm in Togo
M. Esseh-Yovo (DRA, Togo), S.K. Kim and J.G. Kling

M.5.5 Adoption of improved maize in Nigeria
M. Adenola, J. Iken (Maize Assoc. of Nigeria); J. Akinwumi, M. Fakorede, S.Kim and F. Nweke

M.5.6 Savanna Station, Cote d'Ivoire
J.M. Fajemisin, A. Diallo (CIMMYT), S.K. Kim, J. Kling, B. Badu-Apraku, K. Goli and A. Koffi (IDESSA)

M.5.7 Collaborative research

M.5.7.1 Breeding for *Striga* resistance/tolerance.
S.K. Kim, J. Kling, J. Fajemisin, B. Badu-Apraku, C. Thè, P.Y.K. Sallah and M. Esseh-Yovo. See M.1.5

M.5.7.2 Evaluation of usefulness of IITA germplasm for maize improvement in Togo.
M. Esseh-Yovo, S.K. Kim and J.G. Kling. See M.5.4

M.5.7.3 Breeding for resistance to downy mildew
J.G. Kling, S.K. Kim, K.F. Cardwell, J. Iken and M. Fakorede. See M.2.2

M.5.7.4 On-farm testing of stemborer resistant maize hybrids
H.Tijani-Eniola, Univ. of Ibadan; S.K. Kim and M. Mutsaers. See M.2.11

M.5.7.5 Breeding for husk cover and weevil resistance
J.G. Kling, D.K. Kossou, S.K. Kim and N.A. Bosque-Pérez. See M.2.12

M.5.7.6 Traditional maize processing in Mali - B. Assa Kante, A.E. Okoruwa and J.G. Kling. See M.4.3

M.5.7.7 Potential of maize tortillas in Nigeria
V. Obatolu, A.E. Okoruwa and J.G. Kling. See M.4.7

M.5.7.8 Collection of local varieties in Guinea and Nigeria
N.Q. Ng, F.L. Guilavogvi, El Sanonssy Bay, J.G. Kling and S.K. Kim

M.5.8. Graduate training

M.5.8.1 Genetic control of *Striga* resistance in inbred lines - V. Aditimirin (Ph.D. thesis), M.E. Aken'Ova and S.K. Kim. See M.1.8

M.5.8.2 Genetic study to determine potential for improving resistance to *Striga* resistance breeding and infestation technology course will be offered to African national scientists. The course will be held jointly with FAO Pan-African *Striga* Control Network (PASCON). - Coordinator, Prof. S. Lagoke, IAR-ABU, Zaria, Nigeria

GRAIN LEGUME IMPROVEMENT PROGRAM

L.1 Cowpea Breeding Project for Savanna Ecologies

L.1.1 Study of cropping systems
Gerben van Ek, Jantien van Ek 9VSO), B.B. Singh, S.F. Blade, D. Florini, H. Bottenberg, Sule Gaya (KNARDA)

L.1.2 Cropping systems in the dry savanna
A. Moutare, M. Nouhou (INRAN), S. Blade, B.B. Singh, D. Florini and H. Bottenberg

L.1.3 Collection and evaluation of local varieties
B.B. Singh and M. Ishiyaku

L.1.4 Breeding value of local varieties
B.B. Singh, Sanusi Gaya, S. Blade and NARS scientists

L.1.5 Local variety x improved line crossing
B.B. Singh, D. Florini, H. W. Rossel

L.1.6 Farmers' participatory evaluation of advanced breeding lines
Sanusi Gaya, S. Blade, B.B. Singh, D. Florini, H. Bottenberg and E.C. Odion (IAR)

L.1.7 Multilocation testing of improved photoperiod sensitive varieties under various intercropping systems
S. Blade, B.B. Singh, D. Florini and H. Bottenberg

L.1.8 Multilocation testing of advanced lines
B.B. Singh, M. Ishiyaku, D.F. Florini, S. Tarawali (ILCA), A. Moutare (INRAN), M. Nouhou (INRAN), J. Detongnon (Maroua)

L.1. 9 Selection methods for intercropping performance
B.B. Singh

L.1.10 Screening for intercropping performance
S.F. Florini

L.1.11 Pest resistance breeding of local varieties
B.B. Singh, Bottenberg, D. Florini and A.M. Emechebe (IAR)

L.1.12 Screening for field resistance to insects
B.B. Singh and H. Bottenberg

L.1.13 Screening for insect resistance
B.B. Singh and H. Bottenberg

L.1.14 Screening for disease resistance
B.B. Singh, D. Florini, H.W. Rossel, A.M. Emechebe (IAR)

L.1.15 Additional sources of pest resistance
B.B. Singh, D. Florini, N.Q. Ng, A.M. Emechebe (IAR) and A. Moutare (INRAN)

L.1.16 Breeding for combined resistance to different viruses
B.B. Singh and H.W. Rossel

L.1.17 Inheritance of blight resistance
B.B. Singh and D. Florini

L.1.18 Genetic studies in cowpea
B.B. Singh and M. Ishiyaku

L.1.19 Screening against *Striga* and *Alectra*
B.B. Singh and A.M. Emechebe (IAR)

L.1.20 Genetics of *Striga* and *Alectra* resistance
B.B. Singh, I.D.K. Atokple and A.M. Emechebe (IAR)

L.1.21 Effect of resistant lines on the *Striga* population over time
B.B. Singh and I.D.K. Atokple and K.F. Cardwell

L.1.22 *Striga* yield loss assessment
B.B. Singh and I.D.K. Atokple

L.1.23 Possibility of new biotypes of *Striga gesneriodes*
B.B. Singh, Remi Adeleke, S.R. Voudouhe (Benin Rep.) and
A. Lane (Long Ashton)

L.1.24 Screening cowpea varieties for higher leaf yield and quality for human use
B.B. Singh, Jantien van Ek, Hajia Aliu (KNARDA), Y.W. Jeon, L. Halos, S. Das-Gupta (UNICEF)

L.1.25 Breeding cowpea for grain quality
B.B. Singh, S. Nielsen (Purdue Univ.) and M. Bokanga

L.1.26 Cowpea performance under irrigation
B.B. Singh and S.F. Blade

L.1.27 Screening cowpea varieties for Rice Fallows in Fadamas
B.B. Singh, B.N. Singh (WARDA), Remi Adeleke

L.1.28 Screening cowpea varieties for harvest and post-harvest characteristics
B.B. Singh, Y.W. Jeon, L.S. Halos, Sule Gaya (KNARDA)

L.1.29 Breeding for drought tolerance
B.B. Singh and T. Terao (TARC)

L.1.30 Induced genetic variability for resistance to Maruca.
B.B. Singh and H. Bottenberg

L.1.31 Ecological analysis of interaction in a millet-cowpea intercrop
T. Terao

L.1.32 Variation in photosynthetic adaptation of leaves in low light conditions
T. Terao

L.1.33 Analysis of on-farm physiological constraints
S.F. Blade and T. Terao

L.1.34 Analysis of water stress

L.1.35 Evaluation of organic/inorganic fertilization in savanna cropping systems
S.F. Blade and T. Terao

L. 2 Wide Crossing for Insect Resistance in Cowpea

L.2.1 Cowpea wide cross biotechnology research working group
N.Q. Ng, G. Myers, S. Schnapp, L.E.N. Jackai, G. Thottappilly, S. Padulosi, H. Mignouna, P. Petrilli, S.Y.C. Ng, external collaborators at Purdue and three Italian research institutes/Universities.

L.2.2 Outcrossing mechanism in wild cowpea
N.Q. Ng and J. Apeji

L.2.3 Cowpea interspecific hybrids for insect resistance
N.Q. Ng, T. Mesfin, L. Jackai, H. Rossel and D.A. Florini

L.2.4 Interspecific hybridization in *Vigna*
S.R. Schnapp and N.Q. Ng

L.2.5 Embryo rescue of *Vigna* hybrids
S.R. Schnapp

L.2.6 Interspecific hybridization of *Vigna* tetraploids (combines L.2.7, 2.8, 2.9)

L.2.7 Cytogenetics of putative tetraploids
R.E. Ugborogho, F. Saccardo (Italian Institutes)
N.Q. Ng, G. Thottappilly and S.R. Schnapp

L.2.8 Resistance bioassay for pod-sucking bugs
L.E.N. Jackai, R.E. Shade (Purdue Univ.) and S.R. Schnapp

L.3 Soybean Improvement

L.3.1 Soybean improvement for the savanna
K. Dashiell, L. Jackai and C. Akem

L.3.2 Multilocational evaluation of soybean
K. Dashiell, L. Jackai, C. Akem, and L. Bello (Univ. of Agric., Markurdi).

L.3.3 Genetics of resistance to soybean insect pests
G. Semakula-Nankinga, K. Dashiell, I. Fawole (Univ. of Ibadan) and L.E.N. Jackai

L.3.4 Breeding for resistance to frog-eye leaf spot (*Cercospora sojina*) in soybean (*Glycine max* (L.) Merrill)

L.3.5 Changes in seed quality of soybeans during seed production and storage
D.K. Ojo and K.E. Dashiell

L.3.6 Inheritance of promiscuous nodulation and resistance to bacterial pustule disease in soybean
B.M. Yeti and K.E. Dashiell

L.3.7 Screening against the root-knot nematode
I.C. Charles, K. Dashiell, G. Atiri and C. Akem

L.3.8 Soybean breeding in southern Africa
K. Dashiell, C. Akem, and NARS scientists in Zambia
(E.Munsanje and F. Javaheri), Zimbabwe, Tanzania and Mozambique

L.4 Nodulation in IITA Soybean Lines

L.4.1 Evaluation of promiscuous soybeans in diverse soils
R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NiFTAL)

L.4.2 Field trials: Evaluation of promiscuous soybeans in farmers fields
R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NiFTAL)

L.4.3 Nitrogen response and nitrogen use efficiency of promiscuous soybeans
R. Abaidoo, K.E. Dashiell, H. Keyser and P.Singleton (NiFTAL)

L.4.4 Survival and persistence of introduced *Bradyrhizobia*
R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NiFTAL)

L.4.5 Characterization of *Bradyrhizobia* isolates
R. Abaidoo, K.E. Dashiell, H. Keyser and P. Singleton (NiFTAL)

L.5 Soybean Utilisation

L.5.1 Comparative analysis of acceptability and nutrient content of milk substitutes processed from two cultivars of cowpea and two cultivars of soybean
S.M. Osho

L.5.2 The nutritive evaluation and the preparation of some Nigeria based food from soybean shaft
S.M. Osho

L.5.3 The processing of canned soybean milk
S.M. Osho

L.5.4 The development of powdered soybean milk in Nigeria
S.M. Osho

L.5.5 Nutritional and quality assessment of cowpea leaves as human food
S.M. Osho

L.5.6 Formulation, evaluation and optimizaition of tortilla containing different forms of cowpea, maize and soybean flours
S.M. Osho and J. Kling

L.5.7 Production and nutritional assessment of soybean fortified puffed-corn using extrusion-cooking process
S.M. Osho

L.5.8 Characterization of crude and refined soybean oil including studies of quality variation relative to shelf life
S.M. Osho

L.5.9 Soy-fufu
S.M. Osho and M. Bokanga

L.5.10 Maize/soy tortilla
S.M. Osho and J. Kling

L.5.11 Malted maize/sorghum/soy breakfast food
S.M. Osho and J. Kling

PLANTAIN AND BANANA IMPROVEMENT PROGRAM

P.1 Gaining Insight into the *Musa* Genome

P.1.1 Inheritance studies using *Musa* germplasm
R. Ortiz and D. Vuylsteke

P.1.2 Linkage groups in *Musa* using conventional genetic markers
R. Ortiz

P.1.3 Genotype-by-environment (year, location) interaction and broad sense heritability for growth and yield parameters in *Musa* hybrids
R. Ortiz and D. Vuylsteke

P.1.4 Molecular linkage map of *Musa*
R.L. Jarett (USDA/ARS), R. Ortiz, D. Vuylsteke and S. Schnapp (BRU)

P.1.5 The effect of major genes in plant growth and fruit development
R. Ortiz

P.1.6 Genetics of fruit quality
R. Ortiz and S. Ferris

P.2 Developing *Musa* Breeding Capability and Strategy

P.2.1 *Musa* germplasm working collection
D. Vuylsteke, R. Ortiz, R. Swennen (KUL) and Q. Ng (GRU)

P.2.2 *Musa* germplasm characterization
R. Ortiz, D. Vuylsteke, R. Swennen (KUL) and Q. Ng (GRU)

P.2.3 Environmental effects on male fertility and seed set in plantain
R. Ortiz

P.2.4 Male fertility in *Musa* germplasm
R. Ortiz

P.2.5 Ploidy levels of *Musa* hybrids
H.Vandenhout, R. Ortiz, D. Vuylsteke, R. Swennen (KUL) and S.K. Hahn (BRU)

P.2.6 Diploid *Musa* breeding
R. Ortiz, D. Vuylsteke and R. Swennen (KUL)

P.2.7 Plantain germplasm enhancement at the diploid level
D. Vuylsteke, R. Ortiz and S. Ferris

P.2.8 Outcrossing rates in wild diploid *Musa* spp.
R. Ortiz, R.L. Jarett (USDA/ARS), D. Vuylsteke and M. Mutsaers

P.2.9 Combining ability in plantain germplasm
R. Ortiz and D. Vuylsteke

P.2.10 Production of secondary triploids and tetraploids
R. Ortiz, D. Vuylsteke and R. Swennen (KUL)

P.2.11 Breeding the False Horn plantain gene pool using somaclonal variation
D. Vuylsteke and R. Ortiz

P.2.12 Breeding for dwarfism in plantains
D. Vuylsteke and R. Ortiz

P.2.13 Physiological research to enhance *Musa* breeding
I. Ekanayake (TRIP), R. Ortiz and S. Ferris

P.2.14 Multi-site evaluation trials of selected *Musa* hybrids
P. Ortiz, D. Vuylsteke, and TRIP breeders in Nigeria, Cameroon and Uganda, PHMD scientists and NARS cooperators

P.2.15 Advanced *Musa* Yield Trials
D. Vuylsteke, R. Ortiz, and TRIP breeders in Cameroon and Uganda, NARS cooperators.

P.2.16 Defining an ideotype for plantain breeding
R. Ortiz, R. Swennen (KUL), D. Vuylsteke and S. Ferris

P.2.17 A selection index for multitrait selection in *Musa* hybrids
R. Ortiz

P.2.18 The effect of inbreeding and heterosis on growth and yield parameters
R. Ortiz

P.2.19 Computerization of PBIP breeding program
R. Ortiz

P.3 Breeding for Durable Black Sigatoka Resistance

P.3.1 Development of durable black sigatoka-resistant hybrids by combining different sources of resistance
D. Vuylsteke and R. Ortiz

P.3.2 Clonal evaluation of BSR 4x hybrids - the ratoon cycle
K. Mobambo, D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasbert-Gauhl and R. Swennen (KUL)

P.3.3 Multi-location evaluation trial of BSR 4x hybrids at IITA stations
D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasperg-Gauhl and TRIP breeders in Nigeria and Cameroon

P.3.4 Multi-site evaluation trials of selected *Musa* hybrids
R. Oritz, D. Vuylsteke, and TRIP breeders in Nigeria, Cameroon and Uganda, PHMD scientists and NARS cooperators

P.3.5 Advanced *Musa* Yield Trials
D. Vuylsteke, R. Ortiz, and TRIP breeders in Cameroon and Uganda, and NARS cooperators

P.3.6 International *Musa* Testing Program
D. Vuylsteke, R. Ortiz, F. Gauhl, C. Pasberg-Gauhl, INIBAP and FHIA Scientists

P.3.7 Molecular mapping of black sigatoka resistance genes
R. Ortiz, R.L. Jarett (USDA/ARS), D. Vuylsteke and S. Schnapp

P.4. Banana Improvement for Mid to High Altitudes

P.4.1 Screening for female fertility in East African *Musa* germplasm
D. Vuylsteke and R. Ortiz

P.4.2 Mid-altitude cooking/beer banana breeding
PBIP breeder in Uganda, D. Vuylsteke and R. Ortiz

P.4.3 Advanced *Musa* Yield Trials
D. Vuylsteke, R. Ortiz, and TRIP breeders in Cameroon and Uganda, and NARS cooperators (ee P.2.15)

P.4.4 Banana seed health
D. Florini, H. Rossel, F. & C. Gauhl and D. Vuylsteke

P.5 Biotechnology for *Musa* Breeding

P.5.1 *Musa* germplasm exchange in vitro
D. Vuylsteke and S. Ng (TRIP)

P.5.2 *In vitro* propagation of selected genotypes
D. Vuylsteke and NARS collaborators

P.5.3 Embryo rescue in *Musa*
G.I. Harry, D. Vuylsteke and U.U. Ebong (RSUST)

P.5.4 Somaclonal variation studies in *Musa*
D. Vuylsteke and KUL cooperators

P.5.5 Molecular linkage map of *Musa*
R.L. Jarett (USDA/ARS), R. Ortiz, D. Vuylsteke and S. Schnapp

P.5.6 Molecular mapping of black sigatoka resistance genes
R. Ortiz, R.L. Jarret (USDA/ARS), D. Vuylsteke and S. Schnapp (see P.3.7)

P.5.7 Molecular methods in *Musa* breeding: marker assisted selection
R. Ortiz, R.L. Jarret (USDA/ARS), D. Vuylsteke, F. Rosales (FHIA), R. Rowe (FHIA) and S. Schnapp

P.5.8 Development of a secure and rational management system for in vitro propagated and conserved germplasm of *Musa*
D. Vuylsteke, L. Withers (IPGRI), R. Swennen (KUL), R. Ortiz and possibly other cooperators.

P.5.9 Transmission of chloroplasts in *Musa*
R.L. Jarret (USDA/ARS), R. Ortiz and D. Vuylsteke

P.5.10 Application of recombinant DNA technology for the development of disease-resistant plantains and bananas using antifungal proteins
B. Cammue (KUL), R. Swennen (KUL), D. Vuylsteke, R. Ortiz and BRU

P.5.11 Molecular and biochemical studies of *Musa* fruit ripening
G. Tucker, Vendrell (Univ. Nottingham), S. Ferris, R. Ortiz and M. Bokanga (See P.6.10)

P.6 Post-Harvest Quality of Plantains

P.6.1 Fruit quality evaluation of *Musa* hybrids
S. Ferris, M. Bokanga, R. Ortiz and D. Vuylsteke

P.6.2 Sensory evaluation of *Musa* hybrids
S. Ferris and NARS cooperators

P.6.3 Fruit damage susceptibility evaluation
S. Ferris

P.6.4 Effects of black sigatoka disease on post-harvest qualities
S. Ferris and NARS cooperators

P.6.5 On-farm assessment of yield and quality loss from black sigatoka
S. Ferris, F. Gauhl, D. Vuylsteke, R. Ortiz, C. Pasberg-Gauhl and NARS (see P.8.3)

P.6.6 Physiology of fruit maturation
S. Ferris and I. Ekanayake

P.6.7 Fruit storage
S. Ferris and NARS cooperators

P.6.8 Plantain utilisation
S. Ferris and NARS cooperators

P.6.9 Genetics of fruit quality
R. Ortiz and S. Ferris (see P.1.6)

P.6.10 Molecular and biochemical studies of *Musa* fruit ripening
G. Tucker, Vendrell (Univ. Nottingham), S. Ferris, R. Ortiz and M. Bokanga

P.6.11 On-farm cassava processing technology
Y.W. Jeon, L. Halos, S. Ferris

P.7 Genotype by Cropping Systems Interaction

P.7.1 The perennial yield potential of plants
D. Vuylsteke, R. Swennen (KUL) and R. Ortiz

P.7.2 Crop management practices for sustainable and perennial plantain production
M. Gichuru (RCMD), D. Vuylsteke, R. Ortiz and PHMD scientists

P.8 NARS Collaboration and Training

P.8.1 Multilocational trials
R. Ortiz, D. Vuylsteke and cooperators at IITA and NARS
(see P.2.14)

P.8.2 Advanced *Musa* yield trials
D. Vuylsteke, R. Ortiz, IITA and NARS cooperators
(see P.2.15)

P.8.3 On-farm assessment of yield and quality loss from black sigatoka
S. Ferris, F. Gauhl, D. Vuylsteke, R. Ortiz, C. Pasberg-Gauhl and NARS

P.8.4 Cooking bananas for Nigeria
D. Vuylsteke, R. Ortiz, S. Ferris and NARS

P.8.5 Black sigatoka resistant plantains for Nigeria
R. Ortiz, D. Vuylsteke, C. Anojulu (FACU) and other IITA and Nigerian NARS cooperators

P.8.6 Graduate training
D. Vuylsteke and R. Ortiz (supervisors)

1) In vitro embryo rescue for the enhancement of hybrid plantain production - G.I. Harry (see P.5.3)
2) Determination of ploidy levels and ploidy effects on growth and yield parameters in plantain-banana hybrids. H. Vandenhout (see completed studies in P.1 & P.2 and P.2.5 & P.3.5)

P.8.7 Plantain research and technology transfer training course
M.T. Ajayi (Training Program), R. Ortiz (technical coordinator) and other IITA and NARS scientists

P.8.8 Major constriants to *Musa* production in Africa
NARS, D. Vuylsteke and R. Ortiz

P.8.9 Training materials
Training program, NARS and PBIP scientists

P.9 Onne Station

P.9.1 Upgrading of physical facilities at the Onne High Rainfall Station of IITA in support of plantain and banana research and training
D. Vuylsteke and P.D. Austin.

GENETIC RESOURCE UNIT

G.1 Germplasm collection

G.1.1 Collecting cassava and maize germplasm in Nigeria
P.Q. Ng, T.G. Nandang, COSCA team and NRCRI and IAR&T scientists

G.1.2 Germplasm collecting expedition to Uganda
S. Padulosi

G.I.3 Collecting germplasm in Guinea-Conakry
El Sanoussy Bah, Sekouna Camara, N.Q. Ng, J. Kling, S.K. Kim and M.D. Winslow

G.1.4 Wild *Manihot* species collecting expedition in Brazil
S.K. Hahn, A.C. Allem and N.Q. Ng

G.2 Germplasm Conservation, Documentation and Distribution

G.2.1 Cowpea germplasm conservation
N.Q. Ng, A.E. Adegbite and T. Odega

G.2.2 Cowpea core collection
N.Q. Ng

G.2.3 Virus clean-up of cowpea germplasm
N.Q. Ng, H. Rossel, A.E. Adegbite and B.B. Singh

G.2.4 Wild *Vigna* germplasm conservation
S. Padulosi, N.Q. Ng, K. Sotonwa and J.O. Apeji

G.2.5 Yam field genebank
N.Q. Ng, D. Ogunsola and S.Y.C. Ng

G.2.6 *In vitro* yam germplasm conservation
S.Y.C. Ng, N.Q. Ng and R. Asiedu

G.2.7 Yam germplasm characterization
N.Q. Ng, R. Asiedu, H. Rossel, C. Akem, A.E. Adegbite and NRCRI

G.2.8 Cassava germplasm conservation and characterization
N.Q. Ng, T. Nandang, S.Y.C. Ng and H. Rossel

G.2.9 Rice germplasm conservation
N.Q. Ng and E.S.O. Erimah and T. Odega

G.2.10 Miscellaneous leguminous germplasm and agroforestry species conservation
N.Q. Ng, A.E. Adegbite, T.G. Nandang and T. Odega

G.2.11 Germplasm distribution
N.Q. Ng, J. Obarinde and T. Odega

G.2.12 Storage of yam seeds and pollens
N.Q. Ng

G.2.13 Health status of germplasm collections
N.Q. Ng, D. Florini, H. Rossel and other pathologists

G.3 Genetics and Phylogeny of Germplasm

G.3.1 Phylogeny and Biosystematics of African *Vigna:* hybrid between V. *oblongifolia* and V. *ambacensis*
N.Q. Ng, J. Apeji, A.E. Adegbite and H. Mignouna

G.3.2 Wild African *Vigna* palinological analyse
De Leonardis, S. Padulosi and N.Q. Ng

G.3.3 Phenetic studies in four species of yam (*Dioscorea*)
S.I. Chuckwuma, N.Q. Ng and M.O. Akoroda

G.4 NARS Collaboration and Training

G.4.1 Cassava and maize germplasm collection and characterization in Nigeria
N.Q. Ng, A. Dixon, R. Asiedu, S.K. Kim, J.G. Kling, NRCRI and IAR&T scientists

G.4.2 Cassava genetic resources network
N.Q. Ng, R. Asiedu, A. Dixon, S.Y.C. Ng, CIAT, IBPGR and NARS

G.4.3. Germplasm conservation and distribution
N.Q. Ng, S. Padulosi, D. Vuylsteke, S.Y.C. Ng and other IITA scientists

G.4.4 Inter-center working group on plant genetic resources
N.Q. Ng, IBPGR and other CG centers

G.4.5 Graduate training
Phenetic studies in four species of (*Dioscorea*).
S.I. Chukwuma (M.Sc. thesis) N.Q. Ng and M.O. Akoroda

G.4.6 Plant genetic resources concervation, use and management
N.Q. Ng, J. Gulley, other IITA scientists, IBPGR and FAO.

BIOTECHNOLOGY RESEARCH UNIT

B.1 Molecular Marker Techniques

B.1.1 RAPD to assess the genetic diversity in cultivated cowpea

B.1.2 Molecular characterization of improved IITA elite clones of root crops

B.1.3 Construction of yam genomic library

B.1.4 Analysis of proteins and messenger RNAs

B.2 Non-Sexual Gene Transfer Techniques

B.2.1 Plant regeneration from cowpea protoplasts
G. Thottappilly, A.S. Afolabi, S.Y.C. Ng and G. Ude

B.2.2 Callus induction and plant regeneration in yam
S.Y.C. Ng and S.H. Mantell (Wye college, UK)

B.2.3 Anther culture in cassava
S.Y.C. Ng and K. V. Bai

B.2.4 A polyethylene glycol-mediated protoplast ransformation system for production of fertile transgenic cowpea plants
G. Thottappilly, A.S. Afolabi and S.Y.C. Ng

B.2.5 Protoplast transformation system by electroporation
I. Ingelbrecht, G. Thottappilly, A.S. Afolabi, S.Y.C. Ng and J. Ikea

B.2.6 Gene tranformation in cowpea and plantain by DNA particle gun
G. Thottappilly, S.Y.C. Ng, D. Vuylsteke, J. Ikea and A.S. Afolabi
Collaborations: R.A. Bressan, P.M. Hasegawa, P. Dunn and L. Monti

B.2.7 *Agrobacterium fumefaciens* - mediated gene transfer in cowpea
G. Thottappilly, I. Ingelbrecht, S.Y.C. Ng and A.S. Afolabi

B.2.8 *Agrobacterium* gene transfer in cassava
S.Y.C. Ng, O.J. Adeniyi and E. Filippone (Italy)

B.2.9 Use of a defective replicase gene to induce resistance to cucumber mosaic virus in transgenic banana/plantain
S.Y.C. Ng and R. Asiedu

B.3 New Cytogenetic Techniques

B.3.1 DNA flow cytometry in cassava
S.K. Hahn, K.V. Bai and S.Y.C. Ng

B.4 Molecular Diagnostics

B.4.1 Indexing of tissue culture materials of white yam using PCR, universal potyvirus monoclonals and polyclonal antiserum
G. Thottappilly, S.Y.C. Ng, A. Alonge and A.S. Afolabi

B.4.2 Monoclonal antibody production lab at IITA to produce monoclonal antibodies to viruses, fungi, pathogenic bacteria and symbiotic *Rhizobia*
G. Thottappilly. A. Alonge and H.W. Rossel

B.4.3 Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms within Nigerian populations of whitefly
G. Thottappilly and PHMD Entomologist

B.4.4 Nucleic acid detection of ACMV
G. Thottappilly, H.W. Rossel and S.Y.C. Ng

B.5 Training/Workshop/Collaborative work

B.5.1 Biosafety meeting, Ibadan, IITA, June 1993

B.5.2 Plant cell and tissue culture and biotechnology workshop, Ibadan, IITA, 4-22 October, 1993

B.5.3 Workshop on "Application of monoclonal antibodies for identification of cowpea viruses". October/November, 1993

B.5.4 Safe handling of radioactive materials - date to be fixed. This is mainly for IITA researchers. This will be done in collaboration with the Radiation Protection Agency of Nigeria

B.5.5 Collaboration with national programs on application of monoclonal antibodies. IDRC has agreed to provide funds for a second phase mainly to help the national programs. IITA will coordinate this activity

B.5.6 Development of biosafety guidelines
G. Thottappilly and all involved in Biotechnology research. The priority in 1993 is to develop biosafety guidelines

List of Scientific Staff and Students

Office of the Director, CID

F. Margaret Quin, Ph.D.	Director, CID

Root and Tuber Improvement Program

R. Asiedu, Ph.D.	Leader, Breeder
A. Dixon, Ph.D.	Breeder
I. Ekanayake, Ph.D.	Crop Physiologist
M. Bokanga, Ph.D.	Biochemist
M. Porto, Ph.D.	Crop Physiologist, CIAT/IITA Liaison scientist
S.Y.C. Ng, MSc.	Tissue Culture Specialist

Scientists in Special Project: ESARRN, Malawi

M.N. Alvarez, Ph.D.*	Breeder and network coordinator

Graduate Students

A.O. Akano	University of Ibadan, Ph.D. Nigeria
S. Akparobi	University of Ibadan, MSc. Nigeria
V. Boateng (Ms)	University of Ghana, MSc. Ghana
M.A. Fregene	University of Ibadan, Ph.D. Nigeria
R. Kapinga	University of Ibadan, Ph.D. Tanzania
M. Makame	University of Ibadan, Ph.D. Tanzania
V. Mbuyongha	University of Ibadan, Ph.D.Cameroon
M. Simwambana	University of West Indies, Ph.D. Zambia
N. Wanyera	University of Ibadan, Ph.D. Uganda
C.N. Fokunang	University of Ibadan, Ph.D. Cameroon
G.A. Manga	University of Ibadan, MSc. Cameroon
N.E. Nukenine	University of Ibadan, Ph.D. Cameroon
M. Wouamane	University of Ibadan, MSc. Cameroon
M.T. Lahai	University of Njala, Ph.D. Sierra Leone
B. Aighewi	University of Ibadan, MPhil/Ph.D. Cameroon
O.F. Odeleye	University of Ibadan, Ph.D. Nigeria

Maize Improvement Program

F.Margaret Quin, Ph.D. (from May 7)	Leader, Physiologist/Agronmist
S.K. Kim, Ph.D.	Breeder
J.G. Kling, Ph.D.	Breeder
J.M. Fajemisin, Ph.D.	Pathologist/breeder (joint with ICP)
S.T. Yoon, Ph.D.	Visiting Scientist, Korea funded (end June,1993)
B. Badu-Apraku,Ph.D.	Breeder, SAFGRAD Maize Coordinator(joint with ICP)
A. Diallo, Ph.D.	Breeder, CIMMYT Adjunct Scientist (Cote d'Ivoire

Graduate Students

H.A. Akintoye	University of Ibadan, Ph.D. Nigeria
V. Adetimirin	University of Ibadan, Ph.D. Nigeria

Grain Legume Improvement Program

K.E. Dashiell, Ph.D.	Leader, Breeder
B.B. Singh, Ph.D.	Breeder and Officer-in-Charge, Kano Station
S.F. Blade, Ph.D.	Post-doctoral fellow
T.Terao, Ph.D.	Physiologist (TARC), Kano Station
S.M. Osho, Ph.D.	Food Technologist (IDRC)
R. Abaidoo, M.Sc.	Associate Expert (Niftal)
G. Blahut	CUSO Cooperant

E.K. van Gerben	(VSO)
E.K. van Jantien	(VSO)

Graduate Students

M. Ogbaji	University of Ibadan, Ph.D. Nigeria
B. Kehinde	University of Ibadan, Ph.D. Nigeria
G. Ssemakula-Nankinga	University of Ibadan, Ph.D. Nigeria
I.C. Charles	University of Ibadan, Ph.D. Nigeria
P.M. Kormawa	University of Hohenheim, Ph.D. SierraLeone (with RCMD)
M.E. Ebot	Univewrsity of Nigeria Nsuka, Ph.D. Cameroon
M.Y. Bakoh	University of Ibadan, Ph.D. Cameroon
Mrs. F.Z. Kalan	University of Science & Technology, (Kumasi,) Ph.D. Ghana(with RCMD)
Ms. C.A. Onyejegbu	University of Ibadan, Ph.D. Nigeria
D.K. Ojo	University of Reading, Ph.D. Nigeria
M. Ishaku	University of Reading, Ph.D. Nigeria
O. Adjah	Ahmadu Bello University, MSc. Rep. of Benin
Y. M. Kodomi	Southern University of Illinois, Ph.D.

Scientists in Special Projects

D.M. Naik, Ph.D.	Leader, Breeder (SADCC), Mozambique
R. Amable, Ph.D.	Agronomist (SADCC), Mozambique
A. Doto, Ph.D.	Breeder (SADCC), Mozambique
A.M. Hossain, Ph.D.	Breeder (GGDP), Ghana
J. Detongnon, Ph.D.	Legume Specialist (NCRE), Cameroon
N. Muleba	Breeder/Agronomist SAFGRAD, Burkina Faso

Plantain and Banana Improvement Program

D.R. Vuylsteke, Ir.	Leader, Germplasm enhancer
Rodomiro Ortiz, Ph.D.	Breeder/Geneticist
Shaun Ferris, Ph.D.	Post-doctoral fellow, postharvest research

Graduate Students

G.I. Harry	Rivers State Univ. of Science & Tech. Ph.D. Nigeria
Hilde Vandenhout	KUL Belgium, Ir. Belgium

Genetic Resources Unit

N.Q. Ng, Ph.D.	Head, Genetic Resources Specialist
S. Padulosi, Dott. (left during year)	Plant Explorer

Graduate Students

F.N.U. Agwaranze	University of Ibadan, Ph.D., Nigeria.
A. Bakari,	University of Ibadan, Msc., Nigeria
S.I. Chukwuma	University of Ibadan, MSc., Nigeria

Biotechnology Research Unit

G. Thottappilly, Ph.D.	Head, Virologist
S.K. Hahn, Ph.D.	Director Emeritus
S.R. Schnapp, Ph.D. (left during the year)	Associate Scientist
H.D. Mignouna, Ph.D.	Postdoctoral Fellow
P. Petrilli, Ph.D.	Visiting Scientist, Univ. de Napoli, Italy

Graduate Students

T.E. Njock	University of Ibadan, Ph.D., Cameroon
U.U. Ekuere	University of Ibadan, Ph.D., Nigeria