

MID-TERM REPORT

IFCPAR PROJECT No. 3000 B 1

**GENETIC IMPROVEMENT OF YAM
USING DNA MARKERS (AFLP)**

(Report covering the period from 1 August 2005 to 25 May 2007)

Jointly submitted by

Dr. K. Abraham

Central Tuber Crops Research Institute
India

Dr. Gemma Arnau

Centre de Cooperation Internationale en Recherche
Agronomique pour le Developpement
France

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I - GENERAL

REFERENCE NO:	3000 B1
TITLE OF THE PROJECT	Genetic Improvement of Yam Using DNA Markers (AFLP)
INDIAN PRINCIPAL COLLABORATOR	Dr. K. Abraham, Principal Scientist & Head, Division of Crop Improvement, Central Tuber Crops Research Institute, Trivandrum - 695017, India
FRENCH PRINCIPAL COLLABORATOR	Dr. Gemma Arnau Yam Scientist, CIRAD-CA, Station de Roujol, 97170 Petit Bourg, Guadeloupe, French West Indies
DATE OF PROJECT START	1 August 2005
DURATION OF THE PROJECT	3 years

SUMMARY

The project aims at the genetic improvement of the vegetatively propagated greater yam through the integrated techniques of biotechnology and conventional breeding, developed by the CIRAD and the CTCRI respectively. Selection of fertile parents will be facilitated by identifying tetraploid cultivars through cytological studies and estimating their genetic diversity by DNA fingerprinting (AFLP & SSRs). The genetically distant cultivars will be hybridized to maximize heterosis in the progeny. Pollen grains from elite males will be preserved for pollinating superior females that flower at different periods. Large scale seed production will be effected through the joint efforts of scientists from the partner institutes. Among the resulting sexual progeny, DNA markers will be identified to select superior recombinants at the early stages. The relevance of the project is the integration of two complementary technologies in the genetic improvement of greater yam, for the first time. As this important food crop is dioecious, vegetatively propagated and under-studied, many constraints have so far hindered its genetic improvement. By the complementary strategies of CTCRI and CIRAD, the existing constraints will be easily overcome and the production of genetically improved, superior varieties will be easier and quicker.

OBJECTIVES

1. To overcome the constraints in greater yam improvement by streamlining and strengthening conventional breeding by hybridization
2. To develop and integrate biotechnological tools to facilitate efficient parent and progeny selection
3. To develop genetically improved varieties of greater yam having increased productivity, improved palatability, prolonged storability and enhanced dry matter content of tubers
4. To generate basic genetic information on greater yam for developing efficient breeding strategies and promoting interaction and technology sharing between CTCRI and CIRAD

II - WORK REPORT

A. BRIEF REPORT OF THE WORK DONE ON THE PROJECT TILL NOW (COVERING WORK DONE BOTH IN FRANCE AND IN INDIA)

(Please bring out deviations made, if any, from the originally proposed methodology and work schedule, while implementing the project, and reasons thereof. Also bring out clearly the collaborative element)

- 1. Pollination work at CTCRI:** During the year 2005 (October – December), artificial pollinations were carried out between males and females of greater yam. A total of 11,472 pollinations were done in 110 parental combinations. Fruit set was recorded in 96 combinations, seed set in 74 combinations and seed germination in 22 combinations. Fruit set in intervarietal crosses ranged from 1.7 – 86.8 %, seed set from 1.4 – 55.6 % and seed germination from 0.8 – 81.3 %. A total of 3708 fruits and 4373 seeds were collected. During 2006 (April – May), 426 seedlings from the intervarietal crosses germinated. 345 hybrid seedlings and 396 open pollinated seedlings were transplanted to field. Among the *D. alata* seedlings, 49 flowered, of which 42 were males and 7 females. There were four small plants and one looked like a dwarf. Again during 2006 (October – December) 6249 artificial pollinations were carried out in 81 parental combinations. 3119 fruits were collected and the fruit set and seed set data is being recorded.
- 2. Pollination work at CIRAD:** About 8000 seeds were produced by controlled and open pollination in Guadeloupe during the seasons 2005 and 2006. Crosses were carried out between elite male and female varieties chosen for their agronomic features (yield, tuber quality, resistance to anthracnose etc.) and their genetic distances in order to maximise heterozygosity and heterosis in the progenies.
- 3. Joint pollination work at CIRAD:** Pollination work was done jointly by CTCRI and CIRAD scientists at Guadeloupe, when Dr. K. Abraham, Principal Indian collaborator visited Guadeloupe from 28 November to 22 December

2005. It was the flowering time of *D. alata* and Dr Abraham could assist and involve with the CIRAD scientist and technician in conducting pollinations and especially initiate them into field experience of identifying fertile and sterile female clones by floral observations. The rare octoploid male and female clones were available in flowering stage. The octoploid male was used to pollinate tetraploids and octoploids and they were producing fruits and seeds which is not reported yet. It is a significant finding in greater yam breeding.

4. **Tuber yield and ploidy level:** Greater yam is a polyploid species with varieties having three different ploidy levels of $2n = 40, 60$ and 80 . Hexaploids and octoploids were found to be more vigorous and recorded higher tuber yields than tetraploids. Thirty three varieties of CIRAD's collection were evaluated in Guadeloupe in 2006. Results showed that better tuber yield was obtained for varieties with the higher ploidy levels (Fig. 1 & 2).
5. **Production of hexaploid hybrids:** Hexaploid hybrids were produced in 2005 for the first time by hybridisation between tetraploids and octoploids. A total of 701 controlled hybridisations were carried out between four tetraploid female clones (F5, F27, F53 and F74) and one octoploid male clone (CTRTR-148). The fruit set in the various combinations ranged from 45 to 56 % and the seed set from 33 to 38%. Almost all the seeds were found to contain embryos but with an abnormal development of endosperm tissue. Hence fifty embryos per cross were rescued by vitro culture. On the other hand, no seedling was obtained by normal germination of dry seeds. The success of embryo rescue ranged from 15 to 30%. In 2006, 3000 seeds were obtained by open pollination and 800 immature embryos were rescued by in vitro culture.

Reciprocal crosses between octoploid female clones and tetraploid male clones were attempted for the first time in 2006 by allowing natural pollination between two $8x$ female clones (F112, F56) and two $4x$ male clones. A total of 70 fruits and 100 seeds were collected. The development of the seeds was normal. The seeds will be germinated for raising the hybrids.

6. **Production of octoploid hybrids:** Octoploid hybrids were produced for the first time in 2006 by hybridisation between one octoploid female (198 CTRTR) and one octoploid male parent (CTRTR-148) that are genetically distant. A total

of 273 fruits and 670 seeds were collected. Seeds have been planted recently and some have started germination.

- 7. Production of tetraploid hybrids:** Over 4000 hybridizations were conducted by controlled and open pollination during the seasons 2005 and 2006 involving different tetraploid male and female clones. Since anthracnose is one of the main causes of economic loss in greater yam, one highly resistant male variety of CIRAD germplasm (177 CTRT) was used for natural pollination (in 2006) with 6 distant female of high quality to produce resistant hybrids. A total of 1000 seeds were obtained. They will be germinated for raising the hybrids.
- 8. Evaluation of hybrids for the first time:** Hybrids of greater yam produced in 2005 for the first time in Guadeloupe were evaluated in the field during 2006. The hybrids produced in CTCRI, India were also evaluated during 2006. Those produced in 2006 will be evaluated 2007. It would be possible to identify promising lines from the clonal plants of seedlings.
- 9. Ploidy determination by flow cytometry:** Flow cytometry was used for assessing the ploidy levels of 200 clones of greater yam (99 varieties of CIRAD collection and 101 new hybrids). Measures were performed using a Bryte HS Flow Cytometer, that quantifies the fluorescence emitted by isolated nuclei stained with a fluorochrome. This method allows tetraploids, hexaploids and octoploids to be identified.
- 10. Assessment of genetic diversity of CIRAD germplasm using microsatellite DNA markers:** Allelic diversity among parental lines was characterised using 10 selected microsatellite markers. In a first study a set of 6 varieties was used to select the 10 suitable microsatellite loci out of 40 available. The selection was based on the degree of polymorphism detected and the quality of loci. We excluded those with stuttering effects. Primers selected were labelled with three different fluorochromes (HEX, FAM, TET) and PCR products were multiplexed. Migration was carried out with an automatic sequencer ABI PRISM™ 3100 (Applied Biosystems). This method is very efficient because several microsatellite markers can be pooled and it is highly reliable. **Figure 3** shows a typical electrophoregram obtained with multiple samples loading. The number of alleles recorded on the 96 varieties with the ten selected microsatellite loci Dpr3E10, Dab2D08, Da1D08, Dpr3B12, Da1A01, Dab2D11, Da3G04, Da1F08, Da2F10, Dab2E07 are 5, 6, 8, 8, 8, 9,9, 11, 15 and 17 respectively. A total of 96

alleles were detected at 10 loci. For the purpose of assessing genetic diversity leading to the preparation of a dendrogram, data were scored in binary format, with the presence of an allele scored as unity and its absence scored as zero. The binary data were used to compute pair-wise similarity coefficients (Nei and Li) and the similarity matrix thus obtained was subjected to cluster analysis using the UPGMA algorithm on NTSYS-PC version 2.

The genetic similarity for all the possible 96 pairs of genotypes ranged from 0.55 to 1. The dendrogram prepared through cluster analysis is shown in Fig. 4, suggesting a high level of diversity among the 96 genotypes. Microsatellite analysis showed 4 major clusters, cluster I with 47 genotypes, cluster II with 38 genotypes, Cluster III with 4 genotypes and cluster IV with 6 genotypes. Cluster I is further subdivided into two subclusters, subcluster Ia containing 18 genotypes and subcluster Ib containing 29 genotypes.

- 11. Molecular markers heterozygosity determination using microsatellite markers:** Heterozygosity was estimated by calculating the mean number of alleles per locus on 8 microsatellite markers. Test X² showed that the number of homozygote and heterozygote clones is depends on the ploidy levels. Tetraploid varieties are more homozygous than the hexaploid and octoploid varieties. A significant correlation was noted between tuber yield and the estimated heterozygosity (Fig 5). Hexaploid and octoploid varieties have a higher number of alleles per locus which could explain their superior performance observed in the field. **In conclusion, the production of hexaploids and octoploids by crossing distant genotypes appears promising for the genetic improvement of the greater yam, making it possible to maximise heterozygosity and heterosis.**
- 12. Immature embryos rescuing by vitro culture.** Almost all the seeds from the interploid crosses containing embryos were found to have an abnormal development of endosperm tissue and the seeds on drying were not germinable .A method for rescuing immature embryos was developed that could be used to obtain hexaploid hybrids. The effectiveness of different techniques and media were tested. Fig 6 shows embryos of hexaploid hybrids at different stages of development.
- 13. Mode of inheritance of microsatellite markers in *D. alata* varieties indicating diploid nature of $2n = 40$ chromosome types:** The parents and

progeny of four controlled crosses were used to determine the segregation patterns of microsatellites markers. The inheritance patterns of microsatellites were determined from a comparison of the distribution of progeny genotypes with the expected distribution based on the genotypes of the parents. We adopted a Bayesian procedure to discriminate among the different inheritance hypotheses. It better fits the requirement of testing complex segregation patterns than the X^2 statistics.

Inheritance of the genotypes in the progeny of four crosses was consistent with simple diploid, Mendelian inheritance at six loci tested (Tables 1, 2). **Our results provide first reliable evidence supporting that clones with $2n=40$ chromosomes could be diploid and not tetraploid as earlier presumed (Arnau *et al*, in prep).**

14. **Cytological studies:** Meiosis was studied in *D. alata* male clones to ascertain ploidy status and to check for any abnormalities. 22 males were studied in 2005 and 11 males were studied in 2006 at CTCRI. All the males checked were having $2n = 40$ chromosome constitution without any meiotic abnormalities.
15. **Pollen fertility studies:** Pollen fertility was estimated from 102 male clones. The vast majority of the males had pollen fertility of above 70 per cent. There were only three clones which recorded pollen fertility of less than 50 per cent. It shows that, by and large, the males of *D. alata* are highly pollen fertile.
16. **Joint DNA work at CTCRI and CIRAD:** A modified protocol for the extraction of high quality DNA from yam leaf involving isolation of nucleus followed by DNA extraction using Quiagen kit was standardised. The standardisation and the procedure was helped by Dr. Gemma Arnau of CIRAD during her visit to CTCRI from 27 June – 7 July, 2006. Using the modified protocol, high quality DNA from yam leaf was extracted from 80 greater yam accessions of India. Dr. M. N. Sheela of CTCRI carried purified samples of DNA of Indian *D. alata* accessions to CIRAD lab and compared the same with that of Pacific accessions using SSR markers. The results showed the diversification of Indian and Pacific genetic stocks of greater yam. **The study also helped to compare the molecular profile of Indian and Pacific collections in which majority of the Indian accessions were found to be divergent from Pacific accessions.** The fertile highly divergent males (of Indian germplasm) having good tuber shape *viz.* Da 102, Da 97, Da119, Da, 165, Da

166, Da 175, identified from the DNA study, were used in hybridization programme for developing elite hybrids under this project. The genotyping of the germplasm will help in identifying molecular markers to promote marker assisted breeding in greater yam.

17. Visit of scientists for joint work:

i. Dr. K. Abraham, Indian principal collaborator visited the CIRAD station at Guadeloupe, from 28 November to 22 December 2005 and did joint pollination work and laboratory work of analysis of microsatellites, using purified DNA.

ii. Dr. Gemma Arnau, French principal collaborator worked in CTCRI lab during the period 27 June – 7 July 2006 (11 days) in modifying DNA extraction protocols for getting high quality DNA from yam leaves.

iii. Dr. M. N. Sheela, Senior Scientist of CTCRI (Indian collaborator) visited CIRAD lab at Guadeloupe, French West Indies from 8 – 31 July 2006 (21 days) and worked with Dr. Gemma Arnau. Dr Sheela carried DNA samples of Indian accessions and compared the same with that of Pacific accessions using SSR markers. The results showed diversification of Indian and Pacific genetic stocks of greater yam

iv. Dr. Gemma Arnau, French principal collaborator made a short visit to CTCRI and attended the 14th international symposium of the ISTRC and presented a paper from the results of the IFCPAR project work.

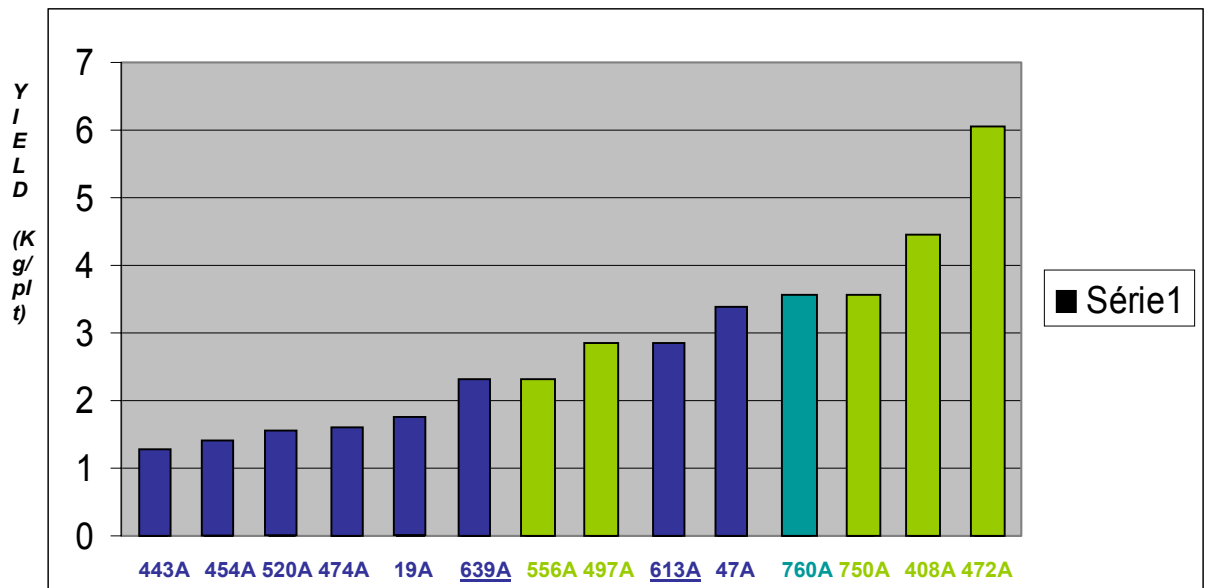
18. Presentation of results in International symposium: Oral presentations were made by Dr. K. Abraham and Dr. Gemma Arnau and a poster presentation was made by Dr. M. N. Sheela in the 14th International Symposium of the International Society for Tropical Tuber Crops (ISTRC) conducted in India during November 2006. The presentations were based on the results of work in the Indo- French project.

19. International award for the presentation of results from IFCPAR project: Dr. M. N. Sheela's poster secured two awards, viz., the prestigious **Pat Coursey**

award for the best yam research work presented in the symposium and also **the best poster presentation** in the 14th International Symposium of the ISTRC.

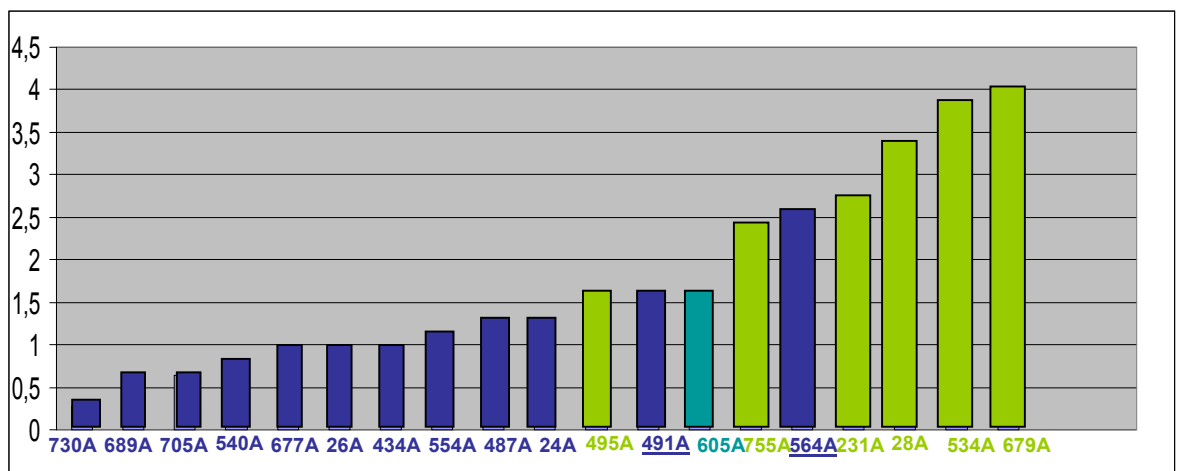
(There has not been any deviation from the originally proposed methodology and work schedule)

Fig .1: Tuber yield of *Dioscorea alata* accessions with different ploidy levels



Accessions with 2n= 40, 60, 80 chromosomes

Fig 2: Tuber yield of *Dioscorea.alata* accessions with different ploidy levels



Accessions with 2n= 40, 60, 80

Fig. 3: Typical electrophoregram produced by the automated DNA sequencer after multiple sample loading

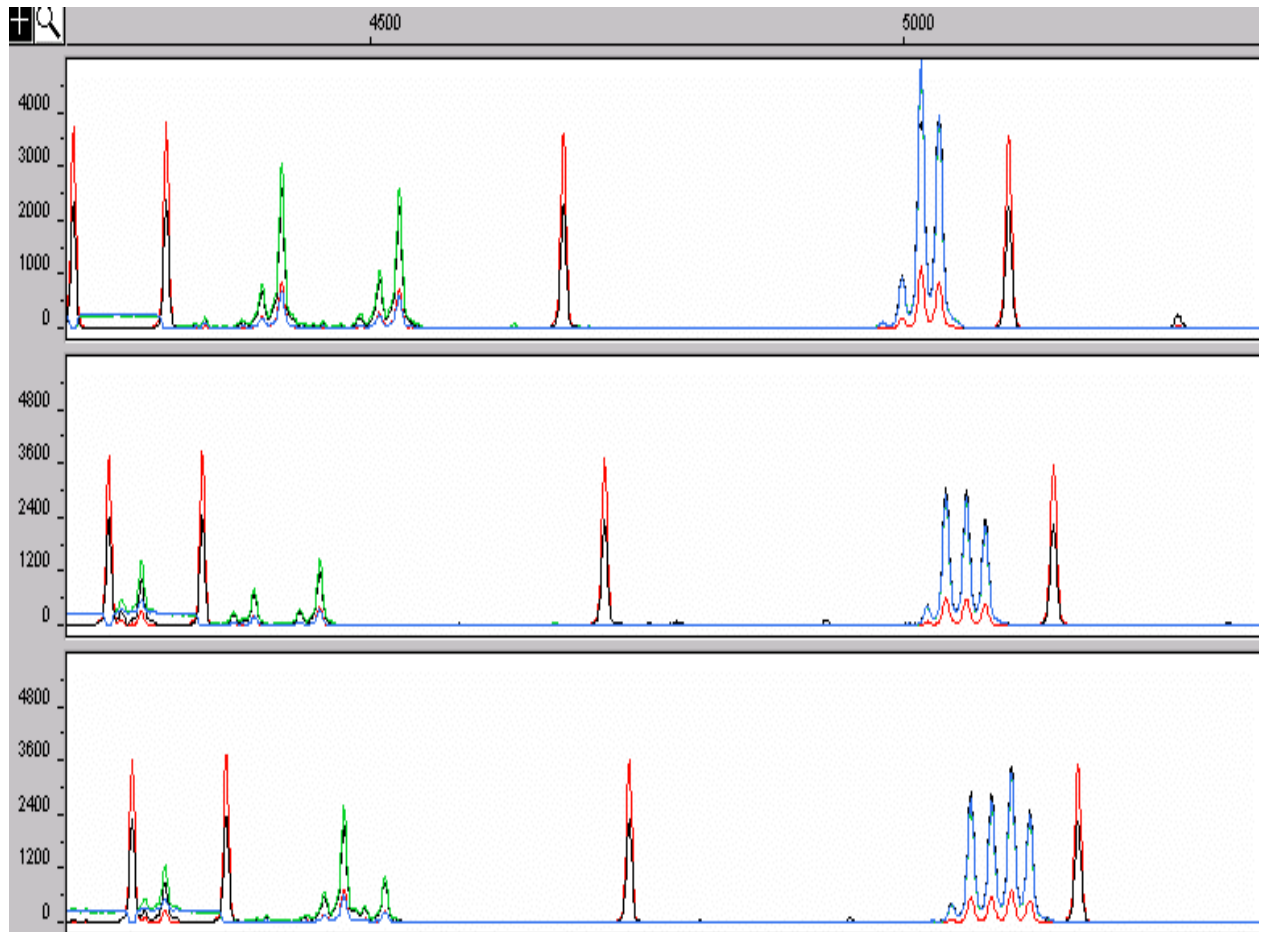


Fig 4 : Dendrogram showing the associations among 96 *D. alata* varieties derived from a UPGMA cluster analysis using the NEI and LI genetic distance coefficient based on alleles present at 10 SSR loci.

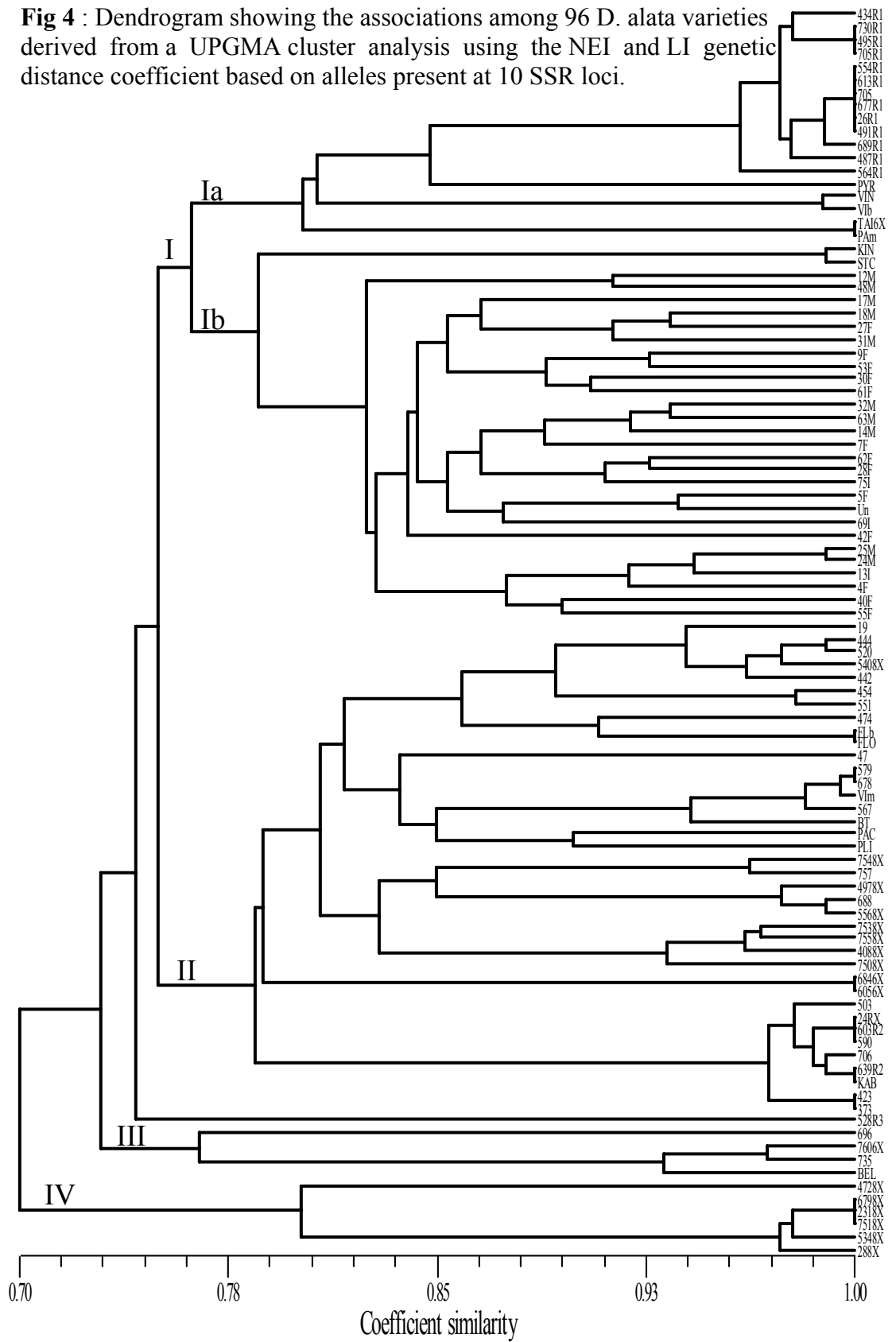


Fig 5: Correlation between yield and molecular marker heterozygosity estimated using SSR molecular markers.

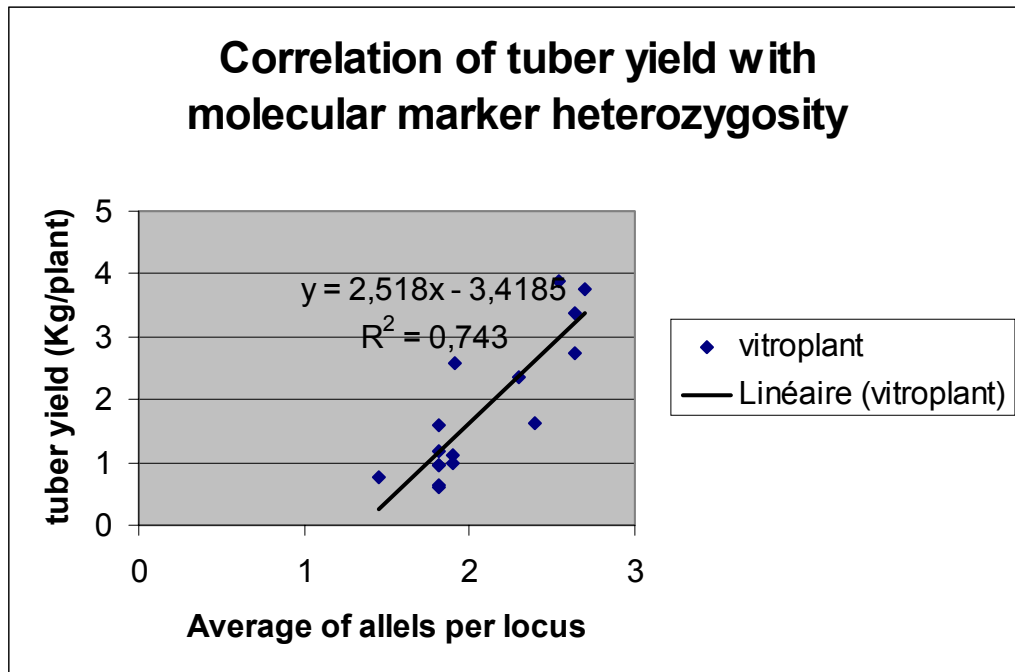


Fig 6: Immature embryos of *D. alata* hexaploid hybrids rescued by *in vitro* culture.

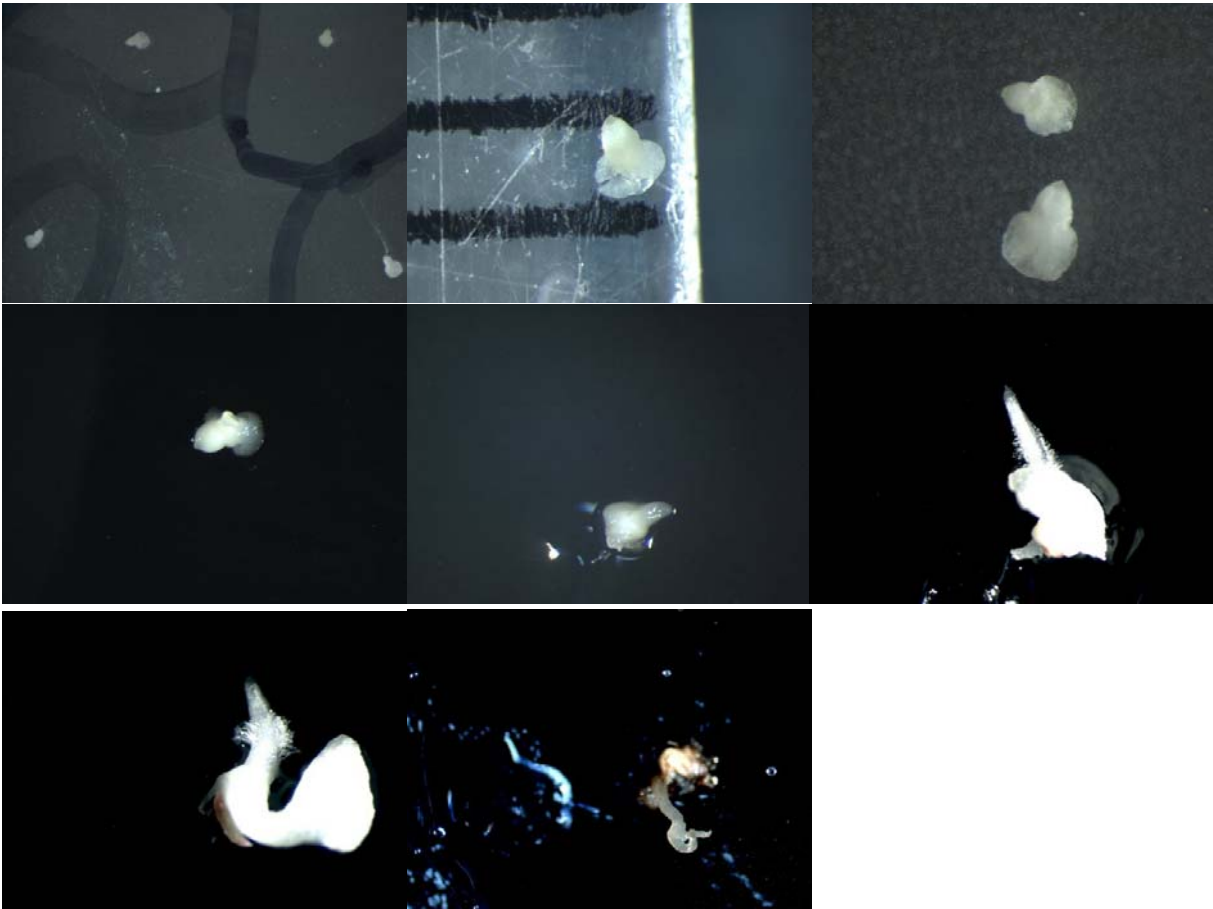


Table 1: Distribution of expected and observed genotypes in the progeny 24M x 27F at the Da2F10 locus under disomic and tetrasomic inheritance. Three different assumptions are tested for 27F genotype assuming that 24M is known without error: (1) bbcc; (2) bccc; (3) bbbc. Logarithms of likelihood for all the possible segregation patterns are given and the Bayes Factor is computed as indicated in the text. For convenience, log Likelihood are given.

Parental genotype	Progeny genotype						Log (L)
	a	b	bc	ac	ab	abc	P=0,01
	Observed genotypes						
	6	17	13	24			
	Distribution of expected progeny for the parental genotypes: ab x bc under disomic inheritance (2x, 1/4)						
ab x bc	1	1	1	1			-6
	Distribution of expected progeny for the parental genotypes: aabb x bbcc under tetrasomic inheritance (4x, 1/36)						
aabb x bbcc	1	5	1	5	24		-34
	Distribution of expected progeny for the parental genotypes: aabb x bbcc under disomic inheritance (4x, 1/16)						
aa/bb x cc/bb						16	-120
aa/bb x bb/cc						16	-120
aa/bb x bc/bc					4	12	-70
bb/aa x bb/cc						16	-120
bb/aa x bc/bc					4	12	-70
bb/aa x cc/bb						16	-120
ab/ab x bb/cc			4			12	-82
ab/ab x bc/bc	1	3	1	3		8	-22
ab/ab x cc/bb			4			12	-82
	Distribution of expected progeny for the parental genotypes: aabb x bccc under tetrasomic inheritance (4x, 1/36)						
aabb x bccc		6	3			27	-24
	Distribution of expected progeny for the parental genotypes: aabb x bccc under disomic inheritance (4x, 1/16)						
aa/bb x bc/cc						16	-120
aa/bb x cc/bc						16	-120
bb/aa x bc/cc						16	-120
bb/aa x cc/bc						16	-120
ab/ab x bc/cc			4	2		10	-57
ab/ab x cc/bc			4	2		10	-57
	Distribution of expected progeny for the parental genotypes: aabb x bbbc under tetrasomic inheritance (4x, 1/36)						
aabb x bbbc	3	3		15	15		-39
	Distribution of expected progeny for the parental genotypes: aabb x bbbc under disomic inheritance (4x, 1/16)						
aa/bb x bb/bc					8	8	-63
aa/bb x bc/bb					8	8	-63
bb/aa x bb/bc					8	8	-63
bb/aa x bc/bb					8	8	-63
ab/ab x bb/bc	2	2			6	6	-38
ab/ab x bc/bb	2	2			6	6	-38
	Bayes factor integrating all the possibilities						6 e+16

Table 2: Bayes Factors testing the likelihood of a 2X versus a 4X inheritance pattern in four segregating populations of *Dioscorea alata* on six microsatellites locus. (see text).

Bold values indicate that 2X is more likely than 4X, in light otherwise.

n = Total number of different allele

bf= Bayes factors

Locus	Population							
	I-27F x I-11M (N=60)		VIN x I-11M (N=23)		I-27F x I-12M (N=17)		I-27F X PYR (N=10)	
	n	bf	n	bf	n	bf	n	bf
<i>Da2F10</i>	3	6 10¹⁶	4	7 10¹³	3	2 10⁴	3	1 10²
Da3G04	-	-	3	4 10⁹	-	-	2	4 10 ⁻¹
Dab2D08	-	-	2	1 10⁰	-	-	-	-
Dab2E07	2	5 10⁰	2	-	2	1 10⁰	2	4 10⁰
Dab2D11	-	-	2	3 10²	-	-	3	1 10³
Da1F08	2	4 10⁰	2	1 10 ⁻¹	2	4 10⁰	2	4 10⁰

B. PLAN OF WORK FOR THE REMAINING PERIOD OF THE PROJECT

(Here again, please mention variations, if any, with reference to the originally proposed methodology and schedule in the implementation of the project with reasons for the same.)

1. Raising of seedlings and clonal plants and the study of their variation with regard to useful characters
2. Study of more greater yam accessions of India with more SSR markers and AFLP markers
3. Hormonal induction of flowering of elite non-flowered accessions to be used as parents in further hybridization
4. Identification of markers (SSRs and AFLP) for efficient progeny selection
5. Pollen preservation for medium and long term storage
6. Development of protocols for the interspecific hybridization of *D. alata* with other edible, minor species for transferring useful traits such as drought resistance, anthracnose resistance etc
7. Marker assisted selection among the sexual progeny

III - PERSONNEL

A. INDIAN SIDE

Details of scientists of the Indian Principal Collaborator's Institution working on this project

1. Dr. M. T. sreekumari, Principal Scientist, CTCRI
2. Dr. M. N. Sheela, Senior Scientist, CTCRI

Details of research staff specifically appointed under this project on the Indian side

(Please give name, designation, qualifications, experience, date and duration of appointment for each person)

Smt. Sreeja Thankappan	Research Associate
Qualifications	M. Sc. (Genetics & Plant Breeding) Diploma in computer applications Experience in tissue culture Research experience of 6 years as Research Assistant/ Research Associate
Date of appointment	6 August 2005
Duration of appointment	3 years

B. FRENCH SIDE

Details of scientists of the French Principal Collaborator's institution working on this project

1. Dr V. Lebot, Chercheur, Programme CALIM, CIRAD
2. Dr. Jean Louis Noyer, Biomolecular Scientist, BIOTROP Unit, CIRAD

Details of research staff specifically appointed under this project on the French side

(Please give name, designation, qualifications, experience, date and duration of appointment, for each person)

Alice Nemorin	Research Associate
Qualifications	M. Sc. (Genetics & Plant Development) Experience in molecular Biology Research experience of 1 year as Research Assistant
Date of appointment	November 2006
Duration of appointment	21 months

IV-FINANCIAL ASPECTS

FUNDS AS APPROVED, RECEIVED AND UTILISED IN THE BUDGET

A. INDIAN SIDE:

DETAILS	<u>BUDGET in Rs. -1st Year</u>			<u>BUDGET in Rs. -2nd Year</u>		
	Approved	Received	Utilised	Approved	Received	Utilised
SALARIES	1,56,000	1,56,000	1,37,110	1,56,000	1,56,000	88,550
RECURRING	3,50,000	3,50,000	3,34,895	3,50,000	3,50,000	1,39,254
EQUIPMENT	6,00,000	nil	nil	6,00,000	5,80,992	5,37,054
TOTAL	11,06,000	5,06,000	4,72,005	11,06,000	10,86,992	7,64,858

B. FRENCH SIDE:

DETAILS	<u>BUDGET in Euro. -1st Year</u>			<u>BUDGET in Euro. -2nd Year</u>		
	Approved	Received	Utilised	Approved	Received	Utilised
SALARIES						
RECURRING	6800	6800	3980	6800	6800	3761
EQUIPMENT	5030					
TOTAL	11830	6800	3980	6800	6800	3761

V - EQUIPMENT

Procurement of Equipment

(Give list of equipment purchased with IFCPAR funds, along with value and date of receipt of the equipment)

A. INDIAN SIDE

<u>NAME OF THE EQUIPMENT</u>	<u>VALUE</u>	<u>DATE OF RECEIPT</u>
1. Scanner	Rs. 12,590	31 July 2006
2. Laser printer	Rs. 9,900	31 July 2006
3. Electronic balance	Rs. 2,644	2 Aug 2006
4. Digital camera	Rs. 57,938	4 Oct 2006
5. Laptop computer	Rs. 97,920	1 Dec 2006
6. Microscope with camera	Rs. 3,56,062	26 Feb 2007
Total	Rs. 537054	

B. FRENCH SIDE

<u>NAME OF THE EQUIPMENT</u>	<u>VALUE IN</u>	<u>DATE OF</u>
<u>RECEIPT</u>		
Lyophilisateur	Euro. 5030	

VI - EXCHANGE VISITS

(Please give names of the scientists who undertook exchange visits with dates and duration of each visit)

A. FROM INDIA TO FRANCE

<u>NAME OF THE SCIENTISTS</u>	<u>DATE & DURATION</u>
Dr. K. Abraham	28 November - 22 December 2005 (25 days)
Dr. M. N. Sheela	8 - 31 July, 2006 (24 days)

B. FROM FRANCE TO INDIA

<u>NAME OF THE SCIENTISTS</u>	<u>DATE & DURATION</u>
Dr. Gemma Arnau	27 June - 7 July 2006 (11 days)
Dr. Gemma Arnau	18 - 25 November 2006 (8 days)

VII- PUBLICATIONS

(Publications in journals & Presentations in conferences)

Note: Please list the publications, giving title of the article & name and date of the journal in which it was published. Also, list any papers presented in conferences. In both these cases, please mention only publications/presentations which have a bearing on the work carried out under the IFCPAR project.

1. Arnau., G. and Maledon, E. 2005. Genetic improvement of yam: what strategy and objectives? Poster presented to the 41st Annual Meeting of the Caribbean Society of Food Crops. July 10 - 16, 2005. Gossier, Guadeloupe, France.
2. Lebot, V., Ivancic, A. and Abraham, K. 2005. The geographical distribution of allelic diversity, a practical means of preserving and using minor root crop genetic resources. *Experimental Agriculture* 41(4): 475 - 489.
3. Abraham, K., Sreekumari, M. T. and Sheela, M. N. 2006. Seed production strategies and progeny selection in greater yam breeding. Paper presented in the 14th Triennial Symposium of the International Society for Tropical Tuber Crops, Trivandrum, India. 20 – 26 November 2006.
4. Arnau., G., Erick Malledon., Isabelle Bachand and Abraham, K. 2006. Production of interploid hybrids and molecular marker heterozygosity determination using microsatellite markers in the greater yam, *Dioscorea alata*: importance for the genetic improvement of greater yam. *Ibid.*
5. Sheela, M. N., Gemma Arnau., Abraham, K. and Sreekumari, M. T. 2006. Comparative analysis of allelic diversity of Indian greater yam. Poster presented in the 14th Triennial Symposium of the International Society for Tropical Tuber Crops, Trivandrum, India. 20 – 26 November 2006.
6. Arnau G *et al.* 2007. Disomic segregation of microsatellites in the yam *D. alata* 2n=40 (In prep).

VIII - ASSESSMENT

(Please give your own assessment of the way the project is going on. We would appreciate receiving your free and frank views. Please mention bottlenecks, if any, faced during the implementation of this project. You may also give any suggestions for improving the method of implementation, of this project in particular and all IFCPAR projects in general).

The project has been going on in a very good manner, with most of the work plans being accomplished. So far we have not yet faced any serious bottle necks. The combined work by the scientists especially during the international visits has given considerable practical knowledge to each other about the crop and its peculiarities. The facilitation of the international visits by the IFCPAR has been smooth and hassle free and they have strengthened the spirit of international cooperation in the context of this project. The synergy of the complementary technologies and combined work by CTCRI, India and CIRAD, France have resulted in two fundamental discoveries (production of higher polyploids by hybridization and the finding of basic chromosome number as 20 in greater yam) even by the short span of the half period of this project.

The overall support of the IFCPAR in this project has been excellent and encouraging.

The philosophy of Indo- French collaboration in research has proved to be excellent. Scientists of both the collaborative institutes have been exposed to new and different situations in the other country during the mutual visits and it has been a learning experience for improving the faculty. CTCRI in India and CIRAD in France have benefited much from each other, due to the collaboration in this project. The gain has been significantly greater than it would have been, if both the partners (scientists and institutes) continued to work alone and independently.

Signature



(Indian Principal Collaborator)

Signature



(French Principal Collaborator)