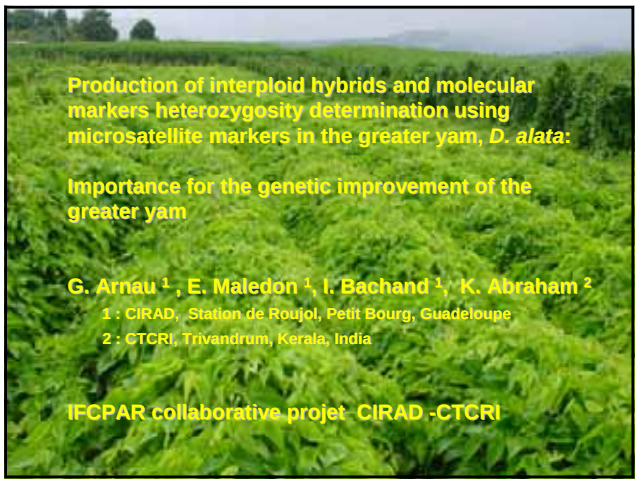
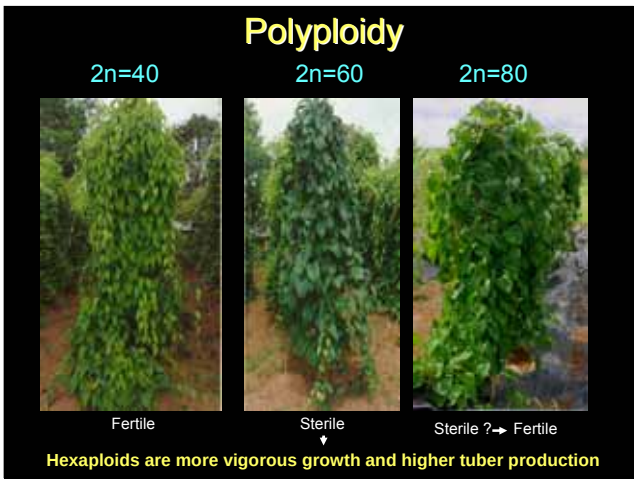
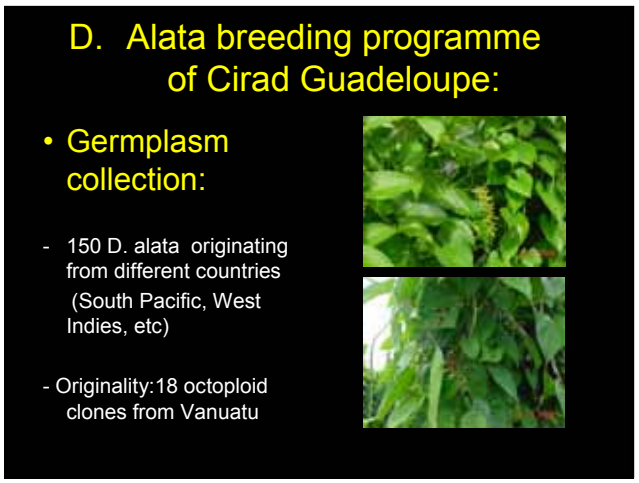
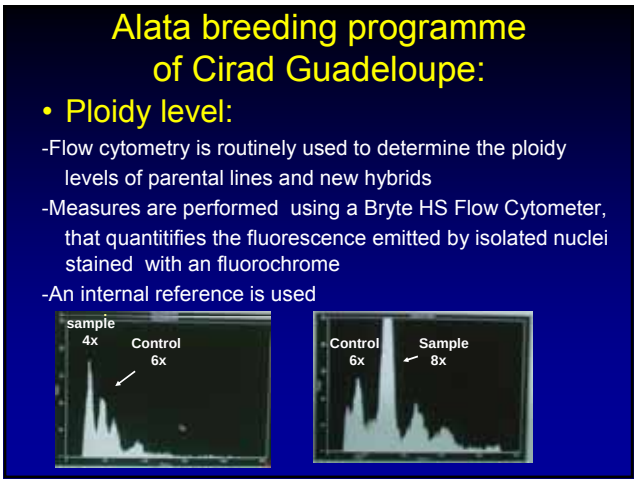

Production of interploid hybrids and molecular markers heterozygosity determination using microsatellite markers in the greater yam, *D. alata*:
Importance for the genetic improvement of the greater yam
G. Arnau [1] , E. Maledon [1], I. Bachand [1], K. Abraham [2]
1 : CIRAD, Station de Roujol, Petit Bourg, Guadeloupe
2 : CTCRI, Trivandrum, Kerala, India
IFCPAR collaborative projet CIRAD -CTCRI


Polyploidy
2n=40
2n=60
2n=80
Fertile
Sterile
Sterile ? Fertile
Hexaploids are more vigorous growth and higher tuber production


D. Alata breeding programme of Cirad Guadeloupe:
• Germplasm collection:
- 150 D. alata originating from different countries (South Pacific, West Indies, etc)
- Originality:18 octoploid clones from Vanuatu


Alata breeding programme of Cirad Guadeloupe:
• Ploidy level:
-Flow cytometry is routinely used to determine the ploidy levels of parental lines and new hybrids
-Measures are performed using a Bryte HS Flow Cytometer, that quantitifies the fluorescence emitted by isolated nuclei stained with an fluorochrome
-An internal reference is used
sample 4x
Control 6x
Control 6x
Sample 8x

D. Alata breeding programme of CIRAD Guadeloupe

- Diversity analysis:

- Allelic diversity among parental lines has been characterised using 15 microsatellites markers
- Primers are labelled with three different fluorocroms (HEX, FAM,TET) and PCR products are multiplexed
- Migration is carried out with an automatic sequencer ABI PRISM™ 3100 (Applied Biosystems)
- This method is very efficient because several microsatellites can be pooled and it is highly reliable

D. Alata breeding programme of CIRAD Guadeloupe

Allelic diversity analysis:

↓

Developed markers are routinely used for choosing distant genotypes for hybridisations to maximise heterozygosity in the progenies.

Microsatellite loci

G	L-2	L-3	L-13	.. ..
♀ 183 2n=80	294 306 313	122 136 138	227 229 231 233	
♂ 177 2n=40	296 306	136 157	231 236	

Cross female ♀ 183 X male ♂ 177

D. Alata breeding programme of Cirad Guadeloupe:

- Inheritance patterns of microsatellite markers were studied on two different progenies and results support the hypothesis that clones with 2n=40 chromosomes could be diploid and not tetraploid (Arnau et al, in prep)

- Microsatellite diversity analysis also suggests that ploidy levels could be different than originally presumed:

  4x, 6x, 8x ⟶ 2x, 3x, 4x

Number of alleles per locus

D.alata 2n=40

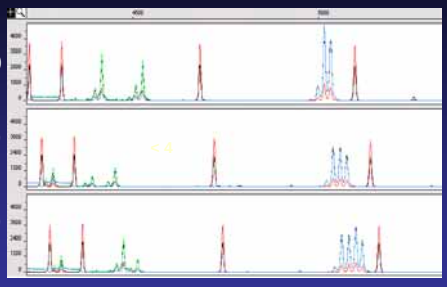
< 2

D.alata 2n=60

< 3

D.alata 2n=80

< 4



Research activities

- **Production of hexaploid hybrids by crossing a male octoploid with different female tetraploids**
  ♀ (2n=40) x ♂ (2n=80)
- **Molecular markers heterozygosity determination using microsatellite markers**

Production of hexaploid hybrids

- A total of 701 controlled hybridisations were carried out between four distinct female clones 4x (F5,F27,F53 and F74) and one male parent 8x (CTRT-148)
  ♀ (2n=40) x ♂ (2n=80)
- Fruit set was recorded three weeks after pollination
- The development of the seed was recorded

Plants flowering

Male 2n=80

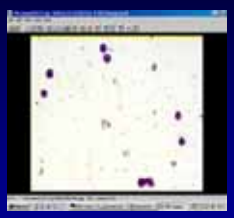
Femelle 2n=40

Pollen fertility

Fertility of clone 8x CTRT 148 was determinated by carmine stainability of pollen:

↓

It was found fertile to 55 %.



Pollinations: pencil pollination technique

Controlled pollinations

Rescue of immature embryos in vitro

- Almost all seeds were found to contain embryos but with an abnormal development of endosperm tissue
- A method for rescuing immature embryos was developed that could be used to obtain seedlings
- Fifty embryos per cross were rescued by vitro culture

RESULTS

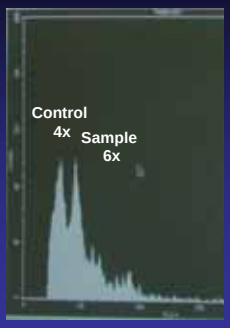
Female genotype	Flowers pollinated	Fruit set	Seeds set	Seedlings /100 seeds	
				Embryo rescue	Nursery
F5	96	56 %	33 %	30%	0%
F27	200	45%	35 %	24%	0%
F53	155	48%	38%	15%	0%
F74	250	53%	32%	27%	0%

- The fruit set in the various combinations ranged from 45 to 56 per cent and the seeds set from 33 to 38 %
- With the embryo rescue, the number of embryos which developed into seedling ranged from 15 to 30%
- non seedling were obtained from dry seeds

Embryo rescue

Control of the ploidy level

- Flow cytometry was used to confirm the hexaploid nature of the hybrids



CONCLUSION

- **Hexaploid hybrids were obtained for the first time by crossing a male 2n=80 with different female clones 2n=40**
- **This required the rescue of immature embryos in vitro**

PERSPESCTIVES

- **Production of hexaploid hybrids crossing female varieties (2n=80) x male (2n=40)**

Not need for embryo rescue?

- **Molecular marker heterozygosity determination using microsatellite markers**

Plant material:
A sample of 96 *D. alata* varieties with different ploidy levels and different gegraphic origins (69 tetraploid,11 hexaploid and 16 octoploid) were selected for this study

Genetic analysis:
Five markers microsatellites was used (Dab2D11, Da1F08, Da3G04, Dpr3E10, Dpr3B12)

Number of homozygote and heterozygote clones depending on the ploidy levels ?

Ploidy	Allelic status	Locis Dab2D11	Da1F08	Da3G04
2n=40	Homozigous Heterozygous	18 51	16 53	4 65
2n=60	Homozigous Heterozygous	0 11	0 11	0 11
2n=80	Homozigous Heterozygous	0 16	0 16	0 16

Number of homozygote and heterozygote clones depending on the ploidy levels ?

Ploidy	Allelic status	Locis Dpr3E10	Dpr3B12
2n=40	Homozigous Heterozygous	37 32	5 64
2n=60	Homozigous Heterozygous	1 10	0 11
2n=80	Homozigous Heterozygous	3 13	0 16

Heterozygote or homozygote state depends on the ploidy level?

	6x/4x	8x/4x
Dab2D11	S	S
Da1F08	S	S
Da3G04	NS	NS
Dpr3E10	S	S
Dpr3B12	NS	NS
Σ locus	S	S

Test X^2 at 5%; S: significatif; NS= non significatif

Number of allels per locus

Loci Da3G04

Ploidy	Average of allels
2n=40	1,7 ± 0,42
2n=60	2,8 ± 0,43

➔ Hexaploid varieties are more heterozygotic than tetraploid varieties and have a higher number of allels per locus which could explain their superior performance observed in the field

CONCLUSION

➔ Production of hexaploid by crossing distant genotypes with different ploidy levels (4x-8x) appears promising for the genetic improvement of the greater yam, making it possible to maximise heterozygosity and heterosis.

PERSPESCTIVES:

- Production of new 2n=80 clones, which will be able to use as parent in the hybridation programme:

1/by doubling chromosomal stock from 2n=40 elite varieties using traitement colchicine

2/ Selecting octoploids naturally created by screening progenies using flow cytometry

Production of new 2n=80 parental lines by doubling chromosomal stock from 2n=40 elite varieties using traitement colchicine

2n=40 heterozygote elite varieties (ab) ➤ 2n=80 varieties (aabb)

Interploid hybrids production:

- when crossing 2n=80 variety (aabb) X 2n= 40 variety (cd)

↓

66 % of ab hybrids

- If using very heterozygote 2n=80:
higher variability and biggest work

2n=80 varieties (abcd) X 2n= 40 varieties ef

↓

ab,ac, ad, bc, bd cd hybrids

Screening progenies by flow cytometry analysis and microsatellite markers

- Plant material:
  One progeny (fifty seedlings) obtained from crossing one male parent 4x 2n=40 (8M) and one female clone 4x 2n=40 (27F)
- Results :
  Two hexaploid and one octoploid hybrids were found

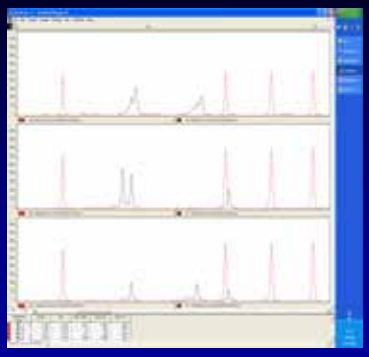
Microsatellite Loci Da2F10

Parent female
2n=40

Parent male
2n=40

Hybrid
2n=60

→ Production by 2n gametes



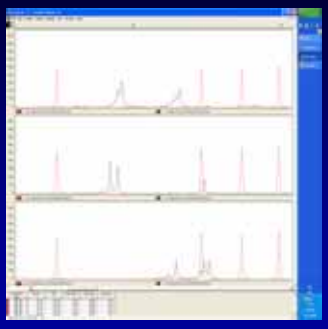
Microsatellite Loci Da2F10

Parent female
2n=40

Parent male
2n=40

Hybrid
2n=80

→ Production by doubling chromosomal or second Division restitution (SDR)



Thank you