First genetic diversity analysis in greater yam (*Dioscorea alata* L.) of a representative word germplasm using microsatellite markers

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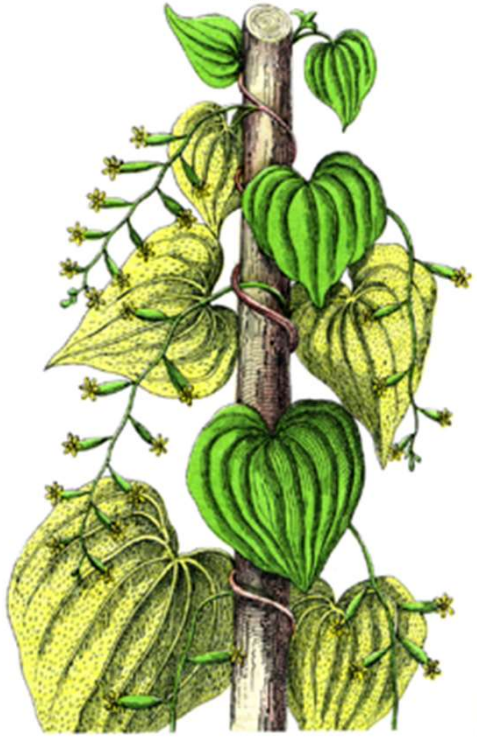
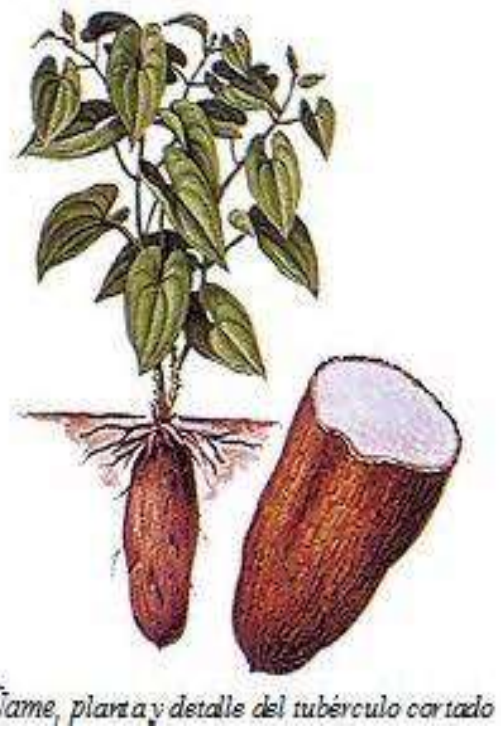




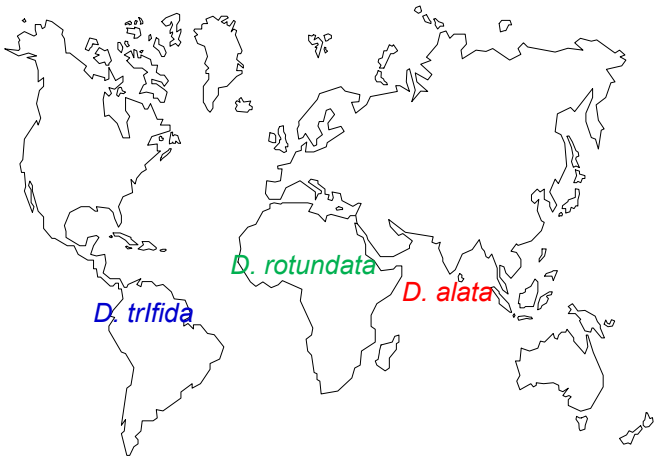
Flesh color

Tuber shape

Projects AIP-Bio-Resources INRA & FEDER Guadeloupe CARAMBA-VALEXBIOTROP

Introduction

- *D. alata* is one of major yam cultivated species
- It is supposed to be originated from Asia-Pacific
- but it's not known in its wild state
- studies on *D.alata* using different molecular markers (RAPD, AFLP, SSRs) involved a limited number of cultivars and none was conducted at the global scale
- the lack of knowledge on its origin and genetic diversity limits the efficacy of genetic improvement
- *D*. alata is a low fertile, dioecious and polyploid species (2n=40,60,80)



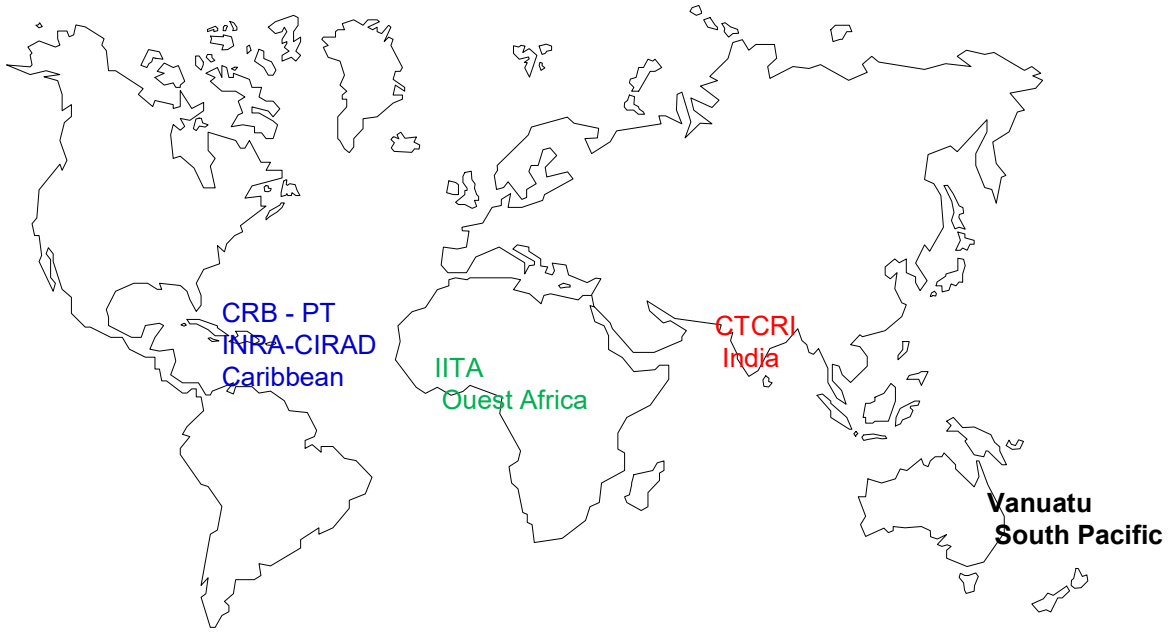
Objectifs

- Genotyping of four different collections of *D. alata (*384 accessions)

  ⇨ South Pacific
  ⇨ Asia
  ⇨ Africa
  ⇨ Caribbean

- Using a common set of 24 microsatellite markers

- Identification of
  Worldwide genetic diversity
  Worldwide structure
  D. alata evolutionary history
  Duplicates

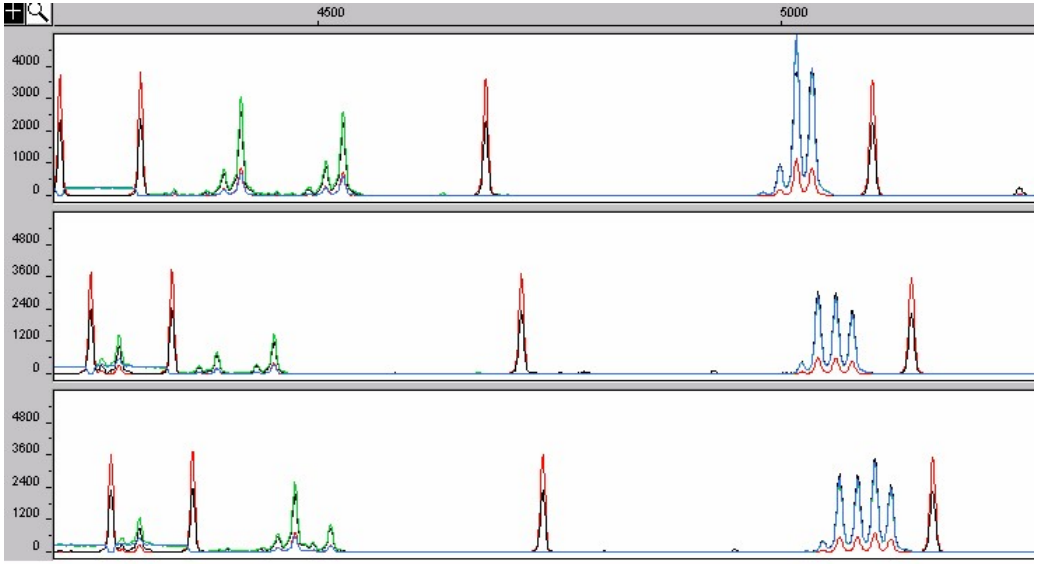


Methods

Genotyping

Multiplex panel of microsatellite markers labeled with fluorescent dyes (FAM, VIC, NED, PET).

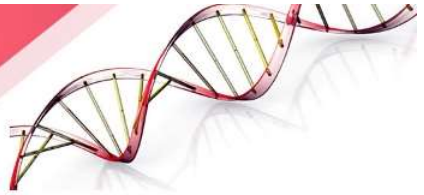
PCR products were visualized using capillary gel electrophoresis (ABI Prism 3100 DNA Sequencer)



Data analysis

- Genetic parameters estimated: n°of alleles per locus, n° of rare alleles, H0, He and Fis
- Distances between each pair of accessions were calculated with Dice dissimilarity coefficients, In order to compare accessions and their relationships,
- Diversity structuration was assessed via :1/PCoA with the full study sample 2/ Cluster analyses UPGMA with diploid cultivars 3/Bayesian approach implemented in STRUCTURE

Results



- **Table 1. Genetic diversity detected in D. alata using 24 microsatellite markers**

Origin [1]	SSR	EMBL[2]	Motif	Min.–max. size (bp)	Total alleles	Al < 1%[3]	Main allele frequency[4]	Ho[5]	He[6]	Fis[7]
D. A	Da3G04	AJ880369	(AC)12	282–306	9	1	0.80	0.83	0.86	0.03
D. A	Da1F08	AJ880368	(TG)13	161–185	9	3	0.88	0.32	0.39	0.19
D. A	Da2F10	[51]	(TG)14	108–151	14	2	0.46	0.65	0.67	0.04
D. A	Da1A01	AJ880381	(GT)8	201–222	7	1	0.85	0.52	0.51	-0.02
D. AB	Dab2D11	[51]	(TC)19	227–247	9	2	0.66	0.83	0.67	-0.25*
D. PR	Dpr3E10	[51]	(TCT)13(CTC)4	170–194	10	1	0.84	0.16	0.32	0.16*
D. PR	Dpr3B12	AJ880376	(TG)8	132–150	8	2	0.81	0.74	0.65	-0.15
D.J	DIJ034	AB201419	(AG)17	198–267	15	2	0.48	0.90	0.80	-0.14
D.J	DIJ443	AB201420	(AG)17	257–285	12	0	0.45	0.73	0.82	0.11*
D.J	DIJ0461	AB201423	(GA)16	120–144	8	3	0.64	0.78	0.71	-0.10
D.J	DIJ1045	AB201422	(TG)19	251–281	13	4	0.52	0.78	0.71	-0.10
D.A	mDaCIR2	FN677762	(CA)10	243–268	9	0	0.44	0.78	0.71	-0.10
D.A	mDaCIR11	FN677767	(AC)10	165–183	8	4	0.79	0.47	0.54	0.12*
D.A	mDaCIR13	FN677768	(GA)17	186–214	14	4	0.49	0.56	0.79	0.28*
D.A	mDaCIR17	FN677770	(AC)7	228–236	5	2	0.96	0.18	0.20	0.11*
D.A	mDaCIR20	FN677773	(GA)16	172–206	11	1	0.69	0.67	0.62	-0.08
D.A	mDaCIR26	FN677776	(TG)15(GA)14	171–219	12	2	0.59	0.60	0.74	0.18
D.A	mDaCIR57	FN677784	(TG)9	143–152	5	0	0.86	0.83	0.55	-0.28*
D.A	mDaCIR59	FN677786	(TC)11(CA)9	186–224	12	5	0.55	0.75	0.75	-0.01
D.A	mDaCIR60	FN677787	(CA)11	132–159	12	1	0.56	0.62	0.81	0.23
D.A	mDaCIR25	[38]	(AC)14	142–184	14	1	0.39	0.49	0.81	0.34*
D.A	mDaCIR61	FN677788	(AG)21	179–221	18	5	0.61	0.81	0.74	-0.09
D.A	mDaCIR63	[38]	(AG)12	155–180	8	1	0.68	0.53	0.65	0.18*
D.A	mDaCIR116	FN677800	(AG)8(AG)7	83–126	14	3	0.70	0.61	0.65	0.06*

[1] D. A, D. alata; D. AB, D. abyssinica; D. PR, D. praehensilis; D. J, D. japonica
[2] Registration number on EMBL database or publication reference
[3] Rare alleles with a frequency lower than 1%
[4] Highest frequency of an allele observed at this locus
[5] Observed heterozygosity.
[6] Expected heterozygosity
[7] Fixation index, *P <0.001

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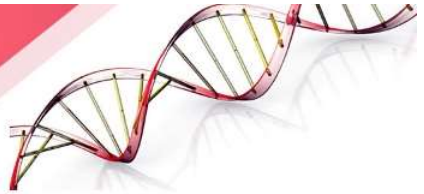
→ A mean genetic diversity (He)= 0.66 Indicating moderate to high levels of polymorphism in *D.alata*

Results



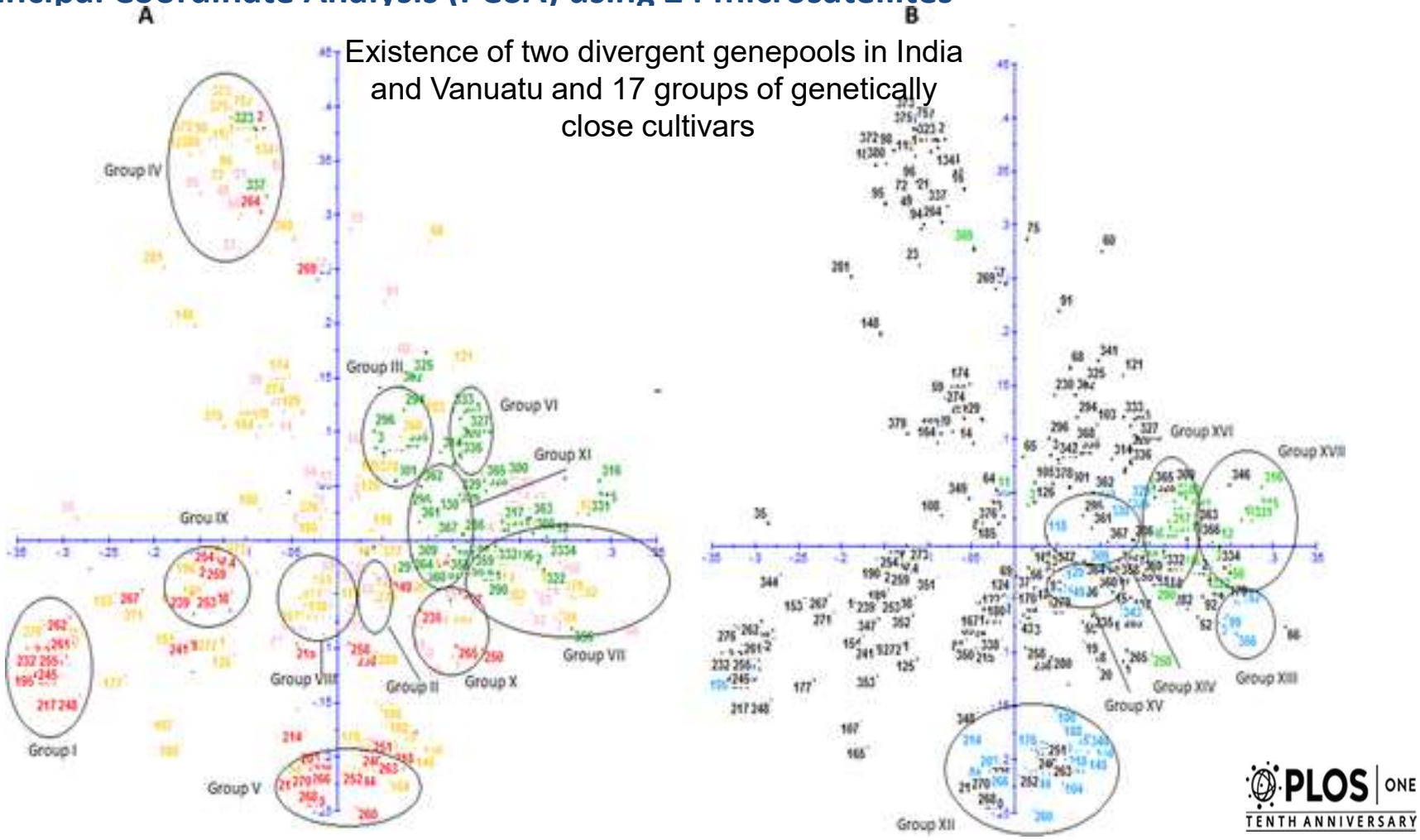
- **Table 2 Details on duplicates from each collection based on genotypic profile across 24 SSR markers.** The accessions grouped together presented identical allelic profiles at 24 SS loci.

Collection	Accession code	Geographic origin	Local name	Study code
CTCRI	Da322	[4]India	Unknown	212
CIRAD	VU579	[3]Vanuatu	Letslets Bokis	318
CIRAD	VU567	[3]Vanuatu	Letslets Bolos	323
CRB-PT	PT-IG-00040	[2]Puerto Rico	59_Vino white forme	151
CRB-PT	PT-IG-00052	[2]Puerto Rico	71_Smooth Statia	168
CRB-PT	PT-IG-00395	Unknown	452_Fafadro bis	173
IITA	TDa-1427	[1]Ghana	Alamun Gaga	54
IITA	TDa-1437	[1]Ghana	Adidianmawoba	66
CTCRI	Da40	[4]India	Elivalan	192
CTCRI	Da73	[4]India	Muramchari	223
CTCRI	Da28	[4]India	Kachil	194
CTCRI	Da39	[4]India	Poolakachil	253
CTCRI	Da143	[4]India	Gutu	233
CTCRI	Da78	[4]India	Kachil	247
CTCRI	Da95	[4]India	Kudakachil	234
CTCRI	Da22	[4]India	Chuvanna Maveran	199
CTCRI	Da100	[4]India	Parisakodan	220
CTCRI	Da70	[4]India	Thekkan Kachil	222
CTCRI	Da120	[4]India	Kaduvakkayyan	228
CTCRI	Da105	[4]India	Chenithakizhangu	261

Results

- **Fig 1A. Diagram showing the relationships among D. alata accessions based on Principal Coordinate Analysis (PCoA) using 24 microsatellites**

Existence of two divergent genepools in India and Vanuatu and 17 groups of genetically close cultivars



Clusters of diploids

Clusters of triploids and tetraploids

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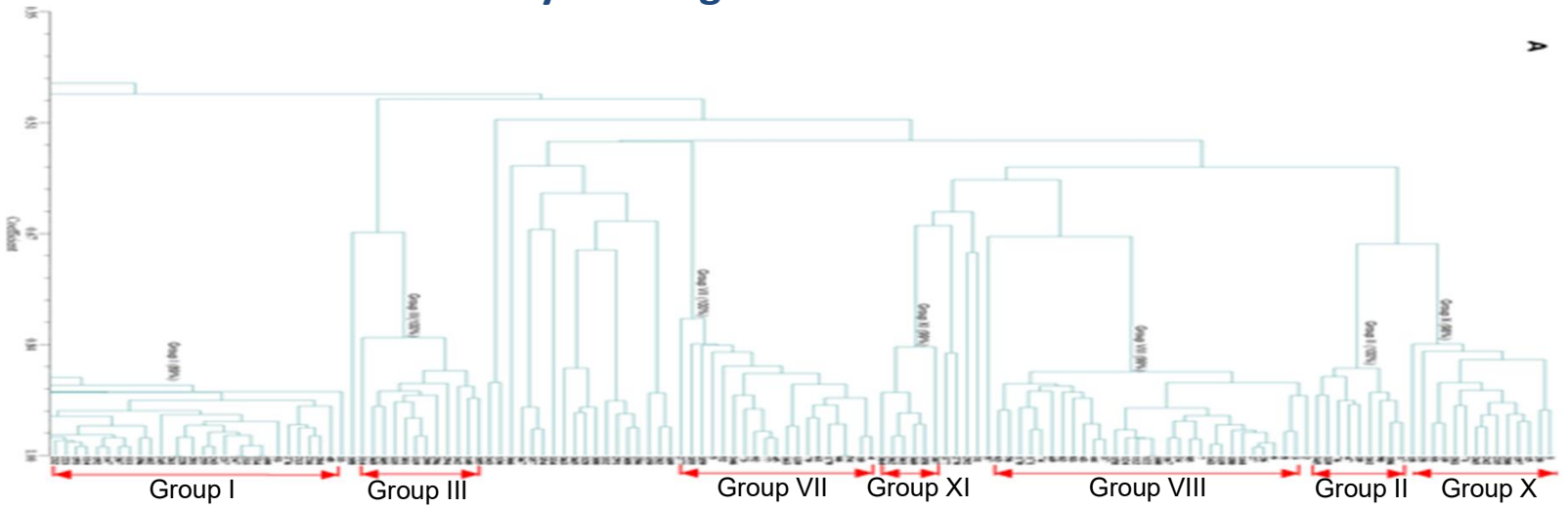
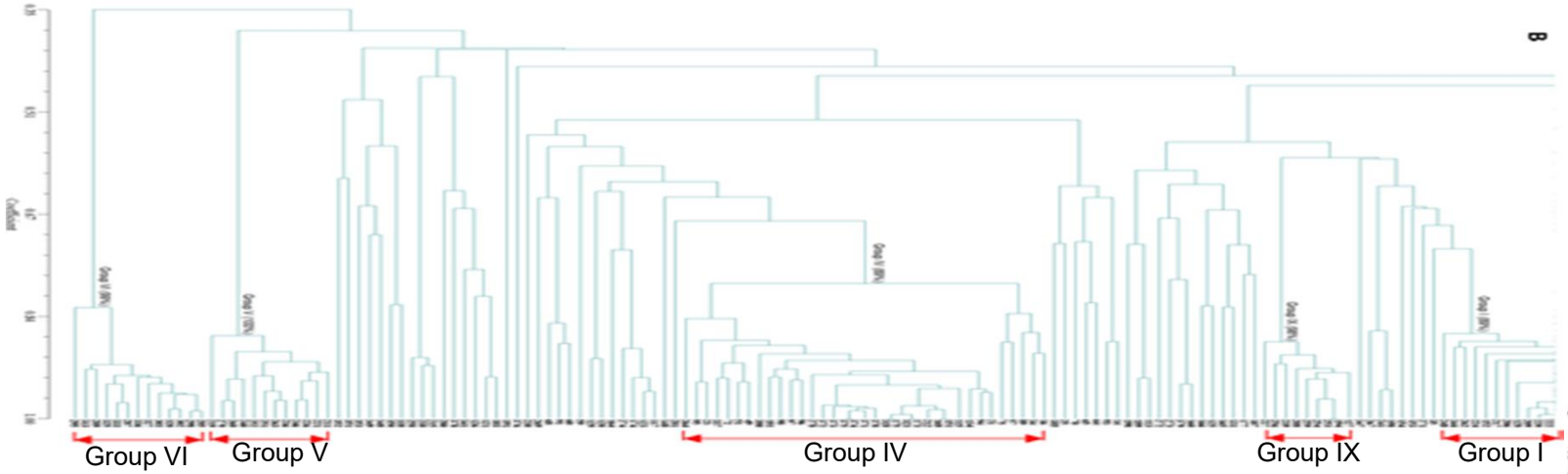
Results

- **Fig 2. Dendrogram showing the relationships among 284 diploid accessions of D. alata based on UPGMA analysis using 24 microsatellites**





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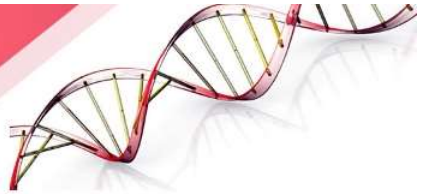


- **Table S1. Details of accessions with their accession code, geographical origin, local name, ploidy level and accession type and included in the study. Identified groups based on PcoA and UPGMA are also indicated**

Collection	Accession code	Geographic origin	Local name	Type of accessi	level ploid	Study code	Identified groups
IITA	TDa-1209	[1]Togo	Tsrokpa 784	C	2	16	IV
IITA	TDa-3207	[1]Togo	Tifiou Bh-32 1125	C	2	21	IV
IITA	TDa-3912	[1]Benin	Hawai	C	2	23	IV
IITA	TDa-1065	[1]Togo	Dodganon Tsrokp	C	2	49	IV
IITA	TDa-1190	[1]Benin	Be 114	C	2	51	IV
IITA	TDa-3226	[1]Ivory Coast	Sakata Koumonkc	C	2	57	IV
IITA	TDa-1196	[1]Ivory Coast	Ic 28	C	2	75	IV
IITA	TDa-1387	[1]Nigeria	Obuneyi	C	2	89	IV
IITA	TDa-3902	[1]Benin	Boniyouro	C	2	94	IV
CRB-PT	PT-IG-00036	[2]Martinique	54_St Vincent bla	C	2	72	IV
CRB-PT	PT-IG-00095	[2]Puerto Rico	115_Sea 144	C	2	96	IV
CRB-PT	PT-IG-00065	[5]French Guyana	84_DA 28	C	2	98	IV
CRB-PT	PT-IG-00016	[2]St. Lucia	34_St Vincent Yan	C	2	115	IV
CRB-PT	PT-IG-00374	[2]Haïti	429_Ti Joseph	C	2	134	IV
CRB-PT	PT-IG-00040	[2]Puerto Rico	59_Vino white fo	C	2	151	IV
CRB-PT	PT-IG-00052	[2]Puerto Rico	71_Smooth Statia	C	2	168	IV
CRB-PT	PT-IG-00043	[2]Haïti	62_Bacala 2	C	2	175	IV
CRB-PT	PT-IG-00024	[2]Martinique	42_St Vincent bla	C	2	181	IV
CRB-PT	PT-IG-00556	[2]Guadeloupe	620_St Vincent m	C	2	278	IV
CRB-PT	PT-IG-00045	[2]Martinique	64_St Vincent Vio	C	2	370	IV
CRB-PT	PT-IG-00050	[2]Cuba	69_Cuba 1	C	2	371	IV
CRB-PT	PT-IG-00051	[2]Cuba	70_Morado	C	2	372	IV
CRB-PT	PT-IG-00060	[2]Guadeloupe	79_Grand Etang	C	2	373	IV
CRB-PT	PT-IG-00067	[3]New Caledonia	86_Wénéféla bis	C	2	374	IV
CRB-PT	PT-IG-00073	[2]Puerto Rico	92_Purple Lisbon	C	2	375	IV
CRB-PT	PT-IG-00333	[2]Guadeloupe	386_Sainte Cathe	C	2	380	IV
CTCRI	Da322	[4]India	Unknown	C	2	212	IV
CIRAD	VU579	[3]Vanuatu	Letslets Boki	C	2	318	IV
CIRAD	VU567	[3]Vanuatu	Letslets Bolos	C	2	323	IV
CIRAD	VU678	[3]Vanuatu	Nowanao	C	2	337	IV

[1]Africa, [2]Caribbean, [3]South Pacific, [4]Asia, [5]South America

Discussion :

- Results revealed the existence of a significant structuring associated with geographic origin, ploidy levels and morpho-agronomic characteristics

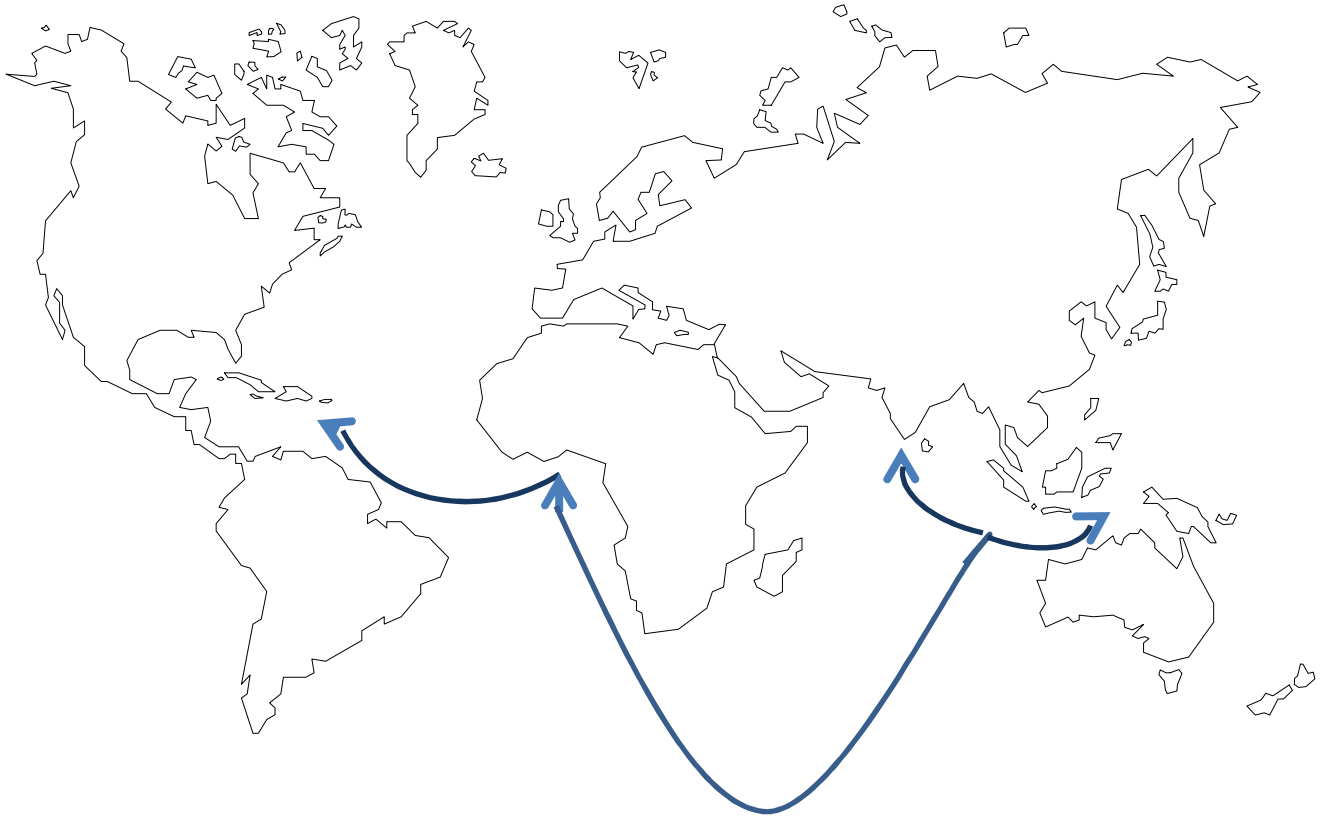
- The clear genetic differentiation revealed between cultivars from India and Vanuatu is consistent with the existence of different secondary diversification centers in Asia and the South Pacific

- Results showed also that cultivars from Africa and New World are very similar to Asian and /or South Pacific cultivars

- Data obtained will be useful for selecting genetically distant parent to maximize allelic diversity and heterosis in breedings programs and improving germplasm conservation methods

Discussion :

Domestication of *D. alata*
about 6000 years
in Southeast Asia → Dispersion → India
Diversificaton → South Pacific (Vanuatu)
→ XV^{eme} Introduction in Africa → XVI^{eme} Introduction in New world



Perspectives

- **Projects CRP-NextGen and FEDER-CAVALBIO**

- CRP-NextGen: Much larger evaluations of *D. alata* genetic resources existing in Asia and South Pacific by Genotyping by sequencing (> 600 accessions)

- FEDER-Guadeloupe CAVALBIO: Selection by Genotyping by sequencing (GBS) of a panel of 200 SNP markers, well distributed on 20 chromosomes useful for:

  - Genetic and diversity analysis (Kaspar technology)
  - Collection management