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Molecular diversity in pineapple assessed by RFLP markers

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Abstract Pineapple, *Ananas comosus* (L.) Merr, is the third most important tropical fruit cultivated in all tropical and subtropical countries. Pineapple germplasm includes all seven species of the genus *Ananas* and the unique species of the related genus *Pseudananas*. A knowledge of its diversity structure is needed to develop new breeding programs. Restriction fragment length polymorphism (RFLP) was used to study molecular diversity in a set of 301 accessions, most of which were recently collected. This sample was analysed using 18 homologous genomic probes. Dissimilarities were calculated by a Dice index and submitted to Factorial Analysis. The same data were represented as a diversity tree constructed with the score method. *Pseudananas sagenarius* displayed a high polymorphism and shares 58.7% of its bands with *Ananas*. Within *Ananas*, variation appears continuous and was found mostly at the intraspecific level, particularly in the wild species *Ananas ananassoides* and *Ananas parguazensis*. As for the cultivated species, *Ananas comosus* appears relatively homogeneous despite its wide morphological variation and *Ananas bracteatus*, which is grown as a fence and for fruit, appears still much less variable. By contrast *Ananas lucidus*, cultivated by the Amerindians for fiber, displays a high polymorphism. This tree displayed a loose assemblage of numerous clusters separated by short distances. Most species were scattered in various clusters, a few of these being monospecific. Some accessions which had not been classified, as they shared morphological traits typical of different species, re-group with one or the other, and sometimes with both species in mixed clusters. No reproductive barrier exists in this germplasm and these data indicate the existence of gene flow, enhancing the role of effective sexual reproduction in a species with largely predominant vegetative mutiplication.

Keywords Pineapple · *Ananas* · *Pseudananas* · Genetic diversity · RFLP markers

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Introduction

Pineapple, *Ananas comosus* (L.) Merr., is the bromeliad with the highest economic importance. It is cultivated in all tropical and subtropical countries and ranks third in production among tropical fruits. It was domesticated long before its first historic mention by Christophorus Columbus in 1493 (Morrison 1973). Pineapple genetic resources are generally considered to include the bromeliads whose flowers and fruits are fused in a syncarp (Coppens d'Eeckenbrugge et al. 1997). According to the last classification by Smith and Downs (1979), which was amended by Leal (1990), this corresponds to the genera *Pseudananas* and *Ananas,* both native to South America. The former is monospecific. *Pseudananas sagenarius* (*Arruda da Câmara*) *Camargo* has been used by the Amerindians as a fiber source, but grows generally wild. The latter includes seven species. In addition to *Ananas comosus*, *Ananas bracteatus* (Lindley) Schultes is cultivated as a fence or for fruit and *Ananas lucidus* Miller for fiber, although all three species can be found in a wild state. The wild species are *Ananas ananassoides* (Baker) L.B. Smith, *Ananas nanus* (L.B. Smith), which was previously considered as a dwarf variety of the former (Smith 1961), *Ananas parguazensis* Camargo & L.B. Smith., and *Ananas fritzmuelleri* Camargo.

The placement of these taxa at the specific or generic level has been seriously questioned (Loison-Cabot 1992; Duval et al. 1998). Indeed the current taxonomic key is mainly based on traits that depend on single genes or vary greatly with the environment. Numerous accessions collected recently could not be classified because they

combine typical traits of distinct species according to the botanical classification. Neither discontinuous morphological variation nor reproductive barriers exist in the genus *Ananas*, which would justify the regrouping of all the current *Ananas* species into a single species (Leal et al. 1998).

Isozymic polymorphism has been studied in pineapple to clarify classification and for phylogenetic analysis (García 1988; De Wald et al. 1992; Aradhya et al. 1994). Up to 37 systems have been studied but only nine could be used and these revealed 31 alleles at ten loci. Obviously, this is a limited number of markers in a genus possessing many chromosomes (x=25) (Collins and Kerns 1931).

RFLPs are potentially more numerous and efficient in revealing polymorphism. In sorghum, RFLP markers proved to be efficient for the racial classification of cultivated landraces (Deu et al. 1994; de Oliveira 1996). In the genus *Musa,* nuclear RFLPs demonstrate strong relation between A-genomes in wild clones and diploid and triploid cultivars (Jenny et al. 1999). In pineapple, a first attempt conducted by Noyer (1991); with five heterologous and nine homologous RFLP probes on sets of respectively 21 and 14 *Ananas* accessions, displayed poor variability with one polymorphic marker out of five in the first set and two out of nine in the second set. Polymorphism was higher in *A. ananassoides*. A further study conducted with the polymorphic DNA ribosome marker on DNA restricted by eight endonucleases separated 92 accessions into six clusters which do not match up to species, with the exception of *A. bracteatus* (Noyer et al. 1997).

In the present work, molecular markers were developed and used for the genetic-diversity analysis of the genera *Ananas* and *Pseudananas*. The results are discussed in relation to the level of differentiation observed.

Materials and methods

Plant material

Three hundred and one accessions belonging to the seven mentioned species of the genus *Ananas* (also including some unclassified accessions) and to the unique species of the genus *Pseudananas* were used for DNA analysis. Most of these were recently collected in Brazil, French Guiana, Venezuela and Paraguay, and maintained *ex-situ* in the field collections of the French Cirad-Flhor or the Brazilian Embrapa-Mandioca e Fruticultura. The other accessions came from the French collection, including accessions collected by Baker and Collins (1939) and obtained through exchanges with the USDA National Clonal Germplasm Repository in Hawaii. The sample composition and origin of the material are detailed in Table 1.

Probe development

Total pineapple DNA from accession CO42 (*A. comosus* cv Manzana) was digested with the endonuclease *Pst*1, ligated into plasmid pUC 18 and transformed into XL1 Blue bacteria. Five hundred colonies were transformed with plasmids containing pineapple DNA inserts. Potential probes were multiplied using bacterial cultures, extracted using a Miniprep Boehringer kit and tested along with mechanically broken pineapple DNA labelled with d(CTP)-^{32}P. A subset of 60 inserts, assumed to be low copy number sequences, was selected, labelled and tested on a subsample of the plant material restricted with nine endonucleases. Twenty five inserts revealed polymorphism.

RFLP

As each accession is a clone, DNA was extracted from young leaves of only one plant following Gawell and Jarret (1991). Pineapple DNA probes were labelled with d(CTP)-^{32}P applying the random primer method (Feinberg and Vogelstein 1983). RFLP procedures were performed as described in Noyer et al. (1997). The 25 selected probes were hybridized with DNA digested with four 6-base restriction enzymes: *Eco*RV, *Sst*1, *Hind*III and *Eco*RI.

Data analysis

Only some RFLP markers revealed a single locus, the others displayed numerous bands that could not be attributed to a particular locus. Consequently, for each accession, bands were scored only for presence (1) or absence (0). The objective for the analysis of these data was to describe the organisation of this set of accessions in terms of genetic diversity. A similarity measure is calculated between each pair of accessions to describe at best their genetic proximity. For co-dominant markers such as RFLPs, a shared band absence is not significant. Through indices based on presence/absence (0/1), only those based on shared presences are pertinent. Among them, the Dice indice was reported to give a good approximation of the genetic dissimilarity which could be calculated when scoring alleles (Perrier et al. 1999).

For two accessions, i and j, $d_{i-j}=2\ n_{11}/(2\ n_{11}+n_{10}+n_{01})$, where n_{11} is the number of bands present in both accessions i and j, with n_{10} and n_{01} the number of bands present for one accession and absent for the other.

First, a factorial analysis was carried out on the Dice dissimilarity matrix. A projection of the global struture was constructed on the first factorial plane. This representation gives a good overview of the general organization of the set, but is much less adequate to represent its lower levels. Consequently, the same matrix was used to construct a diversity tree with the scoring method of (Sattath and Tversky 1977), which can be viewed as an ordinate and thus is a more robust version of the frequently used Neighbor Joining method (Saitou and Nei 1987). This representation is more pertinent to study the lower levels of organization, although the global structure representation may be less accurate and is a good complement to the factorial analysis. Both analyses were carried out with the DARWIN 3.5 software (Perrier et al. 1999).

Results

Levels of polymorphism

Five out of the 25 selected polymorphic probes showed complex band patterns. Two did not hybridize with the DNA of some species. All seven probes were therefore discarded to avoid bias. The 18 selected probes revealed a variable number of bands (from 3 for G 421B to 16 for G 104) with a total of 143 bands for the whole set, i.e. an average of 7.9 bands per probe (Table 2). The polymorphism was important. A total of 135 (94.4%) variable band levels were recorded with 116 (out of 126, i.e. 92.1%) for *Ananas* and 75 (out of 101, i.e. 74.3%) for *Pseudananas*. *P. sagenarius* displays an average number

Table 1 Material studied

Genus	Species	Origin		Number of accessions
Ananas	*comosus*	Caribbean		4
		French Guiana		27
		Guyana		3
		Brazil	Amapá	19
			Rio Negro	33
			Rio Solimões	23
			Acre	13
			Rondônia	1
			Mato Grosso	4
			São Paulo	1
			Parana	2
			Rio Grande do Sul	1
		Venezuela		15
		Colombia		3
		Peru		5
		Bolivia		1
		Paraguay		3
		Others (Germplasm collections)		10
Total				168
Ananas	*bracteatus*	Brazil	Parana	1
			Santa Catarina	1
			Rio Grande do Sul	3
		Paraguay		7
		Others (Germplasm collections)		6
Total				18
Ananas	*lucidus*	Brazil	Amapá	1
			Rio Negro	3
		Venezuela		1
		Others (Germplasm collections)		2
Total				7
Ananas	*Intermediate*	French Guiana		11
		Guyana		1
		Brazil	Amapá	8
			Para	3
			Parana	2
		Peru		1
Total				26
Ananas	*parguazensis*	Brazil	Para	3
			Rio Negro	9
		Venezuela		7
Total				19
Ananas	*ananassoides*	French Guiana		17
		Brazil	Amapá	8
			Rio Negro	3
			Acre	1
			Mato Grosso	15
			Minas Gerais	2
		Venezuela		4
		Others (Germplasm collections)		4
Total				54
Ananas	*nanus*	Others (Germplasm collections)		1
Ananas	*fritzmuelleri*	Others (Germplasm collections)		1
Pseudananas	*sagenarius*	Brazil	Bahia	2
			Mato Grosso do Sul	2
			Parana	1
			Rio Grande do Sul	1
		Others (Germplasm collections)		1
Total				7
Total number of accessions				301

Table 2 Polymorphism of 18 RFLP probes within the genera *Ananas* (7 species, 294 accessions) and *Pseudananas* (1 species, 7 accessions). A: total number of bands; B: number of variable bands; C: mean number of bands per accession. Polymorphism is estimated as the percentage of variable bands for the whole set and for each of the genera

Probe	Whole set		*Ananas*			*P. sagenarius*			Bands shared between
	A	B	A	B	C	A	B	C	*Ananas* and *Pseudananas*
N 4	5	4	3	3	1.6	4	4	2.1	2
N 5	4	4	4	4	2.1	4	2	2.7	4
G 5B	10	10	9	9	1.4	8	7	2.6	7
G 19	8	8	8	8	2.3	5	4	2.4	5
G 22	5	5	5	5	1.2	2	1	1.1	2
G 26	10	10	7	7	2.1	7	7	3.3	4
G 104	16	16	15	15	2.4	8	7	3.3	7
G 124	8	8	8	8	2.5	4	3	2.9	4
G 126	12	12	12	11	4.3	9	6	7.5	9
G 135B	9	9	6	6	2.4	7	5	4.1	4
G 135H	4	4	4	4	1.1	3	3	2.0	3
G 199B	4	4	3	3	1.3	4	3	1.4	3
G 199 H	12	12	10	10	2.6	9	9	4.1	7
G 201	10	10	9	9	1.6	7	7	2.3	6
G 202	6	6	6	6	1.6	2	1	1.9	2
G 259	12	8	9	3	7.0	12	6	7.3	9
G 421B	3	2	3	2	2.0	2	0	2.0	2
G 438H	5	3	5	3	3.9	4	0	4.0	4
Mean	7.9	7.5	7.0	6.4	2.4	5.6	4.2	3.2	4.7
Total	143	135	126	116		101	75		84
Polymorphism	94.4%		92.1%			74.3%			

of 3.2 bands per accession, higher than *Ananas* (2.4), which implies a range consistently from 2.3 to 2.6 depending on the species (Table 3). Within *Ananas*, the polymorphism level varies widely from 28.3% for *A. bracteatus* to 89.3% for *A. ananassoides*.

Genetic diversity

Factorial analysis of Dice dissimilarities was performed on the 134 band levels considered as single variables for the 301 accessions. The two first factors account respectively for 14.4% and 7.9% of the total variation. The principal plan (Fig. 1) displays a continuous dispersion of the whole set. In the genus *Ananas*, species are not clearly separated. *A. ananassoides* is quite as dispersed along factor 1 as the whole genus, and includes the unique *A. nanus*. The main cultivated species, *A. comosus,* mainly regroups in the right part of the plan, opposite to *A. nanus*. Intermediate clones are dispersed along factor 1. *A. parguazensis* and *A. lucidus* are widely scattered along factor 2 in the central part of the figure. The 18 accessions of *A. bracteatus* are also dispersed along factor 2, forming a more distinct group. Even well-grouped species largely overlap with their neighbors. *P. sagenarius* accessions are widely distributed considering the small number of accessions studied (seven). Nevertheless, they form a group in the lower part of the figure, clearly but slightly separated from *Ananas, A. fritzmuelleri* being the tangent point between them and their nearest neighbor *A. bracteatus*. Both species share rare bands with *Pseudananas*.

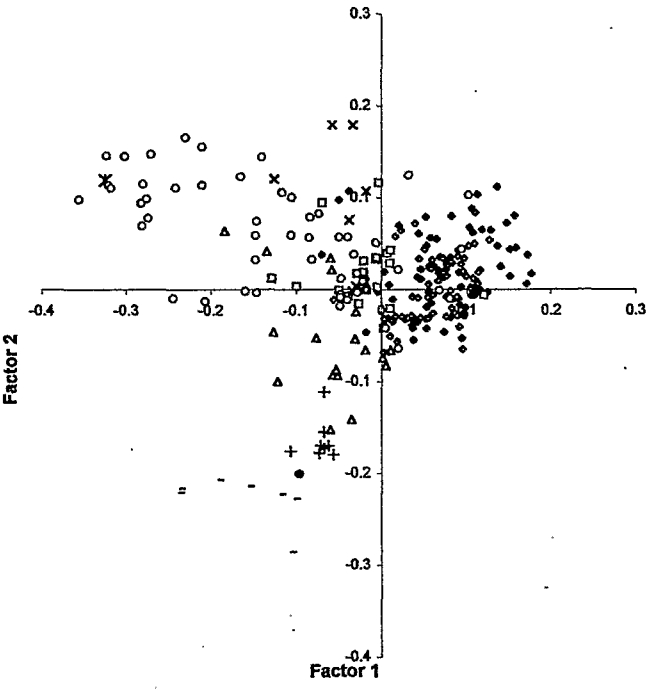

Fig. 1 Representation of the 1–2 plane of the Factorial Analysis (representing 22.3% of the overall variability) performed on 301 clones of pineapple germplasm. ◇ *A. comosus*, □ Intermediate, Δ *A. parguazensis*, × *A. lucidus*, ✳ *A. nanus*, ○ *A. ananassoides*, + *A. bracteatus*, ● *A. fritzmuelleri*, – *Pseudananas*

Table 3 Polymorphism of 18 RFLP probes within the seven species of the genus *Ananas*. A: total number of bands; B: number of variable bands; C: mean number of bands per accession. Polymorphism is estimated for each species as the percentage of variable bands

Probe	Total band number	*A. comosus* 167 acc.			*A. bracteatus* 18 acc.			*A. lucidus* 7 acc.			Intermediate 28 acc.			*A. parguazensis* 19 acc			*A. ananassoides* 57 acc.			*A. nanus* 1 acc.	*A. fritzmuelleri* 1 acc.
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	A
N 4	3	3	3	1.7	1	1	1.0	2	2	1.4	3	3	1.9	2	2	1.4	3	3	1.5	1	1
N 5	4	3	1	2.0	3	1	2.7	3	1	2.3	3	1	2.2	3	2	2.3	4	3	2.2	2	2
G 58	9	5	4	1.3	2	1	1.7	4	3	1.4	5	5	1.6	8	8	1.6	8	8	1.6	1	1
G 19	8	4	3	2.2	3	1	2.1	5	4	2.1	3	2	1.9	6	6	2.3	8	8	2.7	4	2
G 22	5	3	3	1.1	1	0	1.0	2	1	1.4	4	4	1.4	3	3	1.2	5	5	1.4	2	1
G 26	7	3	2	2.1	3	1	2.1	3	2	1.9	4	3	2.1	5	4	2.3	6	6	2.0	2	2
G 104	15	9	9	2.5	2	0	2 0	8	8	3.3	11	11	3.6	8	8	2.4	14	14	2.9	2	3
G 124	8	5	4	2.6	2	0	2.0	5	5	2.6	5	4	2.5	5	4	2.2	6	6	2.4	3	2
G 126	12	10	9	3.8	8	3	6.7	7	4	4.0	11	10	4.4	12	10	5.6	12	11	4.3	5	7
G 135B	6	3	1	2.2	3	1	2.8	4	2	2.6	4	4	2.9	4	4	2.3	4	4	2.4	2	3
G 135H	4	1	0	1.0	2	1	1.8	1	0	1.0	1	0	1.0	4	4	1.5	2	1	1.0	1	1
G 199B	3	2	1	1.2	2	1	1.9	2	1	1.3	2	1	1.5	3	3	1.2	2	2	1.0	1	1
G 199H	10	8	8	2.6	3	0	3.0	4	2	2.6	5	5	2.5	6	6	2.2	8	8	2.6	3	2
G 201	9	5	5	1.5	3	3	1.8	4	4	1.4	4	4	3.0	7	7	1.4	8	8	1.8	1	2
G 202	6	4	4	1.6	2	1	1.1	3	3	1.9	4	4	1.8	5	5	1.5	6	6	1.7	2	2
G 259	9	7	0	7.0	7	0	7.0	7	3	7.0	7	0	7.0	9	3	7.7	8	2	7.0	7	7
G 421B	3	3	2	2.0	2	0	2.0	3	2	2.0	3	2	1.0	3	2	2.0	3	2	2.0	2	2
G 438H	5	5	3	4.0	4	0	4.0	5	3	3.4	5	3	4.0	4	0	4.0	5	3	3.6	3	4
Mean	7.0	4.6	3.4	2.3	2.9	0.8	2.6	4.0	2.8	2.4	4.7	3.7	2.6	5.4	4.5	2.5	6.2	5.6	2.4		
Total	126	83	62		53	15		72	50		84	66		97	81		112	100			
Polymorphism		74.7%			28.3%			69.4%			78.6%			83.5%			89.3%				

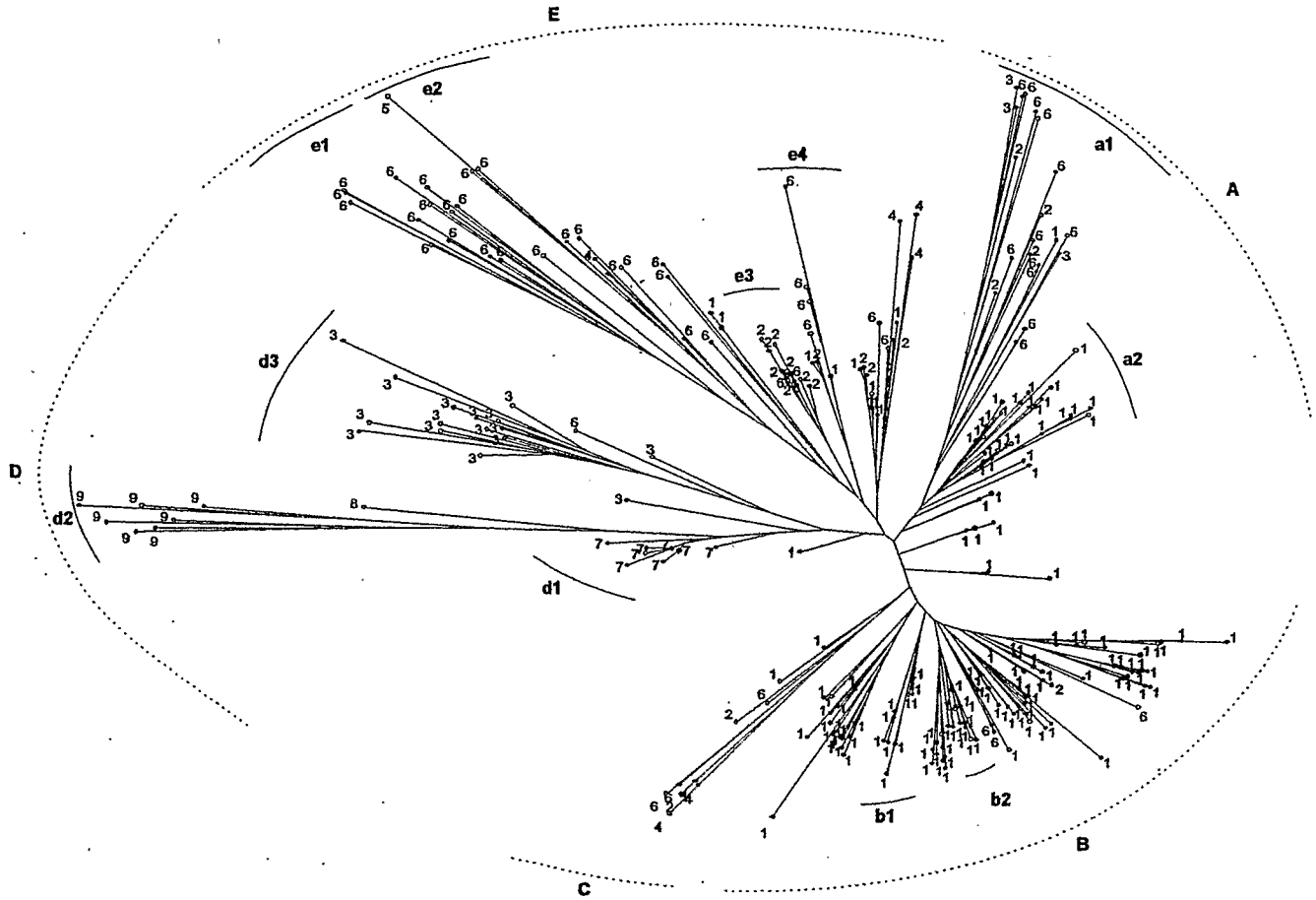

Fig. 2 Diversity tree constructed by Add-Tree method with Dice dissimilarity indices. 1: *A. comosus*, 2: Intermediate, 3: *A. parguazensis*, 4: *A. lucidus*, 5: *A. nanus*, 6: *A. ananassoides*, 7: *A. bracteatus*, 8: *A. fritzmuelleri*, 9: *P. sagenarius*

The genetic diversity tree (Fig. 2) shows a loose assemblage with continuous variation. The majority of *A. comosus* accessions cluster with a few *A. ananassoides* and intermediates in the B region, with short internode distances. Clones of widespread traditional cultivars such as 'Smooth Cayenne' (b1) and 'Pérola' (b2), re-group well. Other sub-clusters or groups are geographically consistent. The closest neighbor is a small cluster (C) re-grouping three *A. lucidus* and two *A. ananassoides* species cultivated for fiber. The other accessions of *A. comosus*, including the cultivar 'Queen', re-group in cluster a2. They divide the A region with a larger cluster (a1) mixing *A. ananassoides, A. parguazensis*, and intermediates collected in the Eastern Guianas (French Guiana and Amapá). Other accessions form a succession of small heterogeneous clusters in the E region, some re-grouping accessions of the same geographical origin (e2, e3, e4). Only sub-cluster e1 is homogeneous and re-groups accessions of *A. ananassoides* from the Brazilian state of Mato Grosso. The more distant and isolated node separates *Pseudananas* (d2) from the unique *A. fritzmuelleri, A. bracteatus* (d1), and a group of *A. parguazensis* from Rio Negro and Venezuela (d3) within the D branch. With the exception of sub-cluster e3, all groups including wild species are separated by significantly longer internodes than the cultivated species *A. comosus* and *A. bracteatus*. However clones of *A. lucidus*, scattered in the regions C and E, are also separated from each other and from other accessions by longer internodes.

Discussion

In our study, 25/60 (41%) of the probes reveal polymorphism (143 variable bands). This is a good level compared to the results obtained with enzymatic markers in *Ananas*, i.e. eight systems (31 alleles) out of 24 tested by García (1988) and two (six alleles) out of 37 by De Wald et al. (1992). Similar differences in the polymorphism revealed by isozymes and RFLP markers were obtained on maize populations by Dubreuil and Charcosset (1998).

Despite the small number of *P. sagenarius* accessions studied (seven), this species displays a high polymorphism with 101 band levels present out of the 143 found in the whole set of 301 accessions. The average number of bands per accession is also higher (3.2) than in *Ananas* (2.4). These results are consistent with the higher ploidy level (4*x* vs 2*x*). Collins (1960) suggested that *P. sagenarius* represents an amphiploid form from an intergeneric hybrid involving species from *Ananas* and *Brome-*

lia. However, our data do not support this hypothesis. Data (not shown) obtained from the hybridization of the same probes with DNA from five *Bromelia* accessions could not be considered in this analysis as the banding patterns were too different. Although Factorial analysis neatly separates *Pseudananas* clones from *Ananas,* the two genera share 58.7% of bands. In comparison, *A. ananassoides,* which displays a higher polymorphism (89.3%) has 72.5% of common bands with *A. comosus*, and 59% with *A. bracteatus*. This, and the fact that Collins (1960) reports that hybridization between *Pseudananas* and *A. comosus* produces 5 to 10% of predominantly tetraploid fertile hybrids from unreduced gametes, suggest that *P.sagenarius* is more likely to be a tetraploid species that should be classified in the genus *Ananas*.

Although some species appear well-grouped and positioned by Factorial Analysis, no species partition could be observed within *Ananas*. This is confirmed by the species dispersion in the diversity tree. These results agree with the low level (14%) of interspecific differentiation in the genus *Ananas* observed by Aradhya et al (1994) with data from the analysis of seven enzymatic loci. Polymorphism with regards to this probe-sample varies depending on the species. The results are to be considered cautiously with regard to the unbalanced accession numbers for each taxon. Nevertheless, the wild species *A. ananassoides* (100 bands/56 accessions) and *A. parguazensis* (81/19) display a clearly higher variability as compared to the cultivated species *A. comosus* (62/167) and *A. bracteatus* (15/18), which confirms the results obtained by Noyer (1991). Such a difference in polymorphism level between cultivated plants and their wild relatives has been commonly observed in other species but is more remarkable in a plant with largely predominant vegetative propagation, even in wild species. This could be explained by a wide geographical distribution in *A.ananassoides* but not in *A. parguazensis* which has only been collected in the northern part of the Amazon and the Orinoco. It supports the hypothesis of a more important role of sexual reproduction in the wild even if it has only rarely been observed (Duval et al. 1997). Moreover, a geographical component of the variation appears clearly within these wild accessions. In some cases, accessions appear more closely related to germplasm from different species collected in the same location than to conspecific material from other regions, which strongly suggests gene flow.

A. fritzmuelleri appeared to be the closest neighbor to *Pseudananas*, with which it shares some specific bands. This rare species was collected in Southern Brazil (Smith 1934; Camargo 1943; Reitz 1983) and is represented by a unique accession maintained in the Brazilian collection. No other representative could be found by recent collecting expeditions (Duval et al. 1997).

A. nanus is sometimes considered as a dwarf mutant of *A. ananassoides* (Coppens d'Eeckenbrugge et al. 1997). In this study, the unique accession analysed displays distinctive band patterns but re-groups with *A. ananassoides* accessions.

A. lucidus, cultivated for fibers by the Amerindians and always found under cultivation (Leal and Amaya 1991; Duval et al. 1997), displays a high polymorphism (69.4%) despite the few accessions studied. It scatters into three separate clusters, always with *A. ananassoides* accessions, and once (B) with two *A. ananassoides* clones also cultivated for fiber. This species is characterized by erect unarmed leaves (Smith and Downs 1979). The former character is shared with some *A. ananassoides* clones and the latter is a single dominant gene trait (Collins 1960) whose reversion has been observed in the French collection. These data are consistent with a low level of convergent domestication based on a few traits (Coppens d'Eeckenbrugge et al. 1997).

A. bracteatus is cultivated in the South of the subcontinent, mainly as a living hedge. In this study it appears homogeneous and with the lowest polymorphism (28.3%), clearly grouped and positioned but not clearly separated from the other species. It shares some rare bands with *A. fritzmuelleri* and *P. sagenarius*, whose geographical distribution is similar. Previous results obtained by García (1988) and Aradhya et al. (1994) also show some similarities between *A. bracteatus* and *Pseudananas* but more neatly separates *A. bracteatus* from other *Ananas* species. This separation was confirmed by Noyer (1991). These differences with our results could be explained by the higher number of accessions and probes used, and the wider geographical distribution of the recently collected accessions.

Despite their wide morphological variation and the high number of accessions studied, *A. comosus* varieties cultivated for fruit appear relatively homogeneous in this work, well grouped in both the Factorial Analysis and the diversity tree. Despite a non-negligible agronomic variation, clones from the most famous and ubiquitous cultivar, Smooth Cayenne, re-group in the same cluster, whether they are overseas lines or clones from the Guianas. Different clones of the South American cultivar Pérola, collected in various regions of the sub-continent, group in the same cluster. A Tahitian clone of the well-known cultivar Queen has been found identical to landraces collected in French Guiana and Amapa. This low level of molecular diversity supports the hypothesis of a unique origin for each one of these cultivars followed by a clonal selection for agronomical traits. By contrast, varieties sharing the dominant monogenic leaf trait known as 'piping', and previously referred as the 'Mordilona' or 'Maipure group' (Leal and Soule 1977), segregate in various sub-clusters and re-group with accessions of the same geographical origin. The West Amazonian/Andean geographical origin of this trait is confirmed, as well as the intervention of sexual reproduction to disseminate the piping character, which corroborates the results obtained in the statistical analysis of morphological traits (Duval and Coppens d'Eeckenbrugge 1993).

Intermediate clones re-group with clones from *A. comosus*, *A. ananassoides* or both. As these two alleged species are interfertile, these intermediate forms are probably spontaneous hybrids scattered by vegetative re-

production and/or represent intermediate stages of domestication.

In conclusion, as in the previous studies with isozymes, most variation was found at the intraspecific level, particularly in wild species. Genetic variation revealed by this set of RFLP markers appears continuous and no clear separation was observed between the *Ananas* species as defined by Smith and Downs (1979). Factorial analysis showed a clear but only moderate distinction between the genera *Ananas* and *Pseudananas*. These data indicate the existence of gene flow within the genus *Ananas* and at the intergeneric level. Morphological observations are in progress but do not support such a complex classification. Thus the proposition of Leal et al (1998) to simplify the taxonomy by considering one genus and two species appears logical. Further investigations are being conducted at the chloroplast DNA level to elucidate phylogenetic relationships.

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