FIRST RESULTS FROM JOINT EMBRAPA-CIRAD *ANANAS* GERMPLASM COLLECTING IN BRAZIL AND FRENCH GUYANA

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Abstract

FIRST RESULTS FROM JOINT EMBRAPA-CIRAD *ANANAS* GERMPLASM COLLECTING IN BRAZIL AND FRENCH GUYANA

In the frame of a collaborative EMBRAPA-CIRAD project, germplasm of *Ananas* and *Pseudananas* genera was collected in certains areas of major diversity, namely the Amazon Basin (Amapa, Rio Negro, Rio Solimoes, Acre and Northern Mato Grosso), the French Guyana and Southern Brazil. Collecting trips were conducted using herbarium data and information provided by scientists and extension agents, and completed by local inquiries. Rivers as well as existing roads and trails were followed ; air transportation was used in some cases. A total of 434 clones, mostly land races and wild types, was collected. Some duplicates are probably included as morphological observation took place at different growth stages or within different ecological conditions. The distribution of the different species is consistent. Part of the accessions, however, could not be classified following the current taxonomic key, which raises question about the validity of the key. The structure of certain wild populations indicates that sexual reproduction is involved in their multiplication and diversification. Variability within the cultivated species *Ananas comosus* is high, and cannot be described in terms of its usual five groups. Collected material was transferred to the collections of CIRAD-FLHOR in Martinique and EMBRAPA-CNPMF in Cruz das Almas (Bahia). Botanical, agronomical and molecular characterization is in progress.

Résumé

BILAN DE COLLECTES CONJOINTES DE GERMOPLASME D'*ANANAS* AU BRÉSIL ET EN GUYANE FRANÇAISE

Dans le cadre d'un projet de coopération, le CIRAD-FLHOR et l'EMBRAPA ont collecté le germoplasme des genres *Ananas* et *Pseudananas* dans des zones de diversité majeure : le Bassin Amazonien (Amapa, Rio Negro, Rio Solimoes, Acre et Nord du Mato

Proc. 2nd. Internat. Pineapple Symposium
Ed. Pierre Martin-Prével, R.Hugon *et al.*
Acta Horticulturae 425

Grosso), la Guyane Française et le Sud du Brésil. Les expéditions ont été organisées sur la base des données d'herbiers et d'informations reçues des scientifiques et des agents régionaux de développement, complétées par enquête auprès des populations locales. Des moyens de transports routiers, fluviaux et plus rarement aériens ont été utilisés. 434 accessions, en majorité sauvages ou cultivars natifs, ont été collectées. Certaines sont vraisemblablement des doublons, les observations morphologiques ayant eu lieu à des stades de développement ou dans des conditions écologiques différentes. Les différentes espèces présentent une répartition géographique cohérente, mais de nombreux clones sont inclassables selon la clé de détermination usuelle, dont la validité s'avère contestable. La structure de certaines populations observées à l'état sauvage met en évidence le rôle actif de la reproduction sexuée dans la multiplication et la diversification. La variabilité très grande de l'espèce cultivée, *Ananas comosus*, n'a pu être analysée en utilisant la répartition classique en 5 groupes. Le matériel végétal est conservé dans les collection du CIRAD-FLHOR en Martinique, et de l'EMBRAPA-CNPMF à Cruz das Almas. Sa caractérisation botanique, agromorphologique et moléculaire est en cours.

Resumen

PRIMEROS RESULTADOS DE RECOLECCIONES CONJUNTAS DE GERMOPLASMA DE *ANANAS* EN BRASIL Y GUYANA FRANCESA POR EMBRAPA Y CIRAD

En un proyecto conjunto EMBRAPA-CIRAD, se recolectó germoplasma de *Ananas* y *Pseudananas* en ciertas áreas de gran diversidad para estos géneros, como la Cuenca Amazónica (Amapa, Acre y el norte del Mato Grosso, Rio Negro y Rio Solimoes), Guyana Francesa y el sur de Brasil. Las expediciones se organizaron con base en datos de herbario e informaciones recibidas de científicos y extensionistas locales, completadas por encuestas en las poblaciones visitadas. Se usaron medios de transporte viales, fluviales y, en ciertos casos, aéreos. Se recolectaron 434 clones, en su mayoría de tipo silvestre o variedades nativas. Parte de ellos pueden ser dobles puesto que las observaciones morfológicas se hicieron en diferentes fases fenológicas y en diferentes condiciones ecológicas. Las diferentes especies presentan una distribución geográfica coherente ; sin embargo, no fue posible clasificar muchos clones según la clave taxonómica tradicional, cuya validez se mostró cuestionable. La estructura de algunas poblaciones silvestres indica la participación de la reproducción sexual en la multiplicación y diversificación de las mismas. No es posible describir la muy importante variabilidad de la especie cultivada *A. comosus* con base en la división clásica en cinco grupos. El material recolectado, conservado en las instalaciones del CIRAD-FLHOR en Martinica y de la EMBRAPA-CNPMF en Cruz das Almas (Bahia), está en proceso de caracterización botánica, agronómica y molecular.

1. Introduction

As with most Bromeliaceae, the genus *Ananas* is native to South America. In their last revision of the family, Smith and Downs (1979) defined eight species. Their taxonomic key is mainly based on traits that depend either on single genes (e.g. leaf margin traits) or which vary greatly with the environment (e.g. fruit size). One of the species, *A. monstrosus*, distinguished from *A. comosus* by the absence of a crown, was invalidated by Leal (1990) because this trait is not permanent in the crownless clones. In addition, the division of the genus *Ananas* into eight species does not take into account the uniformity of the breeding system among species (Coppens d'Eeckenbrugge et al. 1993) and the absence of interspecific incompatibility (Collins 1960).

The geographical origin of *Ananas* was not well established and different hypotheses have been proposed, locating the areas of origin and diversity in the south or the north of South America (Baker, Collins 1939; Leal, Antoni 1981). On one hand, the two most widespread cultivars, Smooth Cayenne and Singapore Spanish, originate from the Caribbean and the northern coast of South America (Venezuela, Guianas), probably because pineapple was discovered and distributed by the great sea travellers of the 15th and 16th centuries. On the other hand, the Amazon and Orinoco basins have remained quite unexplored, and the pineapple varieties from these regions are still mostly unknown and under-represented in the germplasm collections.

EMBRAPA and CIRAD-FLHOR decided to undertake a programme to collect *Ananas* and *Pseudananas* germplasm in Brazil and French Guyana, (a) to better understand their diversity and structure and (b) to recover part of the variability currently endangered both by the expansion of the cultivar Smooth Cayenne and by the habitat destruction caused by deforestation and mining.

2. Materials and methods

Four collecting trips were conducted in the Brazilian Amazon basin to prospect the states of Amapa (June-July 1992), Acre and northern Mato Grosso (September-October 1992), and Amazonas (Rio Negro in July-August 1993, and Rio Solimoes, the upper arm of the Amazon, in November-December 1993). French Guyana was prospected in March-April 1993 and southern Brazil in May-June 1994.

Collecting sites were determined on the basis of previous bibliographic and herbarium data. Priority was given to areas most threatened by genetic erosion. Itineraries were selected according to available trails or rivers. Some places in Guyana (inselbergs and rock savannas) had to be accessed by a helicopter. Emphasis was placed on collecting wild populations and landraces. Most of the material was collected from peasants and smallholders. Passport data were recorded for each collected clone. Samples consisted of three to ten suckers, slips, crowns or even whole plants if necessary. All clones were submittted to phytosanitary treatment and divided into two sets. One was transferred for quarantine to EMBRAPA-CNPMF in Cruz das Almas (Bahía) and the second to CIRAD-FLHOR in Montpellier (France). After *in vitro* propagation in Montpellier, accessions were transferred to the CIRAD-FLHOR collection in Martinique. Quarantine *in vitro* was needed because many pests and diseases common in South America are not present in Martinique.

3. Results

A total of 414 samples from six of the seven *Ananas* species (not considering *A. monstrosus*), plus two clones of *Pseudananas sagenarius*, and 21 unclassified clones were collected in the six trips (table 1).

P. sagenarius was found only in southern Brazil, although it has been previously reported as far north as the northeastern states of Pernambuco and Bahía in Brazil (Lima 57-2803, IPA and Foster 2467 [US]; in Smith, Downs 1979). It grows wild in the forest and on pasture margins, and propagates by stolons.

A. bracteatus was found in the states of Rio Grande do Sul and Paraná. It is widespread in the area of Missions, covering northern Argentina, Paraguay, and southern Brazil. This species was also more confined to the south than previously reported. It was always found cultivated as a hedge or for fruit juice, sometimes in ancient settlements. The plant is vigorous with long leaves, coarse spines, and abundant suckering. The inflorescence is characterized by its bright pink or red colour and long bracts. *A. bracteatus* is well adapted to cold and altitude (one sample was collected at 1 088 m). The observed samples showed little variability, limited to vigour, leaf colour, fruit length and colour, and slip production.

A. ananassoides is the most widespread species. It has been observed from the north of the dispersion area of *A. bracteatus*, in southern Brazil, to the northernmost explored sites in Amapá and French Guyana. Although it was found in some instances in the dense rain forest (Amapá and French Guyana), it is more common in the cerrados (from open savannas to low-shaded forest) where it grows in populations of highly variable density. It is exceptionally cultivated for fibre by Amerindian tribes in French Guyana. Cultivated clones are very similar to *A. lucidus*, the only perceptible difference lying in spininess. The plant has long and generally narrow leaves and bears a small, globular to cylindrical, syncarp on a long peduncle. The collected samples display variability in all traits, particularly spine size and density, fruit size and shape, slip presence, and seediness. Some clones from Pará and French Guyana exhibit long stems creeping and rooting in the litter, in the same ways as rhizomes.

Most populations of *A. ananassoides* clearly correspond to clones, indicating a strong dominance of vegetative reproduction. Polyclonal populations commonly show numerous seeds in the fruits. An impressive variability observed in northern Mato Grosso and Pará suggested that many plant clusters were segregant clones arising from relatively frequent sexual recombination. The size of some plantlets also indicated that they were seedlings.

A. parguazensis was always found north of the Amazon river. Plants grow wild in the lowland forests, often near water, and even on Rio Negro islands. It occurs under canopies of variable density, ranging from clearings or river banks to dense rain forest. The variability found in these collection tours is relatively low, mainly concerning spine size and distribution, as well as leaf colour. Some variation was also observed in plant habit and peduncle length, but these traits do not constitute reliable criteria as their expression depends highly on the environment. The *A. parguazensis* clones collected in Venezuela, and presently under evaluation in Martinique, display a wider variability.

A. lucidus was always found cultivated for fibre in the Amazon basin and in French Guyana. It is disappearing, because most growers would prefer to buy the stronger nylon string, but they cannot afford it.

Only two clones of *A. nanus* were collected in home gardens.

A. comosus was found in all prospected areas, displaying a wide variability. The

classical division in five horticultural groups is inadequate to describe this variation because most clones do not fit into a group description. The clones were therefore simply classified in nine types, according to leaf margin morphology (piping, smooth, spiny tip ['Cayenne'], half spiny, sandpaper, small spines, medium spines, large spines, and 'Pérola'). 'Pérola' was individualized from the other spiny types because this variety is the most common in Brazil. The distribution of the collected clones among these classes is shown in table 2. Within-class variability may differ widely. The 'spiny tip' class comprises only very few varieties, whereas the 'small spines' type was more common and clearly heterogenous. Additionally, genetic variability can be easily overestimated because of variation due to the environment. Plants observed at different phenological stages and under variable environments may not be properly identified, and some clones may have been sampled twice or more.

The number of *A. comosus* clones and the number of different leaf margin classes was clearly higher in the state of Acre, the Amazon basin, and in French Guyana, than in the south. This cannot be due solely to genetic erosion, which is more severe in the south, because more preserved marginal southern areas, such as the Uruguay river banks, are poor in landraces. Some of the 'leaf margin' types were found in specific areas, the 'spiny tip' clones in the northeast (Amapá and French Guyana), and the 'piping' and 'sandpaper' clones in the west (Acre and north Mato Grosso). Along the Solimoes (upper Amazon), only *A. comosus* was found, with a variety distribution indicating a spread from the upper lands down to Manaus.

Absence of crowns was observed in some clones but, according to growers, this trait was variable in expression. So these observations agree with those of Leal (1990) who invalidated the species *A. monstrosus*.

Numerous clones could not be classified in a particular species, because they combined typical traits from distinct species. As observed in previous analysisi (Loison-Cabot, 1992) an important source of confusion in the taxonomic key is the arbitrary discrimination based on quantitative traits such as fruit size, which varies with the environment. Thus, clones with traits associated with wild species, such as large spines, long peduncle and high crown/fruit weight ratio, should be classified as *A. comosus* if fruit length is over 15 cm. In French Guyana and Amapá, such clones were often found in the wild as well as under cultivation, suggesting a still active domestication process. Other clones found in poor growing conditions could not be classified but produced big fruits when cultivated in the Martinique collection.

4. Discussion

The collection tours permitted us to confirm the geographical distribution of the different taxa in the genera *Ananas* and *Pseudananas*; and the greater variability in the north of the Amazon (Rio Negro) basin and in the Orinoco underlined by Leal and Antoni (1981). *A. bracteatus* and *P. sagenarius* constitute the southernmost component. Their distribution area appears more clearly separated from our results than from those of Bertoni (1919) and Baker and Collins (1939), which is probably due to the rapid transformation of southern Brazil. The genus *Ananas* is represented by *A. ananassoides* in the south of the Amazon, and by *A. ananassoides*, *A. lucidus*, and *A. parguazensis* in the north of the Amazon, with the latter restricted to the northeast (upper Rio Negro and Orinoco).

The river spread of the cultivated types along the Solimoes (upper Amazon) and the Rio Negro was certainly a consequence of human exchange. On the other hand, the very extensive flooded areas all along the Amazon and main rivers acted as a barrier against the natural southward spread of the wild types found in the northern regions, so defining the respective distributions of *Ananas* species on the Guyana and Brazilian shields. More generally, geographical isolation certainly played a role in the differentiation of the genus; however, the high fertility of interspecific crosses and hybrids (Collins 1960) and the great similarity in the reproduction system (Coppens d'Eeckenbrugge et al. 1993) indicate that it did not result in a true speciation.

A regional distribution was also found within *A. comosus*. The 'piping' gene conferring completely smooth and 'sandpaper' leaf margins (Collins, 1960) is common in the western region (Acre and north Mato Grosso), while the 'spiny tip' gene conferring partially smooth leaves is common in the northern regions of the Guyana shield. The most widely cultivated variety worldwide, Smooth Cayenne, appeared in this region. Red Spanish, another important and partially smooth variety, the most common in Venezuela, probably originated in the same region. The regional distribution of some genes may be related to the dominance of vegetative reproduction over sexual reproduction which could be responsible for the slow spread of particular genetic traits.

5. Acknowledgment

This work is part of the TS2A-0196-F project funded by the EEC (STD2 program). We thank Margarita Baena for her valuable correction of the manuscript.

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Table 1 - Species distribution observed.

Region \ Species	*Ananas comosus*	*Ananas parguazensis*	*Ananas nanus*	*Ananas lucidus*	*Ananas ananassoides*	*Ananas bracteatus*	*Pseudananas sagenarius*	Unclassified
Amapa	36	1	-	3	8	-	-	9
Para	3	3	-	-	7	-	-	-
Guyane F	47	-	1	3	25	-	-	12
Rio Negro	64	15	-	5	5	-	-	-
Rio Solimoes	65	-	-	-	-	-	-	-
Acre	37	-	1	-	1	-	-	-
Mato Grosso	9	-	-	-	24	-	-	-
Goias	2	-	-	-	1	-	-	-
Minas Gerais	-	-	-	-	2	-	-	-
São Paulo	3	-	-	-	-	-	-	-
Parana	7	-	-	-	-	2	1	-
Rio Grande do Sul	7	-	-	-	-	4	1	-
Paraguay	6	-	-	-	2	9	2	1

Table 2 - Distribution of collected *A. comosus* clones.

Types / Place	Piping	Smooth	Half Spiny	Cayenne	Pérola	Sand-paper	Small spines	Medium spines	Big spines
Amapa	-	-	3	5	4	-	18	3	-
Para	-	-	-	1	-	-	3	2	-
Guyane Fr.	-	-	5	12	-	-	13	16	1
Rio Negro	23	3	-	-	1	-	26	11	-
Rio Solimões	15	-	1	-	1	-	39	9	-
Acre	6	6	-	-	1	6	11	5	2
Mato Grosso	3	-	-	-	1	-	2	3	-
Goias	-	-	-	-	-	-	2	-	-
São Paulo	-	-	-	-	2	-	1	-	-
Parana	-	-	-	-	2	-	4	1	-
Rio Grande do Sul	1	-	-	1	3	-	2	-	-
Paraguay	-	-	-	-	1	-	4	1	-