Chapter 2
Ananas

Geo Coppens d'Eeckenbrugge, Garth M. Sanewski, Mike K. Smith, Marie-France Duval, and Freddy Leal

2.1 Introduction

Pineapple can be counted among the major New World contributions to global well-being, along with major staples (maize, cassava, potato, and sweet potato), legumes (beans), and vegetables (tomato, peppers). It was a major Amerindian crop, being widely cultivated in neotropical areas (Coppens d'Eeckenbrugge et al. 1997), and Colón (Columbus) observed it as early as 1493 in his second voyage. From then on, its unique and impressive characteristics, as well as the drought resistance of its propagules, ensured its rapid diffusion throughout the tropics, so it became a pantropical crop in less than two centuries and accounted for significant greenhouse production in Europe. Its economic importance further developed along with efficient preservation and transportation (Rohrbach et al. 2003).

The importance of the international pineapple trade has often overshadowed the even more important local and national markets. Indeed, major producing countries are also major consumers. National markets have mostly differed in the predominance of the fresh products and their wider genetic basis. While the international market was long dominated by canned pineapples of the "Smooth Cayenne" cultivar, national and regional markets allowed the maintenance of some cultivar diversity. Recently, the introduction of new cultivars on the international market has boosted global production up to 18 million tons in 2007, increased market share for fresh fruit, and maintained

an interest in genetic diversity of this crop (Loeillet 2008). In addition, a small market for ornamental pineapple has developed on the basis of small, colorful fruit types (Souza et al. 2006, 2009; Sanewski 2009).

Other products derived from the pineapple are a very resistant silk-like fiber, processed into luxury clothes or specialty paper, both of remarkable thinness, smoothness, and pliability (Collins 1960; Montinola 1991). Bromelain, a proteolytic enzyme complex, is used as a meat-tenderizer and as a nutraceutical with potential therapeutic activity on inflammatory changes, blood coagulation, debridement of severe burns, drug absorption, and tumors (Taussig and Batkin 1988).

2.2 Basic Botany of the Species

2.2.1 Morphology

The pineapple, *Ananas comosus* (L.) Merr., is a perennial, herbaceous monocot of the family Bromeliaceae. Pineapple leaves are spirally organized in a dense rosette, around a short stem (Fig. 2.1). They are generally spiny; however, many cultivars show a partial or complete absence of spines. The adult plant is 0.8–2 m high and wide, depending mostly on leaf length. The hormonal shift from vegetative to generative growth is triggered by climatic factors, mainly day length and temperature, the plant receptivity increasing with its size and stress conditions. Flowering may be induced artificially, by the application of ethylene or ethylene-producing chemicals, to ensure crop uniformity. The terminal inflorescence develops into a multiple fruit or sorose, composed of 50–200 fruitlets, disposed around its fibrous axis in a 5/13 (small fruits) or 8/21 (medium

G.C. d'Eeckenbrugge (✉)
CIRAD, UMR 5175 CEFE, 1919 Route de Mende, 34293 Montpellier, France
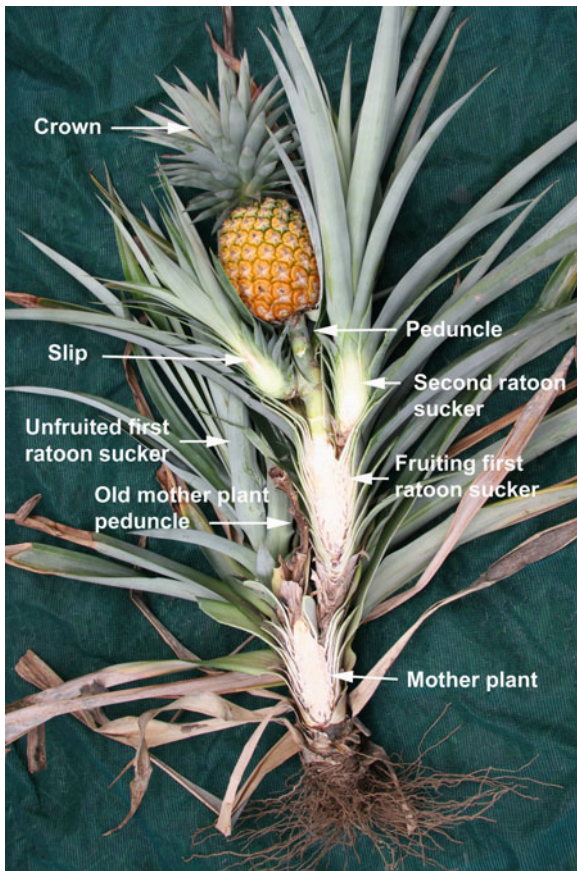e-mail: geo.coppens@cirad.fr

Fig. 2.1 Structure of a pineapple plant (*A. comosus* var. *comosus*) showing the succession of vegetative cycles and different types of planting materials for further vegetative propagation (stem sucker, slip, and crown) (photograph courtesy Garth Sanewski)

to large fruits) phyllotaxy, and borne on a peduncle of variable length. The species reproduces mostly from vegetative propagules that develop on the stem (stem shoots and ground suckers), the peduncle (slips), and the fruit top (crown). The type of planting material determines the initial development of the root system and the duration of the first crop cycle, which usually varies between 12 and 24 months, depending on cultivars and temperature. After fruit maturity, slips can be replanted or suckers may be left on the plant, providing new growth axes for a further production cycle. The latter is cheaper and shorter, as the plant is already established; however, fruit size is reduced and less uniform, so commercial cultivation is generally limited to two or three production cycles.

As with other bromeliads, pineapples have many adaptations related to water economy: CAM metabolism; leaf shape and arrangement, together with aerial roots, favoring rain water collection and absorption; a thick cuticle; and stomata disposed in longitudinal furrows on the abaxial leaf surface, covered with dense shield-shaped trichomes. In addition, the root system is weak, which is also common in a family dominated by epiphytism.

Pineapple flowers are hermaphroditic and trimerous, with three sepals, three petals, six stamens in two whorls, and one tricarpellary pistil. The anthers are bilobed, introrse, and dorsifixed. The style is hollow, trilobed, trifid, almost as long as the petals and equal or longer than the stamens. Petals are free, generally white at their base to violet-blue at their tip. The placentae and numerous ovules are located in the three deep locules of the inferior ovary. These are separated by three nectary glands, whose generous production attracts a range of potential pollinators including hummingbirds, the natural pineapple pollinators. The adjacent ovaries, bracts, and the inflorescence axis coalesce to form the fleshy compound fruit.

2.2.2 Sexual Reproduction, Genome, and Cytology

A. comosus possesses a gametophytic self-incompatibility system, expressed by the inhibition of pollen tube growth in the upper third of the style (Kerns 1932; Majumder et al. 1964; Brewbaker and Gorrez 1967). The most important cultivars of *A. comosus* var. *comosus* are strongly self-incompatible; however, many other cultivars have a weaker self-incompatibility and may produce a few self-seeds. This phenomenon, called pseudo-self-compatibility, is much more common in the other botanical varieties of *A. comosus*, and the self-fertility of some clones is only slightly lower than their cross-fertility (Coppens d'Eeckenbrugge et al. 1993; Muller 1994). *Ananas macrodontes* is highly self-fertile, and self-progenies are homogenous, suggesting that this species is homozygous and autogamous.

The pineapple is diploid, with 50 minute chromosomes. Rare triploids, with 75 chromosomes, have also been observed in *A. comosus* var. *comosus* and *A. comosus* var. *ananassoides*. The meiosis of diploids is normal, with the formation of 25 bivalents, resulting in normal tetrads (Heilborn 1921; Collins and Kerns 1931;

Capinpin and Rotor 1937; Lin et al. 1987; Dujardin 1991; Brown et al. 1997; Gitaí et al. 2005). The formation of a few giant unreduced gametes may result in the production of natural triploids, which produce mostly sterile pollen, and tetraploids (Collins 1933, 1960). Pollen viability is highly variable between varieties, cultivars, and even between clones from the same cultivar (Coppens d'Eeckenbrugge et al. 1993; Muller 1994). Male and female fertility are correlated, and generally lower in pineapples cultivated for fruit (*A. comosus* var. *comosus*), with 0–5 seeds per flower, than in other botanical varieties, where a single ovary may give up to 18 seeds (Coppens d'Eeckenbrugge et al. 1993; see also Leal and Coppens d'Eeckenbrugge 1996). The closest pineapple relative, *A. macrodontes* Morren is a tetraploid (*n* = 100; Collins 1960; Lin et al. 1987). It can be crossed with *A. comosus*, yielding a few fertile seeds that give a majority of tetraploids and some smaller and sterile triploids, whose phenotype is intermediate between parental species (Collins 1960).

Arumuganathan and Earle (1991) estimated the haploid genome size at 444 Mbp for *A. comosus* var. *bracteatus* and 526 Mbp for var. *comosus*.

2.2.3 Taxonomy

The genus *Ananas* belongs to the family Bromeliaceae, a large family of 56 genera and ca. 2,600 species, whose distribution is essentially American, the only exception being the African species of *Pitcairnia feliciana* (Aug. Chev.) Harms & Midbr., native to Guinea. It is further classified in the subfamily Bromelioideae, where it is unique in merging the whole inflorescence into a massive compound fruit. Pineapple is also the most economically important bromeliad. *Aechmea* and *Bromelia*, two other genera of the Bromelioideae, also include species yielding edible fruits, such as *A. bracteata* (Swartz) Grisebach, *A. kuntzeana* Mez, *A. longifolia* (Rudge) L.B. Smith & M.A. Spencer, *A. nudicaulis* (L.) Grisebach, *B. antiacantha* Bertoloni, *B. balansae* Mez, *B. chrysabtha* Jacquin, *B. karatas* L., *B. hemisphaerica* Lamarck, *B. nidus-puellae* (André) André ex. Mez, *B. pinguin* L., *B. plumieri* (Morren) L.B. Smith, and *B. trianae* Mez (Rios and Khan 1998). These minor fruits are consumed locally, under names such as *cardo*, *banana-do-mato* (bush banana), *piñuela* (small pineapple), *karatas*, *gravatá*, or *croata* (generic vernacular names for terrestrial bromeliads). In addition, many bromeliads are cultivated or gathered as ornamentals, fiber extraction, or used in traditional medicine (Corrêa 1952; Purseglove 1972; Reitz 1983; Rios and Khan 1998).

Pineapple taxonomy was recently revised and simplified by Leal et al. (1998) and Coppens d'Eeckenbrugge and Leal (2003), on the basis of new data on reproduction (Coppens d'Eeckenbrugge et al. 1993), morphological (Duval and Coppens d'Eeckenbrugge 1993), biochemical (García 1988, Aradhya et al. 1994), and molecular (Duval et al. 2001b) diversity. The two genera and seven valid species of the previous classification (Smith and Downs 1979) were downgraded to two species and five botanical varieties. Their correspondence is given in Table 2.1.

The tetraploid *A. macrodontes* (Fig. 2.2) is mainly differentiated from *A. comosus* by the lack of a crown at the top of the syncarpic fruit and by its vegetative reproduction by stolons, although it will rarely exhibit a rudimentary crown or produce shoots from the stem. Its strong spines are retrorse at the leaf base, or even

Table 2.1 Correspondence between the current classification (Coppens d'Eeckenbrugge and Leal 2003) and the former one (Smith and Downs 1979)

Coppens d'Eeckenbrugge and Leal (2003)	Smith and Downs (1979)
***Ananas comosus* (L.) Merril**	
A. comosus var. *ananassoides* (Baker) Coppens & Leal	*A. ananassoides* (Baker) L.B. Smith
	A. nanus (L.B. Smith) L.B. Smith
A. comosus var. *erectifolius* (L.B. Smith) Coppens & Leal	*A. lucidus* Miller
A. comosus var. *parguazensis* (Camargo & L.B. Smith) Coppens & Leal	*A. parguazensis* Camargo & L.B. Smith
A. comosus var. *comosus*	*A. comosus* (L.) Merrill
Invalid (Leal 1990)	*A. monstrosus*
A. comosus var. *bracteatus* (Lindl.) Coppens & Leal	*A. bracteatus* (Lindley) Schultes f.
***Ananas macrodontes* Morren**	*Pseudananas sagenarius* (Arruda da Câmara) Camargo

Fig. 2.2 Main distinctive traits of *Ananas macrodontes*: absence of a crown at the apex of the inflorescence and long floral bracts, presence of retrorse spines on the leaves, and vegetative reproduction by stolons (photographs courtesy Garth Sanewski)

higher. The fruit flesh is low in acid and it contains numerous seeds. The plant appears to be highly self-fertile. The natural habitat of *A. macrodontes* corresponds to humid forest areas, under semi-dense shade, in coastal and southern Brazil and in the drainage of the Paraguay and Paraná rivers, from southeastern Paraguay and northeastern Argentina up to Mato Grosso (Coppens d'Eeckenbrugge et al. 1997). The species even tolerates short periods of flooding (Bertoni 1919). Baker and Collins (1939) reported little variation for this species, while Bertoni (1919) distinguished five varieties and Camargo (cited by Reyes-Zumeta 1967) considered the possible distinction between three botanical varieties. Ferreira et al. (1992) reported appreciable variation in a limited sample from Paraguay and southern Brazil, which was confirmed by the restriction fragment length polymorphism (RFLP) study of Duval et al. (2001b). On the other hand, Duval et al. (2003) observed only one chlorotype among six accessions of the species. When selfed, clones of *A. macrodontes* produce uniform progenies (Collins 1960, and field observations by the authors).

Vernacular names for *A. macrodontes* include *yvira* (fiber) in Paraguay, and *gravatá de rede* (fishing net bromeliad), *gravatá de cerca brava* (wild fence-bromeliad), or *nana caçaba* (strong-spine pineapple) in Brazil, thus referring to traditional uses of the plant, mostly as a source of long and strong fibers.

A. comosus var. *ananassoides* is the most common and diverse form of wild pineapple, and it is the most likely ancestor of the cultivated pineapple. It is found in most tropical regions of South America east of the Andes, generally in savannahs or clear forest, growing on soils with limited water-holding capacity (sand, rocks) and forming populations of variable densities. In the Guianas, it can also be found, although rarely,

thriving in dense rain forest. In contrast, it is not found in the seasonally flooded lands along the Amazon and main southern tributaries, which seem to act as a barrier dividing its distribution in two main areas: a northern one corresponding to the Guiana shield, Orinoco Basin, and northern drainage of Rio Negro (i.e., from the Brazilian state of Amapá to eastern Colombia), and a southern one roughly corresponding to the Brazilian shield and northeastern Brazil (from the Brazilian states of Acre, Mato Grosso up to Pernambuco and down to Paraguay, and northern Argentina). A higher morphological diversity is observed in the northern area (Fig. 2.3), where habitats also appear more variable for the wild pineapples (Leal and Medina 1995). In the south, they are mostly restricted to wide areas providing an open and markedly dry habitat (grass savannahs and low open forests) (Fig. 2.4).

Most populations of *A. comosus* var. *ananassoides* are monoclonal, but some are polyclonal, with variation of probably recent sexual origin (Duval et al. 1997). *A. comosus* var. *ananassoides* is characterized by long and narrow leaves, up to 2 m long and less than 4 cm wide, subdensely serrate with wholly antrorse spines. The fruit peduncle is elongate (most often more than 40 cm), slender (usually less than 15 mm wide). Its inflorescence is small to medium in size, globose to cylindrical, and it shows little growth after anthesis, so it has little flesh. The pulp is white or yellow, firm and fibrous, and palatable, with a high sugar and acidity content, with numerous seeds. In contrast, the crown resumes fast growth after fruit maturation, looking disproportionate in comparison to the fruit. Some clones of the Guianas-Orinoco area produce larger, fleshy fruits of intermediate size. They are sometimes cultivated or tolerated in gardens. Such pineapples may have served as a basis for

Fig. 2.3 Variation for *Ananas comosus* var. *ananassoides* in French Guiana: a small dwarf type from the rain forest; a fleshy, relatively large fruit from a wild population growing on a rock savannah; and an intermediate, semi-domesticated type in a home garden (photographs courtesy Geo Coppens d'Eeckenbrugge)

Fig. 2.4 A *Ananas comosus* var. *ananassoides* in Mato Grosso (Brazil) and its typically arid habitat (photographs courtesy Geo Coppens d'Eeckenbrugge)

domestication. At the other end, some dwarf types have recently been cultivated as ornamentals for the cut flower market, at both national and international levels.

Vernacular names include *ananaí*, or *nanaí*, *ananas de ramosa* (Brazil, Pará), *curibijul*, *maya piñon*, *piñuela*, and *ananas do indio*. Since pre-Columbian times, the plant has been known for its digestive properties, as well as a vermifuge, antiamoebic, abortifacient, and emmenagog (Leal and Coppens d'Eeckenbrugge 1996). Its fruits were consumed in the Orinoco (Patiño 2002) and are still occasionally consumed in the Guianas.

A. comosus var. *erectifolius* is very similar to the preceding variety. Plants are medium sized, with abundant shoots, frequent crownlets at the base of the main crown, numerous erect, fibrous leaves, and a small, very fibrous, inedible fruit (Fig. 2.5). In some clones, the fruit appears to be rare. The essential difference with *A. comosus* var. *ananassoides* lies in the smooth leaves of *A. comosus* var. *erectifolius*, a trait which is under monogenic control (Collins 1960). *A. comosus* var. *erectifolius* is not known to occur in the wild. It was cultivated in the West Indies at the time of the contact with the European, and it is still cultivated by the natives in the Guianas, including the

Fig. 2.5 Fruit of *Ananas comosus* var. *erectifolius* (photograph courtesy Garth Sanewski)

Fig. 2.6 *Ananas comosus* var. *parguazensis* (photographs courtesy Geo Coppens d'Eeckenbrugge)

Orinoco basin, and in the north of the Amazon basin, for the strong and long fibers associated with its typical erect habit. Indeed, the dry fibers constitute 6% of the plant weight. They are used to make hammocks and fishing nets (Leal and Amaya 1991), but now suffer competition from synthetic fibers and nylon. Vernacular names include *curagua*, *curauá*, *curaná*, *kulaiwat*, and *pitte*. The typical absence of spines along the leaf margin, as well as its erect habit, is the likely result of artificial selection for high yield of easily extractable fibers among strains of *A. comosus* var. *ananassoides*. The reverse mutation to spiny or partly spiny leaves has been observed under cultivation and in germplasm collections. Genetic diversity studies (Duval et al. 2001b, 2003) indicate that the domestication process that produced *A. comosus* var. *erectifolius* from *A. comosus* var. *ananassoides* has taken place independently at different times and/or places. This variety has recently found a new economic use in the production of cut flowers.

The wild *A. comosus* var. *parguazensis* is also very similar to *A. comosus* var. *ananassoides*, from which it differs by wider leaves, slightly constricted at their base, and larger spines, some of them retrorse (Fig. 2.6). Its distribution mostly corresponds to the basins of the Orinoco and upper Rio Negro, with a few observations in eastern Colombia and in the northeastern Amazon (Coppens d'Eeckenbrugge et al. 1997). It grows in lowland forests, under canopies of variable densities, from clearings or river banks to dense forest. As compared to *A. comosus* var. *ananassoides*, it seems restricted to shadier environments, because of lower water use efficiency (Leal and Medina 1995).

In *A. comosus* var. *comosus*, the most widely cultivated pineapple and the basis of the world trade in fresh and processed fruit, the syncarp grows very significantly after anthesis, so the fruit are generally very large (up to several kilograms in certain cultivars), with many fruitlets ("eyes"); they are borne on a wide and strong, relatively short, peduncle. Seeds are rare in the fruits, because of reduced fertility, conjugated with stronger self-incompatibility and monoclonal cultivation. The plant has numerous wide leaves (40–80), with antrorse spines, generally smaller and denser than in other botanical varieties. They may be suppressed by dominant mutations, as the one leaving only a few spines near the leaf tip of cultivar "Smooth Cayenne" or the one governing the folding of the

lower leaf epidermis over the leaf margin ("piping" phenotype), suppressing all spines but the terminal one. The former mutation is more common in the Guianas, while the latter is mostly found in the western Amazon and in the Andes. These two regions also exhibit a wider overall cultivar diversity (Duval et al. 1997, 2003).

The clearest effects of domestication in *A. comosus* var. *comosus* consist in the reduced susceptibility to natural flowering induction, together with the formation of a larger number of wider, and generally shorter, leaves, a wider and longer stem allowing a larger starch storage capacity, a significant increase in the number of flowers, the enlargement of individual fruits, and reduced seed production through the combination of lower sexual fertility and stronger self-incompatibility. In the cultivars where the reduction of female fertility, i.e., the proportion of ovules producing a seed, is not very severe, it can be counterbalanced by the higher number of flowers. In any case, as vegetative reproduction is largely dominant in the genus, this reduced sexual potential affects the plant survival less than the changes in the vegetative organs and the plant vegetative cycle. Strictly speaking, the domestication syndrome in this botanical variety lies in its lack of adaptation to the natural conditions prevailing for the wild varieties. Pineapple plants from most cultivars can survive when their cultivation is abandoned, resisting competition in sufficiently open vegetation and even dry edaphic or climatic conditions; however, they do not propagate efficiently to form subspontaneous feral populations.

A. comosus var. *comosus* was planted throughout tropical America at the time of the Conquest. Its fruit was widely consumed and particularly appreciated in the form of fermented drinks (Patiño 2002). Other traditional uses are the same as for *A. comosus* var. *ananassoides*. Rotted pineapple was used on arrows and spear heads for poisoning (Leal and Coppens d'Eeckenbrugge 1996).

A. comosus var. *bracteatus* is particular as this variety is an assemblage of two cultivated forms that show the same geographic distribution as *A. macrodontes* and that are morphologically and genetically intermediate between the two *Ananas* species (Fig. 2.7). The most common one, corresponding to *A. bracteatus sensu* Smith & Downs, is a cultigen that was cultivated as a living hedge and harvested for fiber and fruit juice, or for traditional medicine, in southern

Fig. 2.7 The most common form of *Ananas comosus* var. *bracteatus* (photograph courtesy Garth Sanewski)

Brazil and Paraguay (Bertoni 1919). Indeed, its dense, long, and wide leaves are strongly armed by large antrorse spines, forming impenetrable barriers. It is very robust and still thrives in abandoned plantations, but it seems unable to colonize new habitats. The syncarp is of intermediate size (0.5–1 kg), borne by a strong scape, and covered by long and imbricate floral bracts, as in *A. macrodontes*. These bracts are bright pink to red at anthesis, giving the inflorescence a spectacular appearance. Indeed, a variegated mutant has been widely propagated as a tropical garden ornamental. Morphological and genetic variations appear very limited in this first form, being comparable to within-cultivar variations (Duval et al. 2001b, 2003) and suggesting a very narrow origin, possibly a single genotype. The second form, corresponding to *A. fritzmuelleri* Camargo, shares an additional trait with *A. macrodontes*, as it exhibits retrorse spines at the leaf base. According to Camargo (1943) and Smith and Downs (1979), it was also used in living fences. It is a very rare form, whose diversity has not been documented, only one clone being conserved in Brazil, by EMBRAPA and the botanical garden of Rio de

Janeiro. Nuclear and chloroplast DNA data confirm its closer proximity with *A. macrodontes*. The chromosome number is $2n = 2x = 50$ (Camargo 1943).

2.2.4 Capacity for Invasiveness

Although pineapples are hardy plants with good drought tolerance, they achieve this through a high stomatal resistance and hence slow growth rate. Generally, they have a weak root system and have not established as weeds of significance despite being grown commercially in many countries. Reproduction of most commercial cultigens is almost exclusively through vegetative propagules, which can only be distributed through human or animal intervention or extreme environmental events such as flooding. Wild varieties such as *A. comosus* var. *ananassoides* are known to be seedy. However, viability of seed and that of vegetative propagules is substantially reduced after 6 months. All these factors mean it is unlikely that pineapples would become weeds in nature.

2.3 Conservation Initiatives

Pineapple genetic diversity has long been underestimated. Most cultivars that formed the basis of its worldwide cultivation were collected in the Caribbean or near the northern shores of South America. "Smooth Cayenne" was collected in French Guiana and "Queen" in Barbados (although it might have been also brought from French Guiana, where it is a traditional cultivar of the natives). We do not know the origin of "Singapore Spanish", the third common cultivar in Asia. Commercial cultivation in tropical America has been dominated by a few regional cultivars, as "Red Spanish" in the Caribbean (now mostly limited to Venezuela), "Monte Lirio" in Central America, "Perolera" in the Andes of Colombia and Venezuela, and "Pérola" in Brazil. The many cultivars that were collected in the West Indies for glasshouse cultivation in Europe (Loudon 1822) have been lost. To our knowledge, only a handful of them have been recovered in the germplasm collections of Trinidad and Tobago and that of Cuba, and similar systematic collecting should be undertaken in other islands, as well as in Central America, particularly where the commercial planting of "Smooth Cayenne" and, more recently that of "MD-2", has not displaced home garden production.

In South America, the first effort for collecting and conserving pineapple germplasm was initiated by the exploration of southern Brazil and Paraguay, by Baker and Collins (1939). This area was chosen because it was supposed to be the region of origin and domestication, as stated by Bertoni (1919). Baker and Collins confirmed the existence of wild pineapples in this area, but never explored the Amazon basin north of Mato Grosso, which fed a circular reasoning about a southern origin of the pineapple, still biasing very recent literature. This view was seriously challenged in 1981, when Leal and Antoni (1981) showed that northern South America exhibited a larger number of botanical varieties (then considered as different species). This was confirmed by extensive collecting expeditions in Venezuela, Brazil, and French Guiana (Leal et al. 1986; Ferreira et al. 1992; Duval et al. 1997) and preliminary observations in Suriname (Suriname 1995). In addition, national collections were established in Colombia and Peru, gathering cultivars adapted to contrasting conditions, from the Amazon to steep slopes of the Andes (Bello and Julca 1993; Hernández and Montoya 1993).

As could be expected, pineapple genetic erosion has been particularly severe in southeastern Brazil and Paraguay, because of deforestation and agricultural intensification. The situation is very different for wild forms in less densely populated areas of the Amazon or the Orinoco, as they often thrive in open areas, on soils with limited water-holding capacity (sandy hills and shallow soils around rocks), or even in forest conditions, where they do not compete with agriculture. Preservation of cultivated forms depends on the proximity of markets. While Tikuna farmers of the Upper Amazon may cultivate more than a dozen landraces in small plots, for their own consumption or for small village markets, most *caboclos* around Manaus grow two or three more common cultivars in larger fields. Thus, the possibility of in situ conservation of most native germplasm diversity is limited to remote areas, where genetic erosion would be very difficult to monitor. On the other hand, Coppens d'Eeckenbrugge et al. (1997) proposed an effort of rigorous clonal selection in regional cultivars, to optimize their characteristics and enhance their attractiveness for growers

and consumers, in terms of adaptation, resistance, and market diversification. Similarly, the exploration and characterization of germplasm from the "deep" Amazon would certainly be rewarding, allowing the identification of competitive robust cultivars and an expansion of the genetic basis of commercial pineapple cultivation.

Field conservation has constituted the most serious option for pineapple germplasm conservation so far. The most important collections, in terms of numbers and diversity of forms and geographic origins, are those that were established directly from field collecting, i.e., those of EMBRAPA in Brazil, CIRAD in Martinique, INIA in Venezuela, and USDA in Hawaii. Other smaller but important collections are maintained in Côte d'Ivoire, Malaysia, Okinawa, Taiwan, and Australia. All field collections face problems about funding continuity. This may be particularly true for the two smaller collections of Colombia and Peru, which harbor unique materials with particular adaptations.

Concerning procedures for field conservation, Coppens d'Eeckenbrugge et al. (1997) have described the procedures followed at the CIRAD collection in Martinique. Biotechnology, on the other hand, provides alternative or complementary methods for ex situ conservation, through tissue culture and/or cryoconservation. Tissue culture techniques also offer the opportunity for rapid propagation while providing the convenience of medium- and long-term storage of germplasm and facilitating its safe distribution (Smith et al. 2005).

Low temperature (16–20°C) and low sugar (1.5% glucose) culture medium have been used to extend subculture times for up to 4 years (Sugimoto et al. 1991). Zee and Munekata (1992) observed that reducing the nutrient salts in the culture medium to one fourth was successful for medium-term (12 months), low-input maintenance of pineapple cultures. For long-term storage, cryopreservation has been utilized. González-Arnao et al. (1998) demonstrated that pineapple shoot apices could be preserved in liquid nitrogen following pre-treatment and the use of cryoprotectants. A problem with methods only based on tissue culture, however, is the need of regularly controlling the variation induced by mutation, a phenomenon that is particularly important in the pineapple (Collins 1960). Its monitoring is complicated and delayed because the resulting plantlets behave more

like seedlings than field-multiplied material, so a supplementary cycle of traditional multiplication is required.

Pineapple seeds can maintain viability for 2 years or more in dry and cool conditions, opening the possibility of a pineapple seedbank, provided that proper methodology and procedures can be optimized. Seed cryoconservation should also be tested. Economic seed conservation techniques would be particularly interesting for wild germplasm and primitive landraces. For more advanced material, their interest is more limited, as the objective is the conservation of clones, presenting particular genetic combinations.

2.4 Role in Elucidation of Origin and Evolution of Pineapple

The use of molecular biology in recent decades has provided key elements on the origin and domestication of pineapple.

2.4.1 Phylogeography

The first studies were conducted using enzymatic systems and evidenced a high polymorphism in the genus (80–100%), with a strong heterozygosity on a sample including an important number of accessions collected in Venezuela (García 1988). Another study on the USDA collection (missing representatives of wild populations from the north of the Amazon) indicated that 86% of the total variation was found within botanical varieties (then considered distinct species), underlining a moderate genetic divergence (Aradhya et al. 1994). Both studies evidenced a clear separation of a group constituted by *A. comosus* var. *bracteatus sensu* Smith & Downs and *A. macrodontes*.

A first genetic study (Noyer 1991) showed a low cytoplasmic diversity, with only one polymorphic probe–endonuclease combination out of 56 tested on 75 accessions covering a wide *A. comosus* diversity. Polymorphism was then investigated at the nuclear rDNA level and six groups were identified. The largest group includes clones of the varieties *comosus* (all except one), *parguazensis*, and *ananassoides* from Venezuela. *A. comosus* var. *bracteatus* accessions

formed a second group. Other groups correspond to one or two accessions (Noyer et al. 1998).

Following joint French–Brazilian pineapple prospecting expeditions to explore genetic diversity in the genus, a nuclear DNA RFLP analysis was conducted on a sample of 301 accessions, most of these collected in South America with a large distribution range (Duval et al. 2001b). High levels of variation were found within *A. macrodontes* and the wild forms *A. comosus* var. *ananassoides* and *A. comosus* var. *parguazensis*. Genetic diversity varied within cultivated forms, ranging from very low (*A. bracteatus sensu* Smith & Downs), to very high (*A comosus* var. *erectifolius*). The structure of genetic diversity appeared loose. *A. macrodontes* separated well but shared 58.7% of the markers with *Ananas* and was very close to the diploid *A. fritzmuelleri* Camargo. Within *Ananas*, only *A. comosus* var. *parguazensis* accessions form a consistent cluster. The scattering of botanical varieties and the occurrence of intermediate forms indicates a very probable gene flow, which is consistent with the lack of reproductive barriers between them.

Chloroplast restriction site variation was then used to study a subsample of 97 accessions of *Ananas* chosen for their genetic diversity and 14 accessions from other genera of the Bromeliaceae for phylogenetic purposes (Duval et al. 2003). No sister group was evidenced among these bromeliads. *A. macrodontes* and *A. comosus* varieties were represented by 11 haplotypes and formed a monophyletic assemblage with three strongly supported groups. Two of these groups are consistent with the nuclear data analysis and with geographical data.

The first group includes the tetraploid *A. macrodontes*, represented by only one haplotype and the diploid *A. fritzmuelleri* Camargo, both from the south of the subcontinent and adapted to low light conditions. The contrast in *A. macrodontes* exhibiting high nuclear but low cytoplasmic diversity favors the hypothesis of a recent speciation process by autopolyploidization. The nature of its parental relationship with *A. fritzmuelleri* Camargo is difficult to evaluate because of the extreme rarity of the latter (no accession could be recovered during the 1990s prospecting expeditions).

The second group includes the majority of *A. comosus* var. *parguazensis* accessions, all from the Rio Negro region. The third and largest group includes cultivated forms, *A. comosus* var. *comosus* and *A. comosus* var. *erectifolius*, as well as wild forms, *A. comosus* var. *ananassoides*, and the remaining accessions of *A. comosus* var. *parguazensis*, from the whole *Ananas* distribution range.

The comparison of molecular data obtained using uniparentally and biparentally inherited markers indicate hybridization between these groups in the Rio Negro region, as well as the hybrid status of *A. bracteatus sensu* Smith & Downs from the south.

2.4.2 Domestication Processes

Pineapple was domesticated more than 3,500 years ago, as shown by archaeobotanical remains dated from 1200 to 800 BC (Pearsall 1992) and glottochronological data indicate that the crop has been highly significant to Mesoamerican people for more than 2,500 years (Brown 2010). A likely time frame for the divergence between wild and cultivated pineapple lies between 6,000 and 10,000 years BP. Yet, it has not resulted in such a clear morphological or genetic differentiation as to make it a different species.

Molecular studies and morphological observations have suggested a two-phase pathway for the domestication and differentiation of the cultivated pineapple. Indeed, two hot spots for cultivated *A. comosus* var. *comosus* diversity were found. The first one rests in the eastern Guiana Shield and hosts a wide nuclear and cytoplasmic diversity along with a number of intermediate forms between *A. comosus* var. *comosus* and the wild *A. comosus* var. *ananassoides* that is commonly observed in the forest. These intermediate forms are noticeable by their variation in fruit size. These data point out this region as a likely primary center of domestication for the fruit. The second hot spot lies in the upper Amazon. No wild or intermediate forms have been found in this region, which appears as an important center of diversification of agriculture (Schultes 1984; Clement 1989) and could be a center of diversification for the domesticated pineapple. The plant would have been brought there by humans, which allowed for completion of the domestication process while in the absence of counteracting gene flow from wild forms.

A. comosus var. *erectifolius* is cultivated for its fiber and is morphologically very similar to the variety

ananassoides, except for the smooth character of the leaf. Its very high genetic diversity, scattering in the phenetic and phylogenetic trees, and proximity with various *ananassoides* genotypes, generally from the same origins, indicate that the variety *erectifolius* evolved directly from the variety *ananassoides* following convergent domestication processes in various places.

The third cultivated pineapple, *A. bracteatus sensu* Smith & Downs, is limited to the southeast of the subcontinent where it is grown as a fence. This form is very homogenous, displays the most common cytoplasmic haplotype shared with other cultivated forms and the variety *ananassoides*, and shares nuclear markers specific to the southern group constituted by *A. macrodontes* and *A. fritzmuelleri* Camargo. These data point out this form as a hybrid between representatives of these two groups.

2.5 Role in Development of Cytogenetic Stocks and Their Utility

Some commercial cultivars of local importance such as the Puerto Rican "Cabezona" are triploid (Lin et al. 1987). Furthermore, some wild clones such as the *ananas dos indios* population from Aguas Emendadas, near Brasilia, have seedless fruit that appear larger than those of other wild clones in this region (Dujardin 1991). Indeed, the production of 0–6.5% of diploid gametes, in diploid plants, results in the spontaneous formation of triploids and, more rarely, tetraploids. The latter produce about 90% viable pollen, through regular meiosis, and when crossed with diploid plants, they produce a few viable seeds, giving vigorous, healthy tetraploid seedlings (Collins 1960). Despite this vigor, polyploidy in itself does not look promising for pineapple breeding. Autotetraploids of "Smooth Cayenne" have a vegetative growth period 5 weeks longer than diploids and give smaller fruits with fewer, but larger, fruitlets and lower sugar content; however self-incompatibility is not affected (Collins 1960).

Collins (1933) emphasized the possibility of producing triploids by exploiting chromosome non-reduction in the pistillate parent to retain all the characters of a good heterozygote, avoiding sexual recombination, and adding new genes and characters to an existing cultivar. However, spontaneous triploidization appears too infrequent and unpredictable for its exploitation.

When *A. comosus* is crossed with *A. macrodontes*, 5–10% of the seeds formed are viable and give hybrids that are tetraploid, vigorous, highly fertile, and self-fertile. A few rare triploids, which are smaller, sterile, and resemble more the tetraploid parent, are also produced (Collins 1960).

2.6 Role in Classical and Molecular Genetic Studies

2.6.1 Morphological and Agronomic Traits

As observed by Collins (1960), hybridizations between botanical varieties are comparable with crosses between cultivars of *A. comosus* var. *comosus*, so there is no limit in transferring traits from one form to the other. The most investigated one concerns the presence of spines along the leaf margin, a very important trait as far as crop management is concerned. Collins and Kerns (1946) have shown that it is mainly governed by two genes, *S* and *P*, and their results have been corroborated at CIRAD in Martinique and at EMBRAPA in Brazil (Cabral et al. 1997). The recessive *s* allele determines the common spiny phenotypes. The dominant *S* allele determines the "spiny tip" phenotype, with only partial spininess, often concentrated at the leaf tip, as in "Smooth Cayenne". The third allele of the series is *Se*, which is found in *A. comosus* var. *erectifolius*. It is dominant over both *S* and *s*. The *P* gene, only found in cultivars, controls the "piping" character, which consists in the folding of the lower epidermis over the leaf margin, resulting in a complete absence of spines. It is dominant, with an epistatic effect upon the *S* gene. According to Collins (1960), a third gene, named *B*, interferes with the *S* gene. Its dominant allele, present in *A. comosus* var. *bracteatus*, would have determined a 3:1 spiny/"spiny tip" phenotypic ratio and a similar ratio in one F$_2$ family. However, the same cross was repeated in Martinique, producing a 1:1 ratio, as expected from the segregation of the *S* gene only.

Another simple genetic trait concerns the presence of high anthocyanin density, giving the plant a dark red color. Its effect is so clear that it can be easily differentiated from the quantitative effects of minor genes for the red pigmentation. The dominant dark red allele can be found in *A. comosus* var. *erectifolius*, as well as in a few cultivars of *A. comosus* var. *comosus* (e.g., "Roxo de Tefé" and "Red Mundo Nuevo"). The major genes for spininess and anthocyanins are not linked (Cabral et al. 1997).

Acid and sugar content are higher in *A. comosus* var. *ananassoides* than in *A. comosus* var. *comosus*. Their hybrid progenies segregate widely for these traits, with values that are intermediate for acidity, but closer to the wild parent, particularly for sugar content. Their fruits have very pleasant flavors. When *A. comosus* var. *erectifolius* is used as a parent, fruits of the hybrid can be even sweeter, with refractometric indices commonly above 20°Brix. However, these fruits are smaller and highly fibrous. The gain in sugar content is generally lost in subsequent backcrosses onto *A. comosus* var. *comosus*, as fruit size increases to more normal levels. Collins and Hagan (1932) observed that the progeny from a cross between such a wild pineapple and cv. Smooth Cayenne retained its high tolerance to the root-knot nematode *Meloidogyne javanica*. On the other hand, comparable levels of tolerance are found in some cultivars. This and other examples of pest and disease resistance breeding are discussed in Sect. 2.7.

When *A. comosus* var. *bracteatus* is used as a parent, larger fruits are obtained, weighing from 0.56 to 3.20 kg, with a wide range of variation in flavor (Collins 1960; Geo Coppens d'Eeckenbrugge unpublished). In addition, these three varieties present interesting characters of rusticity, such as a strong root system, resistance to nematodes, wilt, heart rot, and root rot, which are transmitted to the hybrid progenies (Collins 1960).

Interspecific hybrids within *Ananas* present an intermediate morphology, with crowned fruits and shoots from the stem as well as from short stolons. Their leaf margins are wavy like those of their *A. macrodontes* parent, some with the same large spines. When the cultivated parent bears a mutation suppressing spines, these tetraploid hybrids segregate for this trait. They are self-fertile, as their *A. macrodontes* parent, and produce very seedy fruits. Backcrossing to *A. comosus* var. *comosus* reduces this high fertility and increases morphological variation, with some plants approaching the backcross parent (Collins 1960).

2.6.2 Molecular Genetics and Genome Mapping

Carlier et al. (2004) published the first pineapple genetic map and used the two-way pseudo-testcross approach to construct two individual maps of botanical varieties *comosus* and *bracteatus* using a segregating population of 46 F_1 individuals from fully fertile crosses between the two varieties. To construct the map, a combination of three different types of markers was used: random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and intersimple sequence repeats (ISSRs). The *A. comosus* var. *comosus* map contained 157 markers (33 RAPDs, 115 AFLPs, eight ISSRs, and the piping locus) with 30 linkage groups, 18 of which assembled four markers or more (Carlier et al. 2004). A relatively large percentage (43%) of markers remained unlinked, a fact perhaps reflecting the small size of the mapping population. This map covered approximately 31% of the *A. comosus* var. *comosus* genome estimated as 4,146 cM with a calculated ratio of 127 kb/cM for the relationship between physical and genetic distance. In the case of *A. comosus* var. *bracteatus*, 50 linkage groups were established containing 335 markers (60 RAPDs, 264 AFLPs, and 11 ISSRs) with 26 linkage groups containing at least four markers. In this case, map coverage is approximately 57.2% of the genome calculated as 3,693 cM with a ratio of 120 kb/cM.

Since the publication of the first *A. comosus* linkage map, selfing of one of the F_1 plants at the CIRAD collection at Martinique has produced an F_2 population for further mapping. J. Leitão's group has greatly improved the quality and resolution of the genetic map and new versions have been published (Carlier et al. 2007; Botella and Smith 2008). The linkage groups shown in the latest map gather a total of 651 markers, with 505 AFLP, 124 RAPD, 20 simple sequence repeats (SSRs), 1 express sequence tag (EST), and 1 morphological trait (piping).

With respect to pineapple genes that have been isolated, cloned, and characterized, these include an

ACC synthase and an ACC oxidase (Cazzonelli et al. 1998); a NAD$^+$-dependent malate dehydrogenase (Cuevas and Podestá 2000); ananain (Carter et al. 2000); a Cu/Zn-superoxide dismutase (Lin et al. 2000); two distinct polyphenol oxidases (Stewart et al. 2001); and the cysteine protease inhibitor cystatin (Shyu et al. 2004). A retrotransposon-like sequence, repeatedly integrated in the genome in multiple variable sequences and still potentially capable of transposing (Thomson et al. 1998), and the genomic sequence coding for bromelain inhibitors (Sawano et al. 2002) have also been isolated and characterized. Moreover, recent studies on genes involved in root development (Neuteboom et al. 2002) and in fruit ripening and nematode–root interactions (Moyle et al. 2005a, b, 2006) have resulted in a very large number of sequenced ESTs.

2.7 Role in Crop Improvement Through Traditional and Advanced Tools

As there are no reproductive barriers among botanical varieties of *A. comosus*, wild and semi-domesticated pineapple germplasm may bring considerable variation for the benefit of pineapple breeding. Hybridization generates a wide variation in most traits, which opened prospects for new shape and colors for the fruit (Fig. 2.8), the introduction of resistance/tolerance traits into the main crop, and also the perspective of exploiting wild pineapple germplasm in the ornamental plant market.

2.7.1 Characters of Interest in Hybrid Breeding for the Fruit

Wild pineapple is highly efficient in its vegetative propagation, multiplying from stem suckers, slips, the crown, and even multiple crowns. Suckering is still important in *A. comosus* var. *bracteatus* and is particularly spectacular in *A. comosus* var. *erectifolius*. However, vegetative multiplication must be limited by the breeder in *A. comosus* var. *comosus* to obtain only a few basal stem suckers, allowing a further cultivation cycle or providing strong material for planting anew.

Tolerance to drought is remarkable in *A. comosus* var. *ananassoides*. However, an ample range of tolerance also exists among cultivars, and the use of a tolerant cultivar such as "Perola" would save the extra cost of several backcross generations.

The highest potential of hybrid breeding between botanical varieties lies in the transfer of resistance/tolerance to major pests and diseases. Thus, clones of *A. comosus* var. *ananassoides*, *A. comosus* var. *erectifolius*, and *A. macrodontes* are thought to exhibit levels of resistance to the nematodes *Rotylenchulus reniformis* and *Meloidogyne javanica*, allowing very low levels of nematode reproduction (Ayala 1961;

Fig. 2.8 Example of variation obtained through hybridization of the large-fruited *A. comosus* var. *comosus* (*upper right*) with small-fruited wild or primitive pineapples (photograph courtesy of Garth Sanewski)

Ayala et al. 1969; Sipes and Schmitt 1994). It is difficult, however, to test for relative differences in genetic resistance to nematodes because of the underlying genetic differences in plant growth parameters and responses to environment. Various methods of quantifying the effect of nematode challenge have been reported for pineapple including assessment of number and distribution of galls or lesions, as well as the effect on root and plant growth. The difficulty of assessment is probably partially responsible for conflicting assessments of genetic resistance and/or tolerance in pineapple.

In early work by Collins and Hagan (1932), an *A. comosus* var. *comosus* x *A. comosus* var. *ananassoides* hybrid progeny called "Lot 520" was considered as highly tolerant of the nematode, *Meloidogyne* sp. Although it was infected by the nematode, there were few galls and plant and root growth were little affected. Ayala et al. (1969) also reported that *A. comosus* var. *ananassoides* has good resistance to infection by *M. incognita* and *R. reniformis*. Sarah et al. (1997) tested *A. comosus* var. *ananassoides*, *A. comosus* var. *parguazensis, A. comosus* var. *bracteatus*, and many *A. comus* var. *comosus* clones for resistance to *Pratylenchus brachyurus* and found none were clearly resistant, whereas *A. comosus* var. *comosus* "Perola" was the least affected. Dinardo-Miranda et al. (1996) tested 13 varieties, mainly clones of *A. comosus* var. *comosus* for resistance to *M. incognita*, and found that only "Huitoto" could be considered as a poor host. Sipes and Schmitt (1994) reported on the most comprehensive study and found *A. comosus* var. *ananassoides* and "Lot 520" supported high levels of both *M. javanica* and *R. reniformis*. In that study, "Smooth Cayenne" was the most tolerant variety. Soler et al. (2009) found the *A. comosus* var. *comosus* hybrid "MD-2" displayed little impact on vegetative growth following infection by *R. reniformis*. One clone of *A. comosus* var. *ananassoides* was also found to tolerate infection very well and another moderately well. Williams and Fleisch (1993) reported that the *A. comosus* var. *comosus* hybrid clone "57-3" displays little growth suppression when infected with nematodes.

A review of the various studies indicates that there are conflicting reports regarding genetic resistance. Usually, the more comprehensive studies suggest that there are probably no genotypes that resist infection by any species of nematode to any extent, but some genotypes tolerate infection better than others. Often the varieties with the least vegetative growth depression will be those with a relatively smaller vegetative mass (Williams and Fleisch 1993). The domesticated *A. comosus* var. *comosus* clones of "Perola" and "Cayenne", as well as many hybrids, appear to offer some tolerance as do some clones of *A. comosus* var. *ananassoides*. The latter is suspected of exhibiting some resistance. Given the large diversity within *A. comosus* var. *ananassoides*, it would not be surprising to find more examples of genetic tolerance to nematode, but the usefulness of this for breeding is questionable given the primitive nature of theses clones. Resistant clones would, however, have significant use in molecular studies.

Resistances to the root pathogens *Phytophthora cinnamomi* and *Phytophthora nicotianae* var. *parasitica* are also reported for wild varieties of pineapple. This work was started by the Pineapple Research Institute (PRI) in Hawaii in 1936. Tests of various hybrids developed by the PRI indicated a level of resistance particularly in hybrids involving *A. comosus* var. *bracteatus* and *A. comosus* var. *ananassoides*. As a consequence, an expedition to South America was undertaken in 1937 to collect wild species and landraces that could be used for resistance breeding (Anderson and Collins 1949). From 1936 to the 1960s, many cultivars with resistance to *P. cinnamomi* and or *P. nicotianae* var. *parasitica* were developed by the PRI from the germplasm collected in South America. Of the germplasm collected, *A. macrodontes* was considered the most resistant, almost immune, but developing commercially acceptable varieties with it as a parent was very slow. No commercial varieties using *A. macrodontes* were ever developed despite a dominant resistance mechanism, mainly because of the poor fruit quality. *A. comosus* var. *bracteatus* was considered highly resistant and progress in breeding commercial types using it was relatively quick. *A. comosus* var. *ananassoides* was moderately to highly resistant and proved to be the most useful parent (Collins 1953). Most of the *P. cinnamomi* and *P. nicotianae* var. *parasitica* resistant varieties developed have approximately one-sixteenth *A. comosus* var. *ananassoides* in their parentage (Williams and Fleisch 1993). *A. comosus* var. *erectifolious* had a similar level of resistance as *A. comosus* var. *ananassoides*, but was a poor parent for other agronomic characteristics. No resistant, commercial varieties

with *A. comosus* var. *erectifolious* parentage were developed. The *A. comosus* var. *comosus* varieties "Red Spanish" and "Pernambuco" were also moderately resistant and contained many good agronomic traits (Smith 1966). Using these varieties, the PRI breeding program incorporated heart rot and root rot resistance into many varieties all with a reasonable complement of other desirable agronomic characters. This was achieved by backcrossing an F_1 progeny onto a commercial variety, usually one with a high proportion of "Smooth Cayenne" genes, over two to three generations (Collins 1953). While none of these varieties are grown commercially today, many would be worthwhile parents for use in breeding where *P. cinnamomi* or *P. nicotianae* var. *parasitica* resistance is a targeted trait. Some of these varieties are held by the University of Hawaii (Williams and Fleisch 1993). Resistant varieties include "PRI-10388" syn. "Spanish Jewel," "PRI-59-656," "PRI-52-323," and "PRI-61-2223" (Smith 1965; Rohrbach and Johnson 2003). Two of these, "PRI-59-656" and "PRI-52-323," were grown commercially on a small scale in Hawaii before improved chemical control methods and high yielding "Smooth Cayenne" clones became available (Williams and Fleisch 1993).

While most resistant varieties developed had *A. comosus* var. *ananassoides* in their parentage, some resistant varieties were derived from two apparently susceptible parents. Resistance was considered quantitative and additive (Smith 1966). Varieties could differ in their susceptibility to both *P. cinnamomi* and *P. nicotianae* var. *parasitica* (Johannessen and Kerns 1964). The variety "59-656" is claimed to possess good resistance to both the pathogens (Smith 1965).

2.7.2 Characters of Interest in Hybrid Breeding for Ornamental Plants

The family Bromeliaceae is well recognized for its extraordinary diversity and ornamental appeal. However, until very recently, *Ananas* has not been exploited significantly as an ornamental, as was the case of a great number of other genera in the family. Small but increasing quantities of *Ananas* plants and blooms are now being marketed in various countries for their ornamental appeal, usually *A. comosus* var.

bracteatus "Tricolor" and *A. comosus* var. *erectifolious* "Selvagem 6" (Fig. 2.5). Both these varieties, while currently commercially exploited, have limitations and do not incorporate the breadth of ornamental potential within the *Ananas* gene pool. There remains exciting potential for further breeding. Breeding programs for ornamental pineapple are reported for Brazil, Australia, France, and Malaysia (Duval et al. 2001a; Chan 2006; Souza et al. 2006, 2009; Sanewski 2009).

Several markets exist for ornamental *Ananas* products, each with an emphasis on different plant characteristics. These markets include the cut-flower market for pre-petal syncarps, miniature fully formed fruit, and attractive cut foliage (F. Vidigal personal communication). The landscape or potted plant market will also take plants with ornamental fruit or foliage characteristics.

For attractive blooms, *A. comosus* var. *bracteatus* is good for imparting a bright red coloration to the syncarp and *A. macrodontes* will impart a pink color. *A. comosus* var. *erectifolious* "Selvagem 6" is a good parent for obtaining smooth reddish leaves, including those in the crown. An example of this hybrid is shown in Fig. 2.9.

For miniature fruit, *A. comosus* var. *ananassoides* is a good parent, as is *A. comosus* var. *erectifolious*. It is important that the small fruit has a strong attachment to a long (50 cm), thin stem and the crown is well formed with no side shoots. Large fruits and fruit on a short stem are less useful in flower arrangements.

Potted or landscape plants should have an attractive foliage, possibly variegated or reddish in color with smooth leaf margins. A dwarf, clumping habit is desirable for potted plants. An attractive syncarp and miniature fruit are also desirable. Again, *A. comosus* var. *ananassoides*, *A. comosus* var. *erectifolious*, and *A. comosus* var. *bracteatus* are excellent parents.

Of all the *Ananas*, *A. comosus* var. *ananassoides* displays considerable diversity in fruit and leaf color and appearance. The collection of *Ananas* held by EMBRAPA holds accessions highly suited as parental stock (Souza et al. 2006). Interspecific crosses also show ornamental interest (Fig. 2.9), and the potential for utilizing other genera might also exist. Most Bromeliaceae contain the same diploid number of 50 chromosomes as *Ananas* (Brown et al. 1997). Successful intergeneric hybrids with *Ananas* are reported for *Aechmea*, *Cryptanthus*, *Neoregelia* (Anonymous

Fig. 2.9 Two smooth-leaved hybrids obtained from crosses between *A. comosus* var. *bracteatus* and *A. comosus* var. *erectifolius* (*left*), or a "piping" leaved *A. comosus* var. *comosus* cultivar with *A. macrodontes* (*right*), both selected for the cut-flower market (photographs courtesy of Garth Sanewski)

2007), and *Tillandsia* (Valds et al. 1998). Many of the other genera of Bromeliacae exhibit greater diversity of foliage morphology and color than do *Ananas*, but none produce an attractive small fruit. The potential for combining the interesting decorative fruit form of *Ananas* and more striking foliage morphology and color might therefore exist.

2.7.3 Advanced Tools for Crop Improvement

Protoplast culture and somatic hybridization, as a tool for introgression of genes, have had no impact on pineapple improvement to our knowledge. There has been a successful attempt to isolate protoplasts of the cultivar "Perolera" (Guedes et al. 1996), but plant regeneration was not achieved.

Pineapple transformation, however, offers the possibility to make small targeted changes to the recipient plant's genome and is seen as an excellent strategy for genetic improvement. A review of pineapple transformation has recently been published (Ko et al. 2008) and methods involving the introduction of recombinant DNA to pineapple cells and tissues via *Agrobacterium tumefaciens*-mediated transformation and direct gene transfer through microprojectile bombardment are reported. Biolistics has been used to deliver genes conferring herbicide resistance (Sripaoraya et al. 2001) and blackheart resistance (Ko et al. 2006) into "Smooth Cayenne." Other groups focused on using *Agrobacterium* to introduce ACC synthase genes to control ripening (Firoozabady et al. 2006; Trusov and Botella 2006).

Despite these advances, consumer resistance to transgenic fresh fruit is limiting wider use of this technology. Incorporation of only native genes from wild relatives and with expression only in plant parts not intended for consumption is the approach worth considering. In addition, before businesses and institutions will have freedom to operate with transgenic lines, intellectual property ownership must be ascertained, and strategies put in place to ensure plants are free from encumbrance, which would otherwise restrict the sale of product.

2.8 Genomics Resources Developed

The amount of genomic data in databases is still scanty, despite the economic importance of pineapple, but has been increasing in the last few years. A search for pineapple genomic data through the National

Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) found about 60 microsatellite and other DNA marker loci from var. *bracteatus* and over 5,700 ESTs from var. *comosus*. About 140 SSR markers have also been published on EMBL database (http://srs.ebi.ac.uk), the main contributors being the Biotechnology Research Institute of Malaysian Sabah University for 76 SSRs (Kumar et al. unpublished) and CIRAD in France with 50 SSRs (Blanc et al. unpublished). Also, recently an entire collection of ESTs was generated during an investigation into fruit ripening and nematode–plant interactions during root invasion (Moyle et al. 2006) and has been made publicly available by an online pineapple bioinformatics resource named "PineappleDB" (http://www.pgel.com.au).

2.9 New Perspectives for Commercial Development

The pineapple yields many products in addition to the edible fruit. Crude extracts from the fruit, stem, and leaves yield several proteinases, mainly bromelain but also ananain (Rowan et al. 1988; Lee et al. 1997) and macrodontain's I and II (López et al. 2001). Bromelain has demonstrated broad bioactivity including antiedematous, anti-inflammatory, antithrombotic, fibrinolytic, immunomodulatic (Maurer 2001), and anthelminthic (Aye et al. 1996; Hordegen et al. 2006). Innovative studies where Chinese cabbage plants were transformed with a bromelain construct demonstrated enhanced resistance to a bacterial soft rot (Jung et al. 2008). Fiber extracted from the pineapple leaf is processed into paper, cloth, and composite plastics (Hepton and Hodgson 2003). The domesticated *A. comosus* var. *comosus* is the predominant source of these products primarily because it is cultivated on a large scale for fruit, making the extraction of additional compounds cost-effective. The primitive forms of pineapple have, however, been traditionally used in similar ways by indigenous people of South America. *A. comosus* var. *ananassoides* is the principal wild pineapple. A drink made from the fruit of the wild *ananassoides* is considered by some indigenous Amazonian tribes to have an abortive effect and this activity has been supported by clinical studies (Nakayama et al. 1993). *A. comosus* var. *ananassoides* is also commonly used in central Brazil for

gastric pain. Recent studies (Silva et al. 2008) have demonstrated antiulcerogenic properties, which support this traditional use. *A. comosus* var. *erectifolious* could be considered as semi-domesticated and is now grown on a commercial basis for its leaf fiber (Leão et al. 2009). The "Curaua" leaf fiber has traditionally been used for twine, cloth, fishing line, nets, hammocks, etc. (Boom and Moestl 1990; Leal and Amaya 1991), but is now being tested in biocomposites with potential for the automotive industry (Zah et al. 2007).

2.10 Concluding Remarks

The indigenous people of South America have led the process of domestication and selection of the pineapple with so much success that only a few hybrid fruit cultivars have been produced through systematic breeding out of tropical America (Leal and Coppens d'Eeckenbrugge 1996). To adapt the crop to intensive cultivation and current standards, modern plant breeders attempt to remove the remaining undesirable "wild" traits such as natural flower initiation, small fruit size, excessive vegetativeness and long peduncles from parental stock. There appears limited scope, however, to revisit the use of most of the wild clones in breeding programs for fruit production as their phytomorph is highly unsuited to modern, efficient fruit production systems. *A. comosus* var. *ananassoides* in particular, while represented by a diverse range of clones, is too primitive in form to be of immediate use in fruit breeding. Its homozygotic tendencies for spiny leaves, vegetative growth characteristic and generally small, often unpalatable fruit are highly unsuited. Hybridization to improve on these characteristics would require many generations and almost all the desirable characteristics are already present in more commercially suited *A. comosus* var. *comosus* clones. Collins (1960) estimated that one generation and four backcrosses taking 20–25 years would be needed to produce commercial types from wild clones. This delay might be slightly shortened if clones with larger and fleshier fruits from northern South America are considered. *A. comosus* var. *bracteatus*, on the other hand, is less homozygotic for wild traits when used as a parent with *A. comosus* var. *comosus* clones and fewer generations are needed so

it might have greater potential for use in breeding. Finally, it must be kept in mind that several hybrids resulting from ancient programs of introgression of genes from wild varieties still exist in germplasm collections.

Many of the useful resistance traits are probably polygenic making gene acquisition difficult. While this might be the current picture, it is also possible that some useful resistance traits remain undiscovered as very few of the wild clones have ever been investigated. Most have only been collected in recent times. The increasing pressure toward more environment-friendly cultivation methods may soon give a much higher priority to the research on mechanisms and sources of resistances.

While the potential use of wild types for fresh fruit breeding awaits detailed characterization of collections, many are immediately useful for other purposes. As an example, many of the *A. comosus* var. *ananassoides*, *A. comosus* var. *bracteatus*, *A. comosus* var. *erectifolious*, and *A. macrodontes* are highly suited to the production of ornamental varieties. The other industrial and pharmaceutical uses are yet to be fully investigated.

There is no doubt that the *Ananas* collections held in various centers are an unexploited resource that needs further investigation to realize their full potential.

References

Anderson EJ, Collins JL (1949) Progress in breeding for resistance to heart rot – root rot. Spl Rep PRI 16:8–13

Anonymous (2007) International Bromeliad Society Website. www.bsi.org/

Aradhya MK, Zee F, Manshardt RM (1994) Isozyme variation in cultivated and wild pineapple. Euphytica 79:87–99

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Ayala A (1961) An analysis of quantitative and qualitative composition of nematodes populations in pineapple fields in Puerto Rico. J Agric Univ Puerto Rico 45:285–299

Ayala A, González-Tejera E, Irizarry H (1969) Pineapple nematodes and their control. In: Peachey JE (ed) Nematodes of tropical crops. CABI, Wallingford, UK, pp 210–224

Aye T, Yi Mon M, Tha SJ, Chit M (1996) Anthelminthic property of Myanmar pineapple (Nanat) on experimental models. Myan Health Sci Res J 8:14–19

Baker KF, Collins JL (1939) Notes on the distribution and ecology of *Ananas* and *Pseudananas* in South America. Am J Bot 26:697–702

Bello S, Julca A (1993) Colección y evaluación de ecotipos de piña *Ananas comosus* de la Amazonía peruana. Memorias Primer Simposio Latinoamericano de Piñicultura, Cali, Colombia, 25–29 May 1993, p 1–13

Bertoni MS (1919) Contribution à l'étude botanique des plantes cultivées. I. Essai d'une monographie du genre *Ananas*. Ann Cient Parag (Ser II) 4:250–322

Boom BM, Moestl S (1990) Ethnobotanical notes of Jose M. Cruxent from the Franco-Venezuelan expedition to the headwaters of the Orinoco river, 1951–1952. Econ Bot 44: 416–419

Botella JR, Smith M (2008) Genomics of pineapple, crowning the king of tropical fruit. In: Moore PH, Ming R (eds) Genomics of tropical crop plants. Springer, New York, USA, pp 441–451

Brewbaker JL, Gorrez DD (1967) Genetics of self-incompatibility in the monocot genera, *Ananas* (pineapple) and *Gasteria*. Am J Bot 54:611–616

Brown CH (2010) Development of agriculture in prehistoric Mesoamerica: the linguistic evidence. In: Staller JE, Carrasco MD (eds) Pre-Columbian foodways: interdisciplinary approaches to food, culture, and markets in Mesoamerica. Springer, New York, USA, pp 71–107

Brown GK, Palaci CA, Luther HE (1997) Chromosome numbers in Bromeliaceae. Selbyana 18:85–88

Cabral JRS, de Matos AP, Coppens d'Eeckenbrugge G (1997) Segregation for resistance to fusariose, leaf colour and leaf margin type from the EMBRAPA pineapple hybridization programme. Acta Hortic 425:193–200

Camargo F (1943) Vida e utilidade das Bromeliáceas. Bol Tec Instituto Agronómico do Norte, Belem, Pará, Brazil

Capinpin JH, Rotor GB (1937) A cytological and morphogenetic study of some pineapple varieties and their mutant and hybrid derivatives. Philip Agric 26:139–158

Carlier JD, Reis A, Duval MF, Coppens d'Eeckenbrugge G, Leitão JM (2004) Genetic maps of RAPD, AFLP and ISSR markers in *Ananas bracteatus* and *Ananas comosus* using the pseudo-testcross strategy. Plant Breed 123:186–192

Carlier JD, Coppens d'Eeckenbrugge G, Leitão JM (2007) Pineapple. In: Kole C (ed) Genomic mapping and molecular breeding in plants, vol 4, Fruits and nuts. Springer, Berlin, pp 331–342

Carter CE, Marriage H, Goodenough PW (2000) Mutagenesis and kinetic studies of a plant cysteine proteinase with an unusual arrangement of acidic amino acids in and around the active site. Biochemistry 39:11005–11013

Cazzonelli CI, Cavallaro AS, Botella JR (1998) Cloning and characterisation of ripening-induced ethylene biosynthetic genes from non-climacteric pineapple (*Ananas comosus*) fruits. Aust J Plant Physiol 25:513–518

Chan YK (2006) Hybridisation and selection in pineapple improvement. The experience in Malaysia. Acta Hortic 702:87–92

Clement CR (1989) A center of crop genetic diversity in western Amazonia. BioScience 39:624–631

Collins JL (1933) Morphological and cytological characteristics of triploid pineapples. Cytologia 4:248–256

Collins JL (1953) Review of breeding for resistance to heart rot and root rot. PRI News 1:73–78

Collins JL (1960) The pineapple. Interscience, New York, NY, USA

Collins JL, Hagan HR (1932) Nematode resistance of pineapples. Varietal resistance of pineapple roots to the nematode *Heterodera radicicola* (Greef) Muller. J Hered 23 (459–465):503–511

Collins JL, Kerns KR (1931) Genetic studies of the pineapple. I. A preliminary report upon the chromosome number and meiosis in seven pineapple varieties (*Ananas sativus* Lindl.) and in *Bromelia pinguin* L. J Hered 22:39–142

Collins JL, Kerns KR (1946) Inheritance of three leaf types in the pineapple. J Hered 37:123–128

Coppens d'Eeckenbrugge G, Leal F (2003) Morphology, anatomy and taxonomy. In: Bartholomew DP, Paull RE, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI, Wallingford, Oxford, UK, pp 13–32

Coppens d'Eeckenbrugge G, Duval MF, Van Miegroet F (1993) Fertility and self-incompatibility in the genus *Ananas*. Acta Hortic 334:45–51

Coppens d'Eeckenbrugge G, Leal F, Duval MF (1997) Germplasm resources of pineapple. Hortic Rev 21:133–175

Corrêa MP (1952) Diccionário das Plantas úteis do Brasil e das Exóticas Cultivadas, vol III. Imprensa Nacional, Rio de Janeiro, Brazil

Cuevas IC, Podestá FE (2000) Purification and physical and kinetic characterization of an NAD+−dependent malate dehydrogenase from leaves of pineapple (*Ananas comosus*). Physiol Plant 108:240–248

Dinardo-Miranda LL, Spironello A, Martins ALM (1996) Host suitability of pineapple varieties for *Meloidogyne incognita* race 1. Bragantia 55:275–278

Dujardin M (1991) Cytogénétique de l'ananas. Fruits 46:376–379

Duval MF, Coppens d'Eeckenbrugge G, Fontaine A, Horry JP (2001a) Ornamental pineapple: perspective from clonal and hybrid breeding. Pineapple News 8:12

Duval MF, Coppens d'Eeckenbrugge G (1993) Genetic variability in the genus *Ananas*. Acta Hortic 334:27–32

Duval MF, Coppens d'Eeckenbrugge G, Ferreira FR, Cabral JRS, Bianchetti LDB (1997) First results from joint EMBRAPA-CIRAD *Ananas* germplasm collecting in Brazil and French Guyana. Acta Hortic 425:137–144

Duval MF, Noyer JL, Perrier X, Coppens d'Eeckenbrugge G, Hamon P (2001b) Molecular diversity in pineapple assessed by RFLP markers. Theor Appl Genet 102:83–90

Duval MF, Buso GC, Ferreira FR, LdB B, Coppens d'Eeckenbrugge G, Hamon P, Ferreira ME (2003) Relationships in *Ananas* and other related genera using chloroplast DNA restriction site variation. Genome 46:990–1004

Ferreira FR, Giacometti DC, LdB B, Cabral JRS (1992) Coleta de germoplasma de abacaxizeiros (*Ananas comosus* (L.) Merril) e espécies afins. Rev Bras Frut 14:5–11

Firoozabady E, Heckert M, Gutterson N (2006) Transformation and regeneration of pineapple. Plant Cell Tiss Organ Cult 84:1–16

García ML (1988) Etude taxinomique du genre *Ananas*. Utilisation de la variabilité enzymatique. Doctoral Thesis, University of Science and Technology, Languedoc, Montpellier, France

Gitaí J, Horres R, Benko-Iseppon AM (2005) Chromosomal features and evolution of Bromeliaceae. Plant Syst Evol 253:65–80

González-Arnao M, Márquez-Ravelo M, Urra-Villavicencio C, Martínez-Montero M, Engelmann F (1998) Cryopreservation of pineapple (*Ananas comosus*) apices. CryoLetters 19:375–382

Guedes NMP, Maria J, Zambolin L, Ventura JA (1996) Protoplast isolation of *Ananas comosus* (L.) Merr. cv 'Perolera'. Acta Hortic 425:259–265

Heilborn O (1921) Notes on the cytology of *Ananas sativus* Lindl. and the origin of its parthenocarpy. Ark Bot 17:1–7

Hepton A, Hodgson AS (2003) Pineapple: processing. In: Bartholomew DP, Paull RE, Rohrbach K (eds) The pineapple, botany, production and uses. CABI, Wallingford, UK, pp 281–290

Hernández MS, Montoya D (1993) Recolección de piña y otras especies de *Ananas* en Colombia. Memorias Primer Simposio Latinoamericano de Piñicultura, Cali, Colombia, 25–29 May 1993 (posters)

Hordegen P, Cabaret J, Hertzberg H, Langhans W, Maurer V (2006) In vitro screening of six anthelmintic plant products against larval *Haemonchus contortus* with a modified methyl-thiazolyl-tetrazolium reduction assay. J Ethnopharmacol 108:85–89

Johannessen GA, Kerns KR (1964) Screening and classifying new varieties for resistance to *P. cinnamomi* and *P. parasitica*. PRI Res Rep 111:96–100

Jung Y, Choi C, Park J, Kang H, Choi J, Nou I, Lee S, Kang K (2008) Overexpression of the pineapple fruit bromelain gene (BAA) in transgenic Chinese cabbage (*Brassica rapa*) results in enhanced resistance to bacterial soft rot. Electron J Biotechnol 11:7

Kerns KR (1932) Concerning the growth of pollen tubes in pistils of Cayenne flowers. Pineapple Quart 1:133–137

Ko HL, Campbell PR, Jobin-Décor MP, Eccleston KL, Graham MW, Smith MK (2006) The introduction of transgenes to control blackheart in pineapple (*Ananas comosus* L.) cv. Smooth Cayenne by microprojectile bombardment. Euphytica 150:387–395

Ko HL, Sanewski GM, Smith MK (2008) Pineapple. In: Kole C, Hall TC (eds) Compendium of transgenic crop plants, vol 5, Transgenic tropical and subtropical fruit and nuts. Wiley-Blackwell, Chicester, Oxford, UK, pp 109–130

Leal F, Amaya L (1991) The curagua (*Ananas lucidus*, Bromeliaceae) crop in Venezuela. Econ Bot 45:216–224

Leal F, Antoni MG (1981) Especies del género *Ananas*: origen y distribución geográfica. Rev Fac Agron, Univ Central de Venezuela, 29:5–12

Leal F, Coppens d'Eeckenbrugge G (1996) Pineapple. In: Janick J, Moore JN (eds) Fruit breeding, vol I, Tree and tropical fruits. Wiley, New York, NY, USA, pp 565–606

Leal F, Medina E (1995) Some wild pineapples in Venezuela. J Bromel Soc 45:152–158

Leal F, García ML, Cabot C (1986) Prospección y recolección de *Ananas* y sus congéneres en Venezuela. Plant Genet Resour Newsl 66:16–19

Leal F, Coppens d'Eeckenbrugge G, Holst B (1998) Taxonomy of the genera *Ananas* and *Pseudananas* – an historical review. Selbyana 19:227–235

Leão AL, Machado IS, de Souza SF, Soriano L (2009) Production of Curauá (*Ananas erectifolius* L.B. Smith) fibers for industrial applications: characterization and micropropagation. Acta Hortic 822:227–238

Lee KL, Albee KL, Bernasconi RJ, Edmunds T (1997) Complete amino acid sequence of ananain and a comparison with

stem bromelain and other plant cysteine proteases. Biochem J 327:199–202

Lin BY, Ritschel PS, Ferreira FR (1987) Número cromossômico de exemplares da família Bromeliaceae. Rev Bras Fruit 9:49–55

Lin MT, Fu MT, Ken CF, Lin CT (2000) Cloning and characterization of a cDNA encoding for Cu/Zn-superoxide dismutase from pineapple. Plant Physiol 122:619

Loeillet D (2008) The European pineapple market. A cure for morosity. FruiTrop 154:7–13

López LMI, Sequeiros C, Trejo SA, Pardo MF, Caffini NO, Natalucci CL (2001) Comparison of two cysteine endopeptidases from *Pseudananas macrodontes* (Morr.) Harms (Bromeliaceae). Biol Chem 382:875–878

Loudon JC (1822) The different modes of cultivating the pineapple, from its introduction to Europe to the late improvements of T.A. Knight esq., Houlgman Hurst Resorme Brown, London, UK

Majumder SK, Kerns KR, Brewbaler JL, Johannessen GA (1964) Assessing self-incompatibility by a pollen fluorescence technique. Proc Am Hortic Sci 84:217–223

Maurer HR (2001) Bromelain: biochemistry, pharmacology and medical use. Cell Mol Life Sci 58:1234–1245

Montinola LR (1991) Piña. Amon foundation, Manila, Philippines

Moyle RL, Crowe ML, Ripi-Koia J, Fairbairn DJ, Botella JR (2005) PineappleDB: an online pineapple bioinformatics resource. BMC Plant Biol 5:21. http://www.biomedcentral.com/1471-2229/5/21. Accessed 16 July 2010

Moyle R, Fairbairn DJ, Ripi J, Crowe M, Botella JR (2005b) Developing pineapple fruit has a small transcriptome dominated by metallothionein. J Exp Bot 56:101–112

Moyle R, Ripi J, Fairbairn DJ, Crowe M, Botella JR (2006) The pineapple EST sequencing and microarray project. Acta Hortic 702:47–50

Muller A (1994) Contribution à l'étude de la fertilité et de l'autofertilité dans le genre *Ananas*. Eng Thesis, ISTOM, Montpellier, France

Nakayama L, Souza JS de, Ohash OM, Vale WG (1993) Abortifacient effects of *Ananas ananassoides* BAK. (Ananai) in rats. Acta Amazonica. 13(5-6), 77–82

Neuteboom LW, Kunimitsu WY, Webb D, Christopher DA (2002) Characterization and tissue-regulated expression of genes involved in pineapple (*Ananas comosus* L.) root development. Plant Sci 163:1021–1035

Noyer JL (1991) Etude préliminaire de la diversité génétique du genre *Ananas* par les RFLPs. Fruits 46:372–375

Noyer JL, Lanaud C, Duval MF, Coppens d'Eeckenbrugge G (1998) RFLP study on rDNA variability in *Ananas* genus. Acta Hortic 425:153–160

Patiño VM (2002) Historia y dispersión de los frutales nativos del Neotrópico. CIAT, Cali, CO

Pearsall DM (1992) The origins of plant cultivation in South America. In: Cowan CW, Watson PJ (eds) The origins of agriculture: an international perspective. Smithsonian Series in Archaeological Inquiries. Smithsonian Institution Press, Washington, DC, USA, pp 173–205

Purseglove JW (1972) Tropical crops. Monocotyledons. Longman, London, 607 p

Reitz R (1983) Bromeliáceas e a malaria – Bromélia endêmica. Flora Ilustrada Catarinense, Santa Catarina, 808 p

Reyes-Zumeta H (1967) Breve nota taxonómica sobre piñas cultivadas *Ananas comosus* (L.) Merr. con mención de dos nuevas variedades silvestres. Rev Fac Agron (Maracay) 39:131–142

Rios R, Khan B (1998) List of ethnobotanical uses of Bromeliaceae. J Bromel Soc 48:75–87

Rohrbach KG, Johnson MW (2003) Pests, diseases and weeds. In: Bartholomew DP, Paull RE, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI, Wallingford, UK, pp 203–252

Rohrbach KG, Leal F, Coppens d'Eeckenbrugge G (2003) History, distribution and world production. In: Bartholomew DP, Paull RE, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI, Wallingford, UK, pp 1–12

Rowan AD, Buttle DJ, Barrett AJ (1988) Ananain: a novel cysteine proteinase found in pineapple stem. Arch Biochem Biophys 267:262–270

Sanewski GM (2009) Breeding *Ananas* for the cut-flower and garden markets. Acta Hortic 822:71–78

Sarah JL, Mesnildrey L, Marguerite E, Boisseau M (1997) Laboratory screening of pineapple germplasm for resistance to the lesion nematode *Pratylenchus brachyurus*. Acta Hortic 425:179–186

Sawano Y, Muramatsu T, Hatano K, Nagata K, Tanokura M (2002) Characterization of genomic sequence coding for bromelain inhibitors in pineapple and expression of its recombinant isoform. J Biol Chem 277:28222–28227

Schultes RE (1984) Amazonian cultigens and their northward and westward migration in pre-Columbian times. In: Stone D (ed) Pre-Columbian plant migration, Papers of the Peabody Museum of Archaeology and Ethnology, vol 76. Harvard University Press, Cambridge, MA, USA, pp 19–37

Shyu DJH, Chyan CL, Tzen JTC, Chou WM (2004) Molecular cloning, expression, and functional characterization of a cystatin from pineapple stem. Biosci Biotechnol Biochem 68:1681–1689

Silva JS, Andreo MA, Tubaldini FR, Varanda EA, Rocha LRM, Brito ARMS, Vilegas W, Hiruma-Lima CA (2008) Differences in gastroprotective and mutagenic actions between polar and apolar extracts of *Ananas ananassoides*. J Med Food 11:160–168

Sipes BS, Schmitt DP (1994) Evaluation of pineapple, *Ananas comosus*, for host-plant resistance and tolerance to *Rotylenchulus reniformis* and *Meloidogyne javanica*. Nematropica 24:113–121

Smith JB (1965) Plantation variety tests 1965. PRI News 13:255–262

Smith JB (1966) Review of the root rot and heart rot problem in pineapple. II. Breeding for resistance. PRI Res Rep 118:50–63

Smith LB, Downs RJ (1979) Bromelioideae (Bromeliaceae) Flora Neotropica. New York Botanical Garden, New York, NY, USA

Smith MK, Ko HL, Sanewski GM, Botella JR (2005) *Ananas comosus* Pineapple. In: Litz RE (ed) Biotechnology of fruit and nut crops. CABI, Wallingford, UK, pp 158–172

Soler AP, Marie-Alphonsine Topart AP, Queneherve P (2009) How to evaluate the resistance or tolerance of pineapple varieties to nematodes. Acta Hortic 822:191–198

Souza FVD, Cabral JRS, dos Santos-Serejo JA, de Matos AP, Reinhardt DH, da Cunha GAP, Ferreira FR, Pereira JA

(2006) Identification and selection of ornamental pineapple plants. Acta Hortic 702:93–97

Souza FVD, Cabral JRS, de Souza EH, MdJ S, Santos OSN, Ferreira FR (2009) Evaluation of F1 hybrids between *Ananas comosus* var. *ananassoides* and *Ananas comosus* var. *erectifolious*. Acta Hortic 822:79–84

Sripaoraya S, Marchant R, Power JB, Davey MR (2001) Herbicide-tolerant transgenic pineapple (*Ananas comosus*) produced by microprojectile bombardment. Ann Bot 88: S597–603

Stewart RJ, Sawyer BJB, Bucheli CS, Robinson SP (2001) Polyphenol oxidase is induced by chilling and wounding in pineapple. Aust J Plant Physiol 28:181–191

Suriname (1995) Country report to the FAO International Technical Conference on Plant Genetic Resources. Ministry of Agriculture Animal Husbandry and Fisheries, Paramaribo, Suriname, 33 p

Sugimoto A, Yamaguchi I, Matsuoka M, Nakagawa H, Kato S, Nakano H (1991) *In vitro* conservation of pineapple genetic resources. Research Highlights, Tropical Agricultural Center, pp 14–16

Taussig SJ, Batkin S (1988) Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application: an update. J Ethnopharmacol 22(2):191–203

Thomson KG, Thomas JE, Dietzgen RG (1998) Retrotransposon-like sequences integrated into the genome of pineapple, *Ananas comosus*. Plant Mol Biol 38:461–465

Trusov Y, Botella JR (2006) Delayed flowering in pineapples (*Ananas comosus* (L.) Merr.) caused by co-suppression of the *ACACS2* gene. Acta Hortic 702:29–36

Valds EA, García RB, Rodríguez NN, Peña AC, Pérez MI (1998) Determination of haploid plants in pineapple and alternative methods in the evaluation of ploidy levels. In: Proceedings of 3rd international pineapple symposium, Pattaya, Thailand, 17–20 Nov 1998, P52

Williams DDF, Fleisch H (1993) Historical review of pineapple breeding in Hawaii. Acta Hortic 334:67–76

Zah R, Hischier R, Leão AL, Braun I (2007) Curauá fibers in the automobile industry – a sustainability assessment. J Clean Prod 15:1032–1040

Zee ET, Munekata M (1992) In vitro storage of pineapple (*Ananas* spp.) germplasm. HortScience 16:495