Chapter 4
Genetic Diversity of Pineapple

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Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is the only edible and most economically important member of the family Bromeliaceae which contains many hundreds of plants that are popular ornamental plants due to their diverse and attractive foliage and inflorescence. Pineapple is cultivated in many tropical and subtropical countries, ranking third in world production among tropical fruits, after banana and mango (Smith and Downs 1979; Loison-Cabot 1992; Botella and Smith 2008; Sripaoraya 2009). A better understanding of the genetic diversity within *Ananas* could present new opportunities for breeding and enhance the effectiveness of current programs.

According to the present classification, all pineapple germplasm are regrouped into one genus *Ananas* Miller with two species: *A. comosus* (L.) Merr. and *A. macrodontes* Morren (Table 4.1) (Coppens d'Eeckenbrugge and Leal 2003). *A. comosus* is a mostly self-incompatible diploid with $2n = 2x = 50$ chromosomes, whereas *A. macrodontes* is a self-fertile tetraploid with $2n = 4x = 100$ chromosomes and lacks the gametophytic incompatibility system of its diploid relative (Marchant 1967; Brown and Gilmartin 1986; Brown et al. 1997). *A. comosus* includes five botanical varieties: *comosus*, *microstachys*, *parguazensis*, *erectifolius*, and *bracteatus*. Pineapple cultivars are normally diploids; however the triploid cultivar 'Gigante de Tarauacá' has been found in the Northern region of Brazil (Table 4.1) (Ferreira and

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Table 4.1 The pineapple species and cultivars defined in the genus *Ananas* based on new classification studies (Smith and Downs 1979; Loison-Cabot 1992; Coppens d'Eeckenbrugge et al. 1997; Kato et al. 2004; Zhang et al. 2014)

Species	Botanical varieties	Common
A. comosus ($2n = 2x = 50$)	*Comosus*	Smooth Cayenne
		Queen
		Red Spanish
		Perola
		Perolera Manzana Singapore Spanish MD-2 CO-2
	Microstachys	
	Parguazensis	
	Erectifolius	Curagua Selvagem 6
	Bracteatus	Tricolor
A. macrodontes ($2n = 4x = 100$)		

Cabral 1993; Scherer et al. 2015). *A. macrodontes* Morren is distinguished from the cultivated pineapples and their wild relatives because its inflorescences and fruits generally lack the crown of leaves and reproduce vegetatively by stolons instead of stem suckers (Coppens d' Eeckenbrugge and Govaerts 2015).

Genetic Diversity in Pineapple Germplasm

Pineapple germplasm characterization and genetics studies indicate the botanical variety *A. comosus* var. microstachys as most likely the wild progenitor of domesticated pineapple *A. comosus* var. comosus (Fig. 4.1) (Coppens d'Eeckenbrugge and Duval 2009; Clement et al. 2010). *A. comosus* is widely considered to be heterozygous, and consequently there is much diversity in plant and fruit characteristics between cultivars. The genetic diversity in pineapple was driven by a system of outcrossing and a high frequency of somaclonal variation. Among them, these cultivars exhibit a wide variety of diverse and useful traits (Py et al. 1987; Wee and Thongtham 1991; Duval and Coppens d'Eeckenbrugge 1993).

'Smooth Cayenne' in Cayenne group is the standard for processing because of its high yields, vigorous robust growth, and suitability for processing and relative ease of flowering control (Fig. 4.2e). Local selections are somaclonal variants that are mostly known by their areas of origin, such as 'Sarawak' in Malaysia and 'Champaka' in India. The 'Smooth Cayenne' cultivar dominates commercial production for

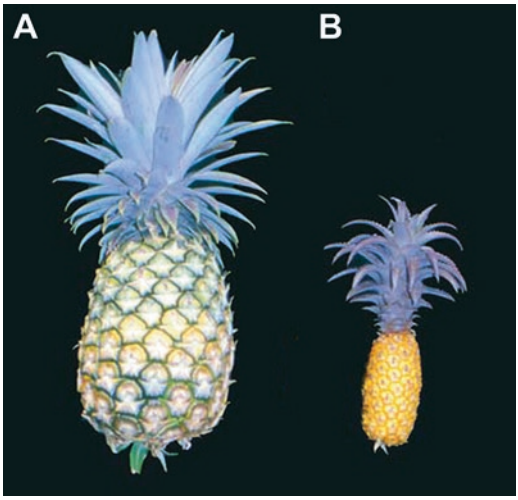

Fig. 4.1 A domestic variety *A. comosus* var. comosus (**a**) and its wild ancestor *A. comosus* var. ananassoides (**b**) (Sanewski 2011)

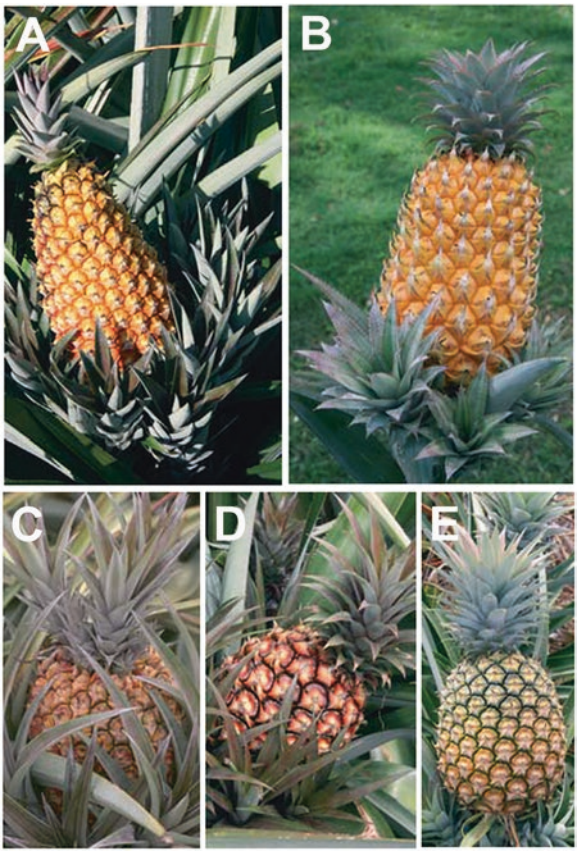

Fig. 4.2 The five cultivars for commercial production: (**a**) Maipure or Perola, (**b**) Queen, (**c**) Abacaxi, (**d**) Red Spanish, and (**e**) Cayenne (Sanewski 2011)

canning and is also one of the major fresh fruit varieties with bright yellow flesh color which is preferred by consumers.

Queen group generally produces smaller plants and fruit with spiny, shorter leaves than 'Smooth Cayenne' (Fig. 4.2b). 'Queen' is grown in South Africa, Australia, Thailand, Vietnam, some Pacific Island countries, and India for the fresh fruit market. 'Red Spanish' in Spanish group is the major cultivar in the Caribbean region (Fig. 4.2d).

Perola is grown only in Brazil and some African countries (Fig. 4.2a). The fruit is not considered suitable for canning or for fresh fruit export, but the juicy, sweet flavor, white flesh character of the fruit is favored in the local markets. Singapore Spanish is grown in some African countries and SE Asia particularly Malaysia.

There are also several piping leaf cultivars including Primavera, Manzana, Monte Lirio, and Perolera that are grown throughout western South America, Central America, and Mexico usually for local markets (Fig. 4.2). These clones may be of interest to breeders in the Western Hemisphere as they constitute a gene pool of adapted forms almost unused in breeding programs.

Similarly with most crops, the important traits in cultivated pineapples are usually related to yield, fruit size and quality, or production efficiency. The pineapple fruit size generally varies from less than 100 g to over 7 kg, but the size is approximately 1400–1600 g (Wells et al. 1928). Fruit quality consists of skin color, flesh color, sweetness, aroma volatile component, and content of bioactives. The pineapple skin color naturally presents yellow-, orange-, green-, cream-, pink-, and red-skinned types; most pineapples seen in world markets possess a yellow to orange skin at maturity. The more usual yellow skin color is due to carotenoids with more yellow varieties containing a higher level (Brat et al. 2004). Although the common 'Smooth Cayenne' pineapple has relatively low vitamin C content at around 10–15 mg/100 mL juice, the commercial cultivar MD-2′ contains 5–6 times the vitamin C level, approximately 91 mg/100 mL (Johannessen and Kerns 1964; Ramsaroop and Saulo 2007).

Wei et al. (2016) analyzed the diversity of aroma volatile compounds and found a high variability of aroma volatile compounds among 12 pineapple varieties. According to cluster analysis, these 12 varieties were separated into four groups when correlation coefficient distance was 19. Principal component analysis suggested 120 compounds identified could be simplified to 3 components including 27 compounds, and Mibao variety reached the highest comprehensive score. Sun et al. (2016) conducted an analysis for the characteristics involved 12 Tainong pineapple varieties. They found that leaf length and leaf width of 12 pineapple varieties ranged from 54.40 to 97.00 cm and 4.28 to 6.48 cm, respectively. Different leaf colors were found in all the varieties. Among 12 varieties, Tainong No. 19 was with the lowest fruit total acid content, while Tainong No. 4 was with the highest vitamin C content. The crude fiber contents of Tainong No. 1, No. 2, and No. 20 were higher than the rest.

The major collections of pineapple germplasm in the world are distributed in the United States Department of Agriculture (USDA)-Agricultural Research Service in Hilo, Hawaii, which maintains over 180 accessions of pineapple cultivars and their

wild relatives, as well as the collections maintained by EMBRAPA/CNPMF in Cruz das Almas, Brazil, and by CIRAD-FLHOR in Martinique. As with many other perennial fruit crops, the pineapple germplasm are almost exclusively maintained by vegetative propagation, by crowns, slips, suckers, or in vitro culture. Vegetative propagation has led to the accumulation of somatic mutations, some of which cause noticeable phenotypic effects, which can become the target of clonal selection. While selected mutants are important in horticultural production, it is necessary to identify them so that breeders and genebank curators can efficiently conserve and use these genetic materials.

Genetic Diversity Analysis in Pineapple

The genetic divergence between *A. macrodontes* and *A. comosus* and the genetic differentiation among the botanical varieties of *A. comosus* were explored by a large panoply of studies using biochemical and molecular marker techniques (Zhang et al. 2014; Zhou et al. 2015). Before DNA molecular markers were used, the genetic diversity of pineapple germplasm was identified using isozyme markers.

De Wald et al. (1992) and Aradhya et al. (1994) have initially used isozyme polymorphism in the genus *Ananas* to identify *A. comosus* cultivars. De Wald et al. identified 15 of 27 *A. comosus* cultivars by enzymatic systems, two peroxidases and three phosphor glucomutases. Aradhya et al. studied isozyme variation in 161 pineapple germplasm, including four different species of *Ananas* and one species of *Pseudananas*, from the Hawaiian collection. Considerable variation within and between species of *Ananas* was explored by six-enzyme system and 66 distinct zymotypes that were able to differentiate all species, and botanical varieties were identified. Their multivariate analyses also indicated that the five genetically diverse groups in *A. comosus* did not consist perfectly with previously traditional phenotypic groupings. Isozyme evidence also showed that *A. erectifolius* is a conspecific variant of *A. comosus* and that among other wild species, *A. ananassoides* is more closely related to *A. comosus* than *A. bracteatus*. It was shown to be genetically distinct between *Pseudananas* and all species of *Ananas*. Their study also suggested that differentiation among the species of *Ananas* may be due to ecological isolation rather than genetic divergence with breeding barriers and therefore may represent a species complex. However, enzymatic systems have limited use for analyzing the genetic diversity of *Ananas* accessions due to the low number of markers (Aradhya et al. 1994).

Up to now, DNA-based molecular markers, such as RAPD, RFLP, AFLP, SSR, and SNP, have been widely utilized in the detection and the evaluation of the genetic diversity in pineapple (Ruas et al. 1995; Kato et al. 2004; Wang et al. 2017). Ruas et al. (1995) used random amplified polymorphic DNA (RAPD) markers to evaluate the relationships among four major pineapple cultivars in Brazil: Perola, Smooth Cayenne, Primavera, and Perolera. Fourty-seven of the 75 RAPD markers used were polymorphic. Smooth Cayenne and Primavera sharing 17 polymorphic fragments

were most closely related, followed by Perolera, which shared 15 fragments with Smooth Cayenne and Primavera. Popluechai et al. (2007) studied nine pineapple cultivars in Thailand with 40 RAPD primers, and the results of RAPD analysis exhibited a high similarity among the cultivars collected.

Duval et al. (2001) utilized restriction fragment length polymorphism (RFLP) markers to analyze 301 accessions of *Ananas* including 168 *A. comosus* accessions, which suggested that *A. comosus* had lower levels of polymorphism than wild *Ananas* species. Kato et al. (2004) used amplified fragment length polymorphism (AFLP) markers to estimate 148 accessions of *A. comosus* and 14 of related species, and the results suggested that there was abundant genetic variation within existing pineapple germplasm for selection and discrete DNA molecular difference for commercial cultivars. However, Paz et al. (2005) explored a low level of genetic diversity in the Mexican germplasm collections based on 169 AFLP markers. Similarly, Cuban pineapple germplasm assessed by AFLP markers were grouped at distances lower than 0.20 and also showed a low level of diversity (Paz et al. 2012).

Shoda et al. (2012) used 18 polymorphic simple sequence repeat (SSR) markers to genotype 31 pineapple cultivars in Japan. With the exception of 'N67–10' and 'Hawaiian Smooth Cayenne', all 31 cultivars could be effectively differentiated by the 18 SSR markers. Their results indicated that genetic diversity was presented in pineapples bred in Japan. Vanijajiva (2012) applied inter-simple sequence repeats (ISSR) markers to assess genetic diversity among 15 accessions of pineapple in Thailand. The data showed that the 15 accessions were successfully classified into 3 clusters with similarity coefficients ranging from 0.316 to 0.968. Feng et al. (2013) utilized SSR marker to carry out germplasm genetic diversity analysis for 48 breeds of pineapple and divided the germplasms into 4 subgroups instead of 3 (Smooth Cayenne, Queen, and Spain) by conventional morphological classification. Rodrígueza et al. (2013) developed effectively 10 SSRs to perform successfully the detection of 26 polymorphic alleles in 6 different pineapple genotypes representing the main groups of varieties of this crop.

Single nucleotide polymorphisms (SNPs) are the most abundant class of polymorphisms in plant genomes. Zhou et al. (2015) developed firstly 213 SNP markers by using expressed sequence tag and nucleotide sequences from public databases and validated 96 SNPs by genotyping the 170 *Ananas* accessions in the USDA pineapple germplasm collection in Hawaii and found only 64 distinctive genotypes (Fig. 4.3). Although the relationship among 64 pineapple accessions was exactly in line with the traditional classification, these SNP markers provide robust and universally comparable DNA fingerprints and can serve as an efficient genotyping tool to assist pineapple germplasm management, propagation of planting material, and pineapple cultivar protection. Moreover, the results suggested that somatic mutation mainly contributed to intra-cultivar variations in pineapple. The increasing use of SNPs as markers could make a good choice for accurate genotype identification and diversity analysis in perennial crops and will also facilitate further studies of the genetic diversity in pineapple.

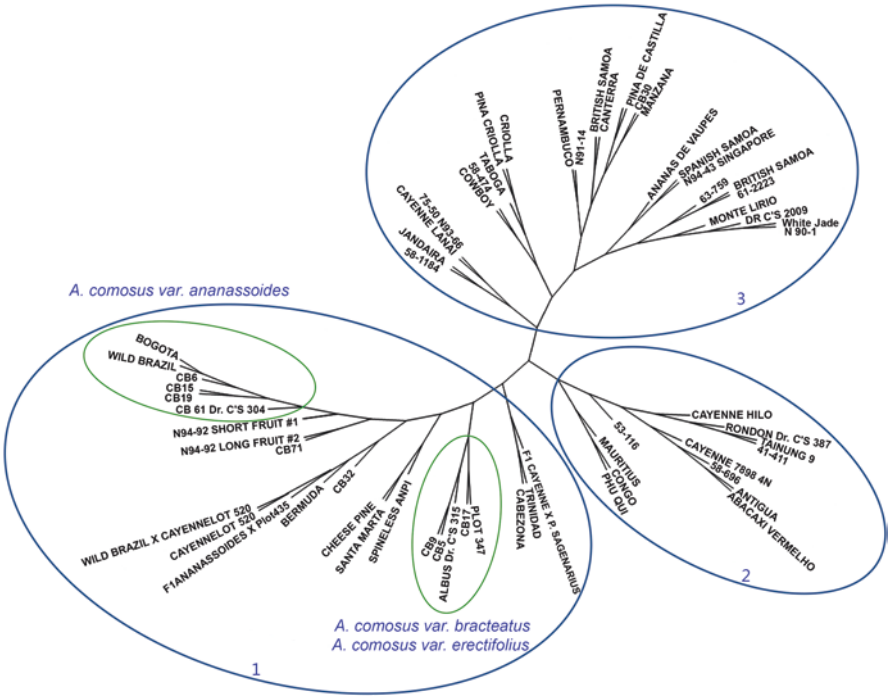

Fig. 4.3 The relationship among 64 pineapple accessions from USDA-ARS by SNP markers (Zhou et al. 2015)

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