18 Pineapple

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18.1 Introduction

18.1.1 Economic Importance

Pineapple (*Ananas comosus* (L.) Merrill) is the third most important tropical fruit crop, after bananas and mangoes (citrus being considered mainly subtropical). Although cultivated in all tropical and subtropical countries, mostly between 30°N and 30°S, minor plantations can be found beyond these latitudes in areas with mild climates, often under protective shelter (Nakasone and Paull 1998). According to the Food and Agriculture Organisation (FAO) statistics (http://apps.fao.org), world pineapple production increased from 3,833,137 tons in 1961 to 15,287,413 tons in 2004. Five countries, namely Thailand (17,000,000 t), the Philippines (1,650,000 t), Brazil (1,435,600 t), China (1,475,000), and India (1,300,000) contributed with about half of the world production in 2004. A second group of significant producers that includes countries as disparate as Nigeria, Mexico, Costa Rica, Indonesia, Kenya, Colombia, Ivory Coast, Venezuela, Vietnam, Malaysia, United States, and South Africa supply about one third of the total world production.

Approximately 70% of the produced pineapple is consumed as fresh fruit in the country of origin (Loeillet 1997). The world pineapple trade consists mainly of processed products as canned slices, chilled fresh-cut chunks and spears, juice and juice concentrates. For example, worldwide exports of concentrated juice represent more than US$ 250 million and the value of exported canned pineapple more than US$ 600 million. Even so, the value of the fresh fruit market is rapidly increasing, particularly the chilled, fresh-cut fruit market (Rohrbach et al. 2003). Pineapple is also a source of bromelain, used as a meat-tenderising enzyme, and high quality fiber. The waste resulting from industrial processing is used for animal feed.

18.1.2 Taxonomy

Pineapple is a perennial monocot belonging to the order Bromeliales, family Bromeliaceae, subfamily Bromelioideae. The Bromeliaceae comprise 56 genera with 2,921 species (Luther 2002), classified into three subfamilies: Pitcarnioideae, Tillandsioideae and Bromelioideae. This last subfamily shows a tendency towards the fusion of floral parts, a trait most developed in *Ananas*, the only genus whose flowers and bracts are completely merged into a single sorose-type parthenocarpic fruit formed by 50 to 200 coalescent berries (Coppens d'Eeckenbrugge et al. 1997).

Pineapple taxonomy was recently revised by Coppens d'Eeckenbrugge and Leal (2003). Until then, the accepted taxonomy and nomenclature of pineapple was that of Smith and Downs (1979), which described nine species distributed in two genera, *Ananas* and *Pseudananas*, the latter being monotypic. It was first amended by Leal (1990) who invalidated *A. monstrosus*.

Most quantitative traits used in the Smith and Down's classification are not clearly discriminative and are excessively dependent on environmental conditions. The qualitative traits used for classification, such as leaf spininess, are determined by one or few loci (Cabral et al. 1997), which mutate and segregate within species as well as between species.

Many distinctions, particularly those related to spininess, fruit size and fertility, appeared to be the direct result of human selection in the course of domestication. In addition, crosses among the

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species of Smith and Down are successful and the resulting hybrids are fully fertile. Even the diploid *Ananas* and the tetraploid *P. sagenarius* (Arruda da Câmara) Camargo can be hybridized experimentally, mainly producing self-fertile tetraploid hybrids and a few triploids. Available data on biochemical and molecular diversity also indicated an incipient speciation process within *Ananas* (Leal and Coppens d'Eeckenbrugge 1996; Coppens d'Eeckenbrugge et al. 1997).

Coppens d'Eeckenbrugge and Leal (2003) proposed a simplification of the classification, downgrading the two genera and eight species into two species (with the restoration of *A. macrodontes* Morren instead of *P. sagenarius*) and five botanical varieties of *A. comosus*. Since then, more recent molecular marker analyses have refined our understanding of pineapple diversity and speciation, and have provided new data on the relationship between the two pineapple species, without affecting the new classification.

A. macrodontes is a vigorous self-fertile tetraploid ($2n = 4x = 100$), with spiny leaves, 2–3 m long and 7 cm wide, propagating by elongate basal stolons. The syncarp lacks the leafy crown typical of *A. comosus*. The latter species is generally diploid ($2n = 50$), self-incompatible, and propagates vegetatively by suckers (borne on the stem), slips (borne on the peduncle), and the fruit crown(s). The pineapple cultivated for the fruit corresponds to the botanical variety *A. comosus* var. *comosus*. Its leaves are relatively wide (more than 5 cm), spiny, partially spiny or smooth, and its strong peduncle bears a fruit whose size may reach several kilograms. *A. comosus* var. *ananassoides* (Baker) Coppens & Leal (formerly *A. ananassoides* (Baker) L.B. Smith and *A. nanus* (L.B. Smith) L.B. Smith) corresponds to the most common wild form, with thinner spiny leaves and a much smaller fruit on a long, slender scape. Another wild form is *A. comosus* var. *parguazensis* (Camargo & L.B. Smith) Coppens & Leal (formerly *A. parguazensis* Camargo & L.B. Smith), with wider leaves, constricted at their base, antrorse and retrorse spines, and a globose fruit. The two remaining botanical varieties are cultivated. *A. comosus* var. *erectifolius* (L.B. Smith) Coppens & Leal (formerly *A. lucidus* Miller *sensu* Smith & Downs) is very similar to *A. comosus* var. *ananassoides*, except for its smooth fibrous leaves, which are used by Amerindians to make hammocks, fishing lines and nets. *A. comosus* var. *bracteatus* (Lindl.) Coppens & Leal (grouping the former *A. bracteatus* (Lindley) Schultes f. and *A. fritzmuelleri* Camargo) is a very vigorous and spiny plant, producing a medium-sized fruit with long bracts. It is cultivated as a living fence. Its fruit was also collected for juice and it is still found as a sub-spontaneous plant in ancient settlements of southern South America. A variegated variant has become a common ornamental of tropical gardens.

18.1.3
Natural Habitat and Origin

Both *Ananas* species (*A. comosus* and *A. macrodontes*) have a natural distribution confined to the South American sub-continent. Nevertheless, long before the arrival of Christopher Columbus to Guadeloupe in 1493 and this first European contact with this crop, the Native Americans had already domesticated and dispersed the pineapple throughout South and Central America, the Antilles and the Caribbean region (Leal and Coppens d'Eeckenbrugge 1996; Coppens d'Eeckenbrugge et al. 1997). The pineapple cultivation very probably initiated with var. *comosus* and var. *erectifolius* evolving from var. *ananassoides* and/or var. *parguazensis* in the region north of the Amazon river (Orinoco and Rio Negro basins, and Guiana shield), where a wider morphological and molecular variability is found in wild and cultivated types (Duval et al. 2003). *A. comosus* var. *bracteatus*, and *A. macrodontes* originated in the South of the continent (Paraguay and southern Brazil) (Leal and Coppens d'Eeckenbrugge 1996).

18.1.4
Chromosome Number and Genome Size

The most common chromosome number for the subfamily Bromelioideae is $2n = 50$, (Cotias-de-Oliveira et al. 2000). It is also the most common among the *A. comosus* varieties and cultivars. Nevertheless, triploid and tetraploid clones have also been identified in var. *comosus* and tetraploid clones in var. *ananassoides* (Sharma and Ghosh 1971; Lin et al. 1987; Dujardin 1991; Cotias-de-Oliveira et al. 2000). *A. macrodontes* is tetraploid ($2n = 100$) (Lin et al. 1987). Arumuganathan and Earle (1991) estimated the haploid genomes size at 444 Mbp for *A. comosus* var. *bracteatus* and 526 Mbp for var. *comosus*.

18.1.5
Propagation and Floral Biology

The propagation of pineapple is mainly vegetative, by stem suckers, peduncle slips, and fruit crown. *A. macrodontes* produce underground stolons. Natural genetic diversity studies indicate that clonal propagation is also the most common mode of multiplication in the wild, as seeds germinate slowly and/or young seedlings rarely survive under natural conditions (Coppens d'Eekenbrugge et al. 1997).

All botanical varieties of *A. comosus* possess a gametophytic self-incompatibility system. The self-rejection reaction is variable in intensity and generally stronger in the cultivated var. *comosus*, which is probably a result of the domestication process and selection for seedless fruits (Coppens d'Eeckenbrugge et al. 1993). In contrast, *A. macrodontes* is self-fertile and the self-progenies are very homogeneous, indicating that this species is highly homozygous and autogamous (Collins 1960). The main vectors for natural cross-pollination are humming birds, while bees and ants may play a secondary role. Wind pollination has never been reported. Seeds lack dormancy and can retain germination capacity for at least six months.

While no apparent depression was detected in formation or germination of self-seeds, severe inbreeding depression has been observed among self-progenies of several *Ananas* cultivars in later stages of development, rendering almost impossible the continuation of the inbreeding process beyond the first or second generation of selfing (Collins 1960; Coppens d'Eeckenbrugge et al. 1993; Cabral et al. 2000).

The success of the intraspecific and interspecific crosses in the genus *Ananas* was referred to above. References to intergeneric hybrids involving *Ananas* and other genera as *Aechmea*, *Cryptanthus* and *Neoreglia*, exclusively for ornamental purposes, can be found in Grant (1998) and, for example, on the web page of the Florida Council of Bromeliad Societies (http://fcbs.org/articles/Bigenerics.htm).

18.1.6
Pineapple Breeding

Hybridization and clonal selection, mainly involving elite cultivars, have been used in modern pineapple breeding for over a century. Nevertheless, the heterozygous nature of pineapple cultivars, and the consequent strong segregation and recombination, have strongly limited the success of hybrid breeding. Until recently, the severe selection among millions of seedlings resulted in cultivars of only average quality or of local importance (Coppens d'Eeckenbrugge et al. 1997). This quite desperate situation ended in the late 1990s with the commercial success of 'Golden Ripe', a new cultivar that stirred the world market of fresh pineapple and awakened the interest in cultivar diversification.

Included among the goals of pineapple breeding programs are the introgression of resistances to diseases such as *Phytophthora* and *Fusarium,* the prevention of disorders such as internal browning (blackheart) and the control of specific traits such as early natural flowering, in elite cultivars. To avoid the lottery of segregation and recombination, genetic engineering appears to be a promising breeding strategy since it allows transferring a single gene, or a few genes, without substantially altering the initial genome. Efficient procedures for genetic transformation (Sripaoraya et al. 2001; Espinosa et al. 2002) and in vitro regeneration and propagation (Escalona et al. 1999; Firoozabady and Gutterson 2003; Sripaoraya et al. 2003) have already been established. The first field and greenhouse trials of genetically transformed pineapple clones exhibiting reduced expression of polyphenol oxidase (PPO) and of 1-aminocyclopropane-1-carboxilate (ACC) synthase or expressing the bialaphos resistance (*bar*) gene have already been carried out (Rohrbach et al. 2000; Sripaoraya et al. 2001; Sripaoraya et al. 2006; Botella and Fairbairn 2005; Trusov and Botella 2006).

In this respect, the construction of dense genome maps of molecular markers is of paramount importance for the further isolation, via positional cloning, of genes of interest for pineapple improvement. This is of particular significance regarding those genes that are uniquely known and uniquely detected by their phenotypic expression in plants (e.g. resistance genes).

18.2
Molecular Systematics

Pineapple molecular studies have been carried out with a variety of techniques, including isozymes (García 1988; Aradhya et al. 1994), RAPD (Ruas et al. 2001), AFLP (Kato et al. 2004; Paz et al. 2005), RFLP (Duval et al. 2001) and cpDNA PCR-RFLP (Duval et al. 2003). All of them clearly support the separation between *A. comosus* and *A. macrodontes*, as well as the low level of genetic differentiation among the former *Ananas* species. Thus, Aradhya et al. (1994) observed that the variation among the five botanical varieties accounted merely for 14% of the total isozymic variation and Duval et al. (2001) reported a continuous RFLP variation within *A. comosus*. Another consistent observation (Duval et al. 2001) was a wide variation within *A. comosus* var. *ananassoides* and a close genetic relationship between this and other varieties, particularly the wild *parguazensis* and the cultivated *comosus* and *erectifolius*, confirming that the cultivated types were directly derived from their wild relatives.

The existence of clones in the Guianas, morphologically intermediate between *comosus* and *ananassoides*, with haplotypes also found in both these varieties, suggests a recent domestication and a continuing introgression process. Concerning the *erectifolius* variety, the RFLP and PCR-RFLP data indicate multiple domestication events, involving convergent selections from different *ananassoides* genotypes for a few morphological traits (smooth and fibrous leaves).

According to isozyme, RFLP and AFLP data, *A. comosus* var. *bracteatus* appears relatively uniform and better differentiated from the other varieties. Isozyme and RFLP markers indicate a particular affinity with *A. macrodontes*. The study of cpDNA by PCR-RFLP identified a unique haplotype for the common representatives of var. *bracteatus* shared with all the other varieties. On the other hand, a unique accession, formerly classified as *A. fritzmuelleri* Camargo, presents a haplotype that is almost identical to the one typical of *A. macrodontes*. Thus molecular data suggest a special position of *A. comosus* var. *bracteatus* in relation to *A. macrodontes*, as it appears to be constituted by two combinations of nuclear genes from both species with chlorotypes from one or the other species. Whether these combinations are the product of a rare introgression event during the evolution of *Ananas*, an "accidental genotype" maintained by vegetative propagation, or the testimony of an intermediate stage in its evolution, is difficult to ascertain.

18.3
Construction of Genetic Maps

Available information on ongoing genome mapping programs in pineapple is very scarce. The unique pineapple genome maps published so far are the genetic maps of molecular markers including the morphological trait 'piping', constructed by Carlier et al. (2004, 2006). To our knowledge no other qualitative trait or QTLs have been mapped, and no results of physical mapping have been reported.

18.3.1
F_1-Based Genetic Maps

As mentioned above, except for breeding purposes, *Ananas* is usually propagated vegetatively either for cultivation or under natural conditions. Genotypes well adapted to a particular natural environment, as well as those selected for human needs, tend to be fixed in a heterozygous state. In addition, heterozygosity is reinforced by different degrees of self-incompatibility and lack of sexual barriers, which promote crosspollination, and by a strong inbreeding depression.

The high level of heterozygosity allows the use of F_1 progenies as segregant populations for genetic mapping employing the "two-way pseudo-testcross" or "double pseudo-testcross" strategy (Grattapaglia and Sederoff 1994; Hemmat et al. 1994), since the alleles of a heterozygous polymorphic locus present in one parent and absent in the other are expected to segregate in a 1:1 Mendelian ratio among the F_1 population.

The first genetic maps of pineapple, one for *Ananas comosus* var. *bracteatus* and a second one for *Ananas comosus* var. *comosus* were recently published (Carlier et al. 2004). The mapping population used for the construction of both maps consisted of 46 F_1 plants derived from a cross between var. *comosus* (cv. Rondon – clone BR 50) and var. *bracteatus* ("Branco do mato" – clone BR 20), carried out in Martinique. Map construction was twice more efficient for var. *bracteatus* than for var. *comosus*, a consequence of the higher average heterozygosity of the former and, also, of the fact that it possesses a large number of specific DNA-markers not present in var. *comosus*.

The F_1-based map of *A. comosus* var. *bracteatus* is constituted by 335 DNA markers (60 RAPD, 264 AFLP and 11 ISSR), assembled in 50 linkage groups: 26 groups gathering at least four markers each, six groups of three markers each and 18 pairs of markers. This map spans over 2,111 cM, which corresponds to 57.2% of the 3,693 cM genome length estimated according to Chakravarti et al. (1991).

The A. *comosus* var. *comosus* map gathers 156 molecular markers (33 RAPD, 115 AFLP and 8 ISSR) in 30 linkage groups spanning over 1,311 cM, corresponding to 31.6% of the 4,146 cM long genome. The locus *P*, whose dominant allele determines the 'piping' morphological trait, was also included in this map. The ratio between physical and genetic distances is approximately 120 kbp/cM for var. *bracteatus* and 127 kbp/cM for var. *comosus* (Carlier et al. 2004).

18.3.2
F_2-Based Genetic Map

One of the F_1 plants used for the construction of the first genetic maps was selfed in Martinique and leaves of 142 F_2 plants were sent to Portugal for the construction of a new, F_2-based, genetic map. Among the 451 molecular markers analysed, 52 molecular markers (16 from var. *comosus* and 36 from var. *bracteatus*) showed a less pronounced distortion $\left(\chi^2_{0.01} > \chi^2 \geq \chi^2_{0.05}\right)$ while 43 other markers showed a more skewed segregation $\left(\chi^2 > \chi^2_{0.01}\right)$.

The first genetic map constructed on the basis of an F_2 segregating population assembles 412 genetic markers (311 AFLP, 66 RAPDs, 34 ISSRs and one morphological trait, piping) in 50 linkage groups. Thirty-nine markers remained unlinked (Carlier et al. unpublished).

In order to estimate the total span of the map, each linkage group was enlarged at each extremity (Marques et al. 1998) with the average distance between adjacent markers 5.32 cM. The total span of the F_2-based map is 2,458 cM, which corresponds to 62.7% of the average pineapple map length calculated on the basis of the above-mentioned F_1-based maps.

Unfortunately, the F_2 population used to construct this map showed a very strong inbreeding depression. Most of the plants exhibited a very weak and unhealthy phenotype and died before fruit setting. Therefore, the segregation analysis of morphological traits, with the exception of the piping phenotype, could not be performed.

18.3.3
Integrated Genetic Maps of Pineapple

One of the main goals of the construction of the F_2-based genetic map was to use it for the construction of an integrated genetic map. Such an integrated map would join the F_2-based and the F_1-based maps and markers that had previously remained unlinked.

The first integrated genetic map of molecular markers in pineapple was published recently by Carlier et al. (2006). This map gathered 574 markers (454 AFLP, 79 RAPD and 41 ISSR) in 46 linkage groups, spanning more than 2,421 cM, and covering 62% of the genome, the genetic size of which, 3,919 cM, was calculated as the arithmetic average of the previous estimations (Carlier et al. 2004) for the genomes of var *bracteatus* and var. *comosus*.

Presently, the integrated map (Fig. 1) assembles 659 DNA markers (506 AFLP, 113 RAPD and 40 ISSR), one isozyme locus (PGM) and one morphological trait locus (piping). Thus far, this map is constituted by: (a) 17 linkage groups integrating molecular markers of var. *bracteatus* and var. *comosus* (15 of these integrate markers from the F_1-based and F_2-based genetic maps, while two of them gather only markers analysed in the F_2 population); (b) 11 linkage groups integrating markers of F_1- and F_2-based maps, but only of var. *bracteatus*; and (c) eight linkage groups that, in the same way, integrate only markers from var. *comosus*.

18.4
Germplasm Resources and GeneBank Data

Pineapple genetic resources (e.g., for plant genetic improvement, production of segregating populations or construction of cDNA or BAC genome libraries) are maintained by CIRAD in Martinique (French West Indies); by the Brazilian National Genetic Resources and Biotechnology Research Centre (EMBRAPA/CENARGEN), in Brasilia; by the National Cassava and Tropical Fruit Research Centre (EMBRAPA/CNPMF), Cruz das Almas, Brazil; and by the US Pacific Basin Agricultural Research Center, USDA, Hawaii. These represent the most diverse germplasm collections. Other important collections are maintained for breeding purposes by national institutions in Malaysia, Australia, Cuba, Japan, and other producing countries, as well as by private companies (Coppens d'Eeckenbrugge and Duval 1999).

Functional genomic studies in pineapple are very scarce but their number is increasing rapidly. Thus far, have been cloned and functionally characterized genes that encode: an ACC synthase and an ACC oxydase (Cazzonelli et al. 1998); a NAD^+-dependent

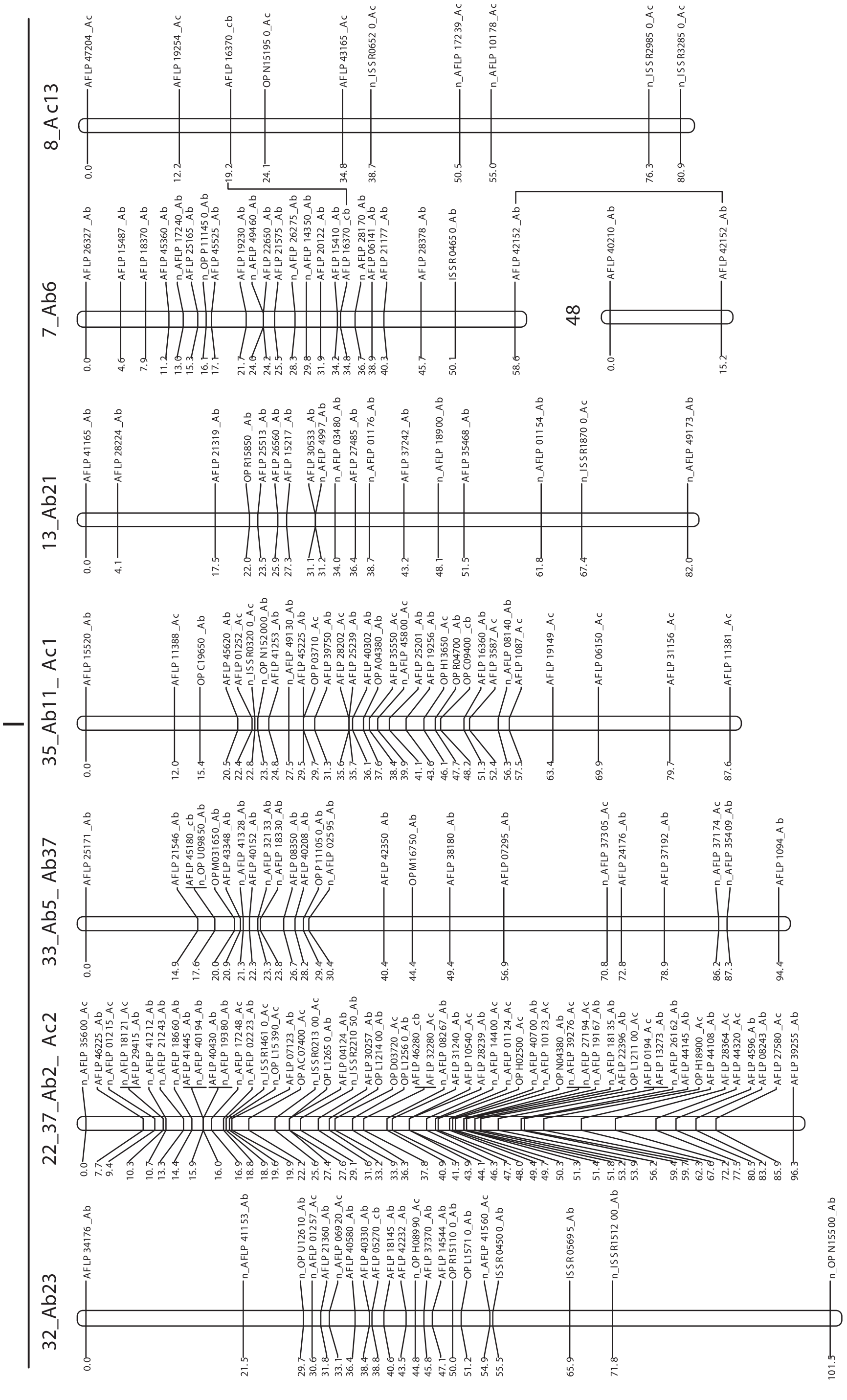


Fig. 1. Integrated genetic map (F_1-based and F_2-based genetic maps) of *Ananas comosus* (pineapple). *I* – Linkage groups that integrate molecular markers of var. *bracteatus* and var. *comosus*; *II* – Linkage groups integrating markers only of var. *bracteatus*; and *III* – Linkage groups that integrate markers only of var. *comosus*. Identification of the integrated linkage groups: e.g. 22_37_Ab2_Ac2 – numerals 22 and 37 refer to the linkage groups 22 and 37 of the F_2-based genetic map; Ab 2 – refers to the linkage group 2 of the F_1-based genetic map of var. *bracteatus*; Ac2 – refers to the linkage group 2 of the F_1-based genetic map of var. *comosus*. Molecular markers are identified as previously described (Carlier et al. 2004)

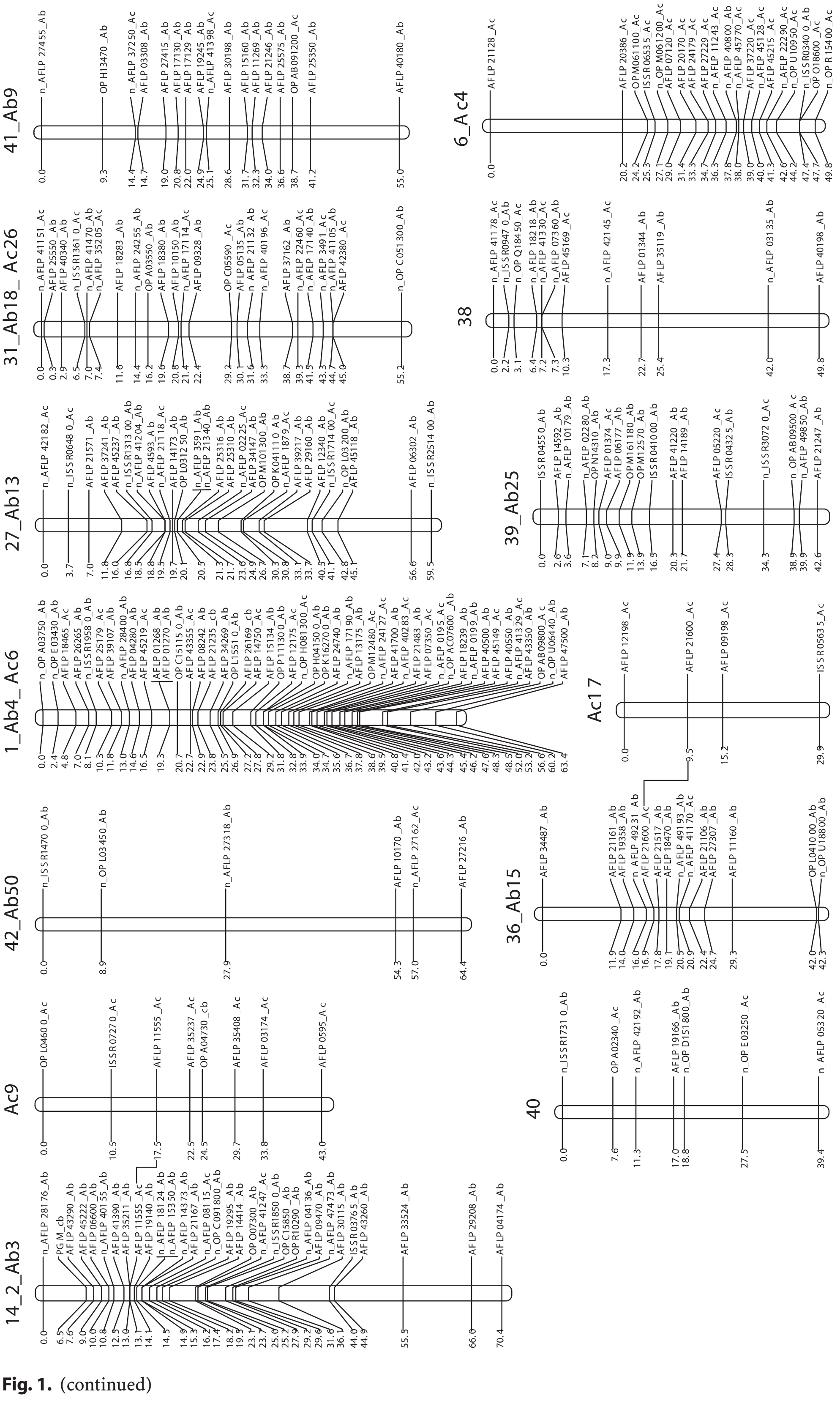
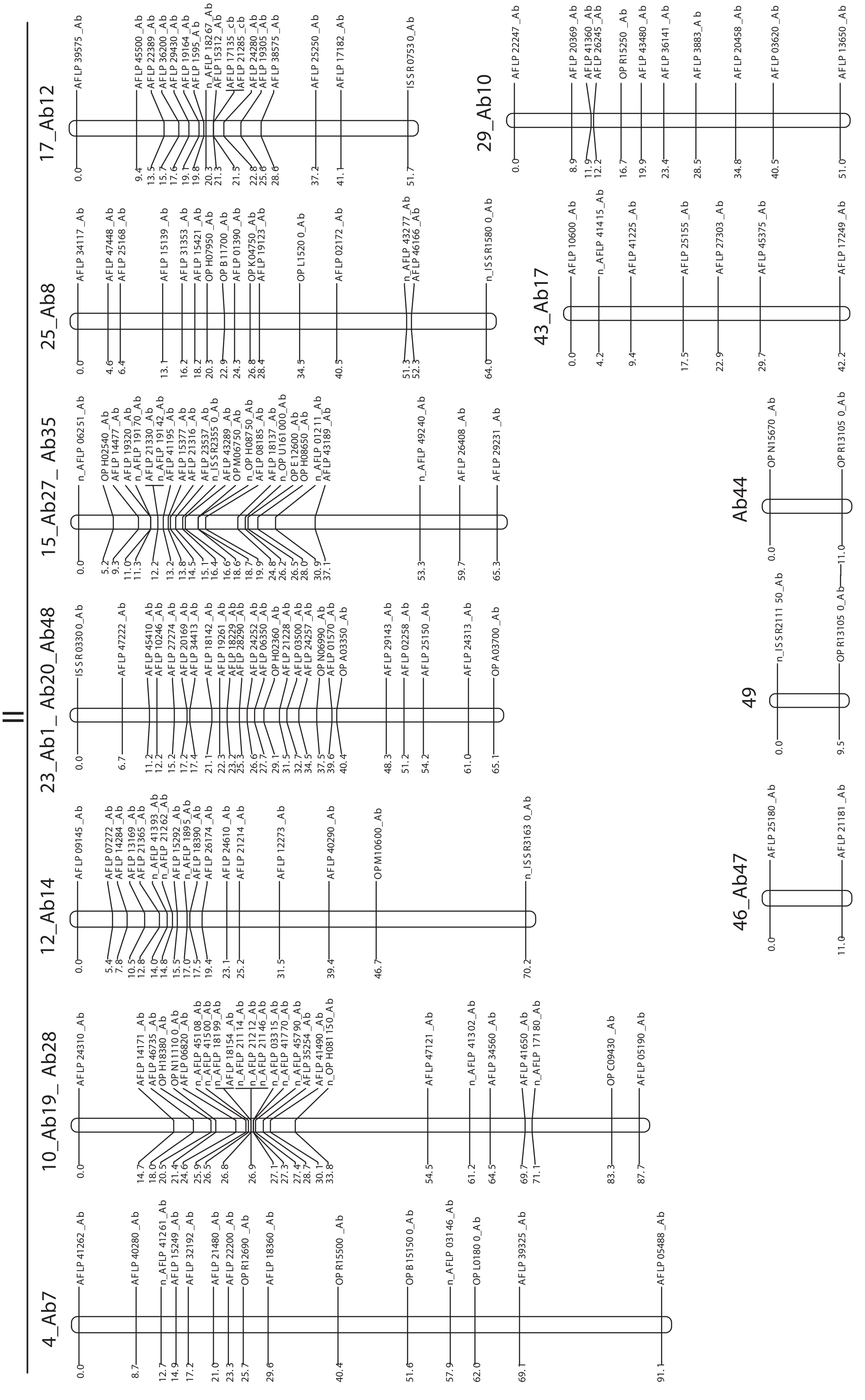


Fig. 1. (continued)



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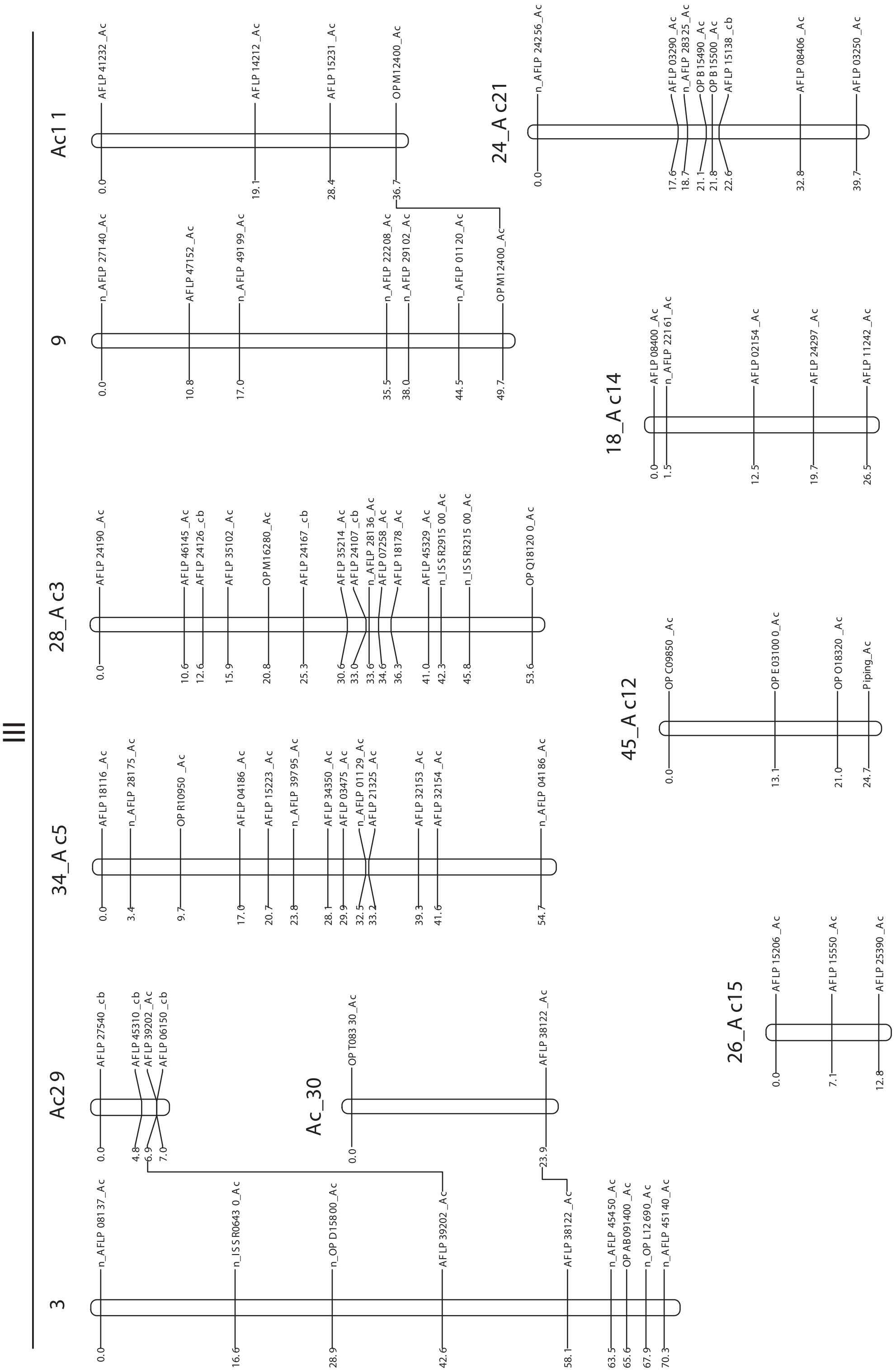

Fig. 1. (continued)

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 cistatin (Shyu et al. 2004). A retroposon-like sequence, repeatedly integrated in the genome in multiple variable sequences and putatively 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 interaction (Moyle et al. 2005a, b, 2006) have resulted in a very large number of sequenced ESTs.

The amount of genomic data in databases is still scarce but has been rapidly increasing, particularly during the last two years. Presently, a search for pineapple genomic data through the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) results in about 60 microsatellite and other DNA marker loci from var. *bracteatus* and over 5,700 ESTs from var. *comosus*. About 5,650 of these EST sequences have been contributed by Moyle et al. (2005a, b, 2006) who have clustered 408 green fruit, 1,140 yellow fruit, 343 root tip, 1,298 early nematode infection and 246 late nematode infection related ESTs into 3,383 contigs. This research group has created an online pineapple bioinformatics resource: PineappleDB (www.pgel.com.au), periodically updated with gene expression data arising from the pineapple microarray project they are implementing.

The TropGENE-DB (http://tropgenedb.cirad.fr) is an information system created by CIRAD, France, to store genetic, molecular and phenotypic data, particularly data on genetic resources, molecular markers, genetic and physical maps, sequences, genes, etc. Presently, information is available only on banana, cocoa, coconut, coffee, cotton, oil palm, rice, rubber tree and sugarcane, but the extension of the database and the inclusion of pineapple and other tropical crop species is expected soon.

18.5
Future Prospects

The pineapple integrated genetic map (Fig. 1) is incomplete and requires further study in order to assemble nearly all analysed markers into 25 linkage groups corresponding to the $n = 25$ chromosomes of the species. Two tasks appear more urgent to accomplish this goal as described below.

The first task is to include in the map some of the already published microsatellite markers, contributing to its further improvement by the integration of new markers and groups of markers from the F_1-based and F_2-based maps in linkage groups established and ordered with higher statistical significance. Additionally, due to the genetic similarity between both *Ananas* species and among the botanical varieties, the microsatellite markers and other polymorphic sequence tagged site (STS) markers are expected to be useful for the integration of genetic markers from other *Ananas* genotypes. Eventually, genetic markers could also be integrated from other *Bromeliaceae* species.

The second task is to clone, sequence and transform into sequence characterized amplified region (SCAR) markers an array of mapped RAPD, AFLP and ISSR markers strategically distributed along the genome.

A very dense and integrated genetic map of molecular markers, complemented by microsatellite and SCAR markers covering almost all the pineapple genome, will constitute a major scientific tool. It will allow the rapid location of any genetic locus and, consequently, the rapid identification of molecular markers linked to any gene of interest. Such markers can be very useful in marker-assisted selection (MAS) and in gene isolation via map-based cloning programs. In addition, such a map would act as a basic framework of the genome in future physical mapping programs or pineapple genome sequencing projects.

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