# Chapter 18
# Genomics of Pineapple, Crowning The King of Tropical Fruits

**Jose Ramon Botella and Mike Smith**

**Abstract** Pineapple [*Ananas comosus* (L.) Merr.] is the third most important tropical fruit in world production after banana and citrus. Nevertheless, and despite its commercial importance, very little genomics research has been performed in this crop. Development of molecular markers has been reported recently to study genetic relationships among the different *Ananas* species and with other members of the Bromeliaceae family. Results from those studies suggest that the existing classification of the seven *Ananas* species needs to be reconsidered. A basic pineapple genetic map is available, although it needs to be developed with the addition of additional markers. Medium scale expressed sequence tag (EST) projects have been undertaken using developing fruits and nematode-infested roots as tissue sources. A bioinformatic resource providing sequence and functional information on all EST clones has been developed. Finally, pineapple microarrays containing in excess of 9,000 EST clones have been produced. Although research in pineapple genomics is taking momentum, much more is needed before the tools developed can be used for the benefit of the industry. An international collaborative effort to develop additional molecular markers and perhaps a genome sequencing initiative is needed.

## 18.1 Introduction

Pineapple (*Ananas comosus*) is native to South America and was first seen by Europeans when Columbus landed on the inhabited island that he named Guadalupe on 4 November 1493 during his second voyage to the New World. It is generally recognized that the indigenous peoples of South America contributed substantially to the domestication of the pineapple (Leal and d'Eeckenbrugge 1996), probably through the selection of spontaneous mutations expressing desirable traits, e.g. improved palatability, improved fruit size, seedlessness, smooth leaves, and in some cases improved leaf fiber properties which are not commonly found in wild

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types (Collins 1951; d'Eeckenbrugge et al. 1997). The pineapple was used not only for fresh fruit consumption, but also for wine making, medicinal purposes, and the rotted fruit for poisoning the tip of arrows (Leal and Amaya 1991; Leal and d'Eeckenbrugge 1996). Crowns, slips. and suckers withstand considerable desiccation and resume growth when planted. Consequently, pineapples have been easily dispersed as the result of mankind's many migrations and are now found throughout the tropics.

Based on the key of Smith and Downs (1979), the genus *Ananas* contains seven species: *A. comosus*, *A. ananassoides*, *A. nanus*, *A. bracteatus*, *A. parguazensis*, *A. fritzmuelleri,* and *A. lucidus*. The closely related genus, *Pseudananas*, contains the monotypic *P. sagenarius*. Molecular studies suggest a revision of the current classification system is needed, which would lead to fewer species within the genus *Ananas*. A new system proposed by d'Eeckenbrugge and Leal (2003) would have the seven valid *Ananas* species downgraded to the level of five botanical varieties of *A. comosus*. *Pseudananas sagenarius* would also become *Ananas macrodontes* under their new classification.

All *A. comosus* have a diploid number of 50 small, spherical chromosomes (2n =2x=50) (Collins and Kerns 1931; Marchant 1967; Brown and Gilmartin 1986; Brown et al. 1997). Within the genus *Ananas* there are triploid, tetraploid, and heteroploid cultivars, while *Pseudananas sagenarius* is a naturally occurring tetraploid with 100 chromosomes (Collins 1960). Most varieties of *A. comosus* are self-incompatible due to the inhibition of pollen tube growth in the upper third of the style (Kerns 1932), which is gametophytically controlled by a single locus with multiple alleles (Brewbaker and Gorrez 1967). Some cultivars exhibit partial incompatibility (Cabral et al. 2000), which may be temperature dependent. The wild types, *A. ananassoides* and *P. sagenarius*, are either partially or completely self-compatible and self-compatibility is common in the other wild pineapples.

Pineapple is highly heterozygous and improvement of many different characters is possible. Breeding programs have made both intraspecific and interspecific crosses and selection has encompassed many aspects of productivity, fruit quality, and pest and disease resistance. In addition, clonal selection has also been utilized with up to 30 different somatic mutations described for the Smooth Cayenne cultivar (Collins and Kerns 1938). Once a desirable cultivar has been bred or selected it is relatively easy to propagate by vegetative means. The pineapple breeding system therefore combines very efficient vegetative reproduction with functional allogamous sexual reproduction.

World production of pineapple is estimated at greater than 14.6 million tonnes annually (FAOSTAT 2005) and more than 70% is consumed locally in the area of production. Although only a third of its output is used for processing (e.g., canned slices, chunks, crush, and juice), pineapple products account for more than two-thirds of the trade in pineapple by value. The processing industry is dominated by a single cultivar, Smooth Cayenne, with export earnings estimated at US$1.2 billion for countries in Asia and parts of Africa and Latin America. A recent trend in the industry has been the development of new hybrids specifically aimed for domestic fresh-fruit markets. A first result of these efforts has been the successful

introduction of a low-acid cultivar by Del Monte from Costa Rica into the European and American markets (Rohrbach et al. 2003).

Pineapple is the third most important tropical fruit in world production after banana and citrus, however, very little is known about the molecular genetics of pineapple. No molecular markers have been used in breeding programs to date, although they could be of tremendous use if they could be linked to important agronomic traits or to disease and pest resistance. Only recently have genes been isolated, described, and utilized in genetic transformation programs (Smith et al. 2005).

## 18.2 Progress in Genomics

Very little progress had been made in pineapple genomics until the last five to six years. The available data on *Ananas* genetic diversity is limited and is mostly based on morphological characters. Most of the initial molecular work focused on the genetic relationships among the seven *Ananas* species and the neighboring monospecific genus *Pseudoananas*, as well as their position within the Bromeliaceae family, to clarify classification and for phylogenetic analysis (Noyer et al. 1995; Terry et al. 1997; Duval et al. 2001; Ruas et al. 2001; Duval et al. 2003). Duval et al. (2001) studied molecular diversity in a set of 301 *Ananas* and *Pseudananas* accessions using restriction fragment length polymorphism (RFLP) and 18 pineapple genomic DNA probes. Factorial analysis differentiated *Pseudananas* from *Ananas*, but nevertheless, the two genera shared 58.7% of all bands, suggesting the existence of intergeneric gene flow. Genetic variation revealed by the set of RFLP markers used by these authors seems continuous with most variation found at the intraspecific level but no clear species partition was evident within *Ananas*. This lack of correspondence between the molecular and the taxonomical data was also observed in previous studies (Noyer 1991; Noyer et al. 1995). A different study by Ruas et al. (2001) was somewhat more successful in grouping different *Ananas* species using a much larger set of 148 RFLP markers but fewer accessions (a total of 16 from four *Ananas* species). Nevertheless, the generated dendogram had a number of abnormalities positioning several accessions in the wrong clusters and splitting species into different branches.

Chloroplast DNA has also been used to study phylogenetic relationships between *Ananas* and related genera (Duval et al. 2003). One hundred fifteen accessions representing the seven *Ananas* species and seven other Bromelioideae were analyzed using polymerase chain reaction-RFLP. Phenetic and cladistic analyses positioned *Ananas* and *Pseudananas* in a monophyletic group, with three distinct sub-groups. Interestingly, these groups do not reflect the different *Ananas* species but the geographical origin of the accessions.

*A. comosus* varieties cultivated for fruit have been divided into a number of groups based on similarity of morphological characters. Phenotypically, these groups are well differentiated and have been extensively characterized (Samuels 1970; Leal and Soule 1977; Dewald et al. 1988; Duval and d'Eeckenbrugge 1993; Noyer

et al. 1995). Nevertheless, and despite their wide morphological variation, RFLP analysis of 168 *Ananas comosus* accessions showed a relatively homogeneous group with low level of polymorphism when compared to wild *Ananas* species (Duval et al. 2001). Sripaoraya et al. (2001b) used random amplified polymorphic DNA (RAPD) to study three commercial cultivar groups, Cayenne, Queen, and Spanish, with the Cayenne and Queen groups appearing as separate clusters in the dendogram but failed to position the Spanish group representative in an independent cluster.

In contrast, amplified fragment length polymorphism (AFLP) markers seem to be more effective than RFLPs for the assessment of genetic diversity. A recent study of 148 *A. comosus* accessions using AFLP markers revealed a high degree of genetic variation within this species (Kato et al. 2004). But even though different DNA patterns could be assigned to each of the commercial cultivars studied, AFLP markers were still unsuccessful in clearly separating major cultivar groups (Kato et al. 2004). In contrast, Paz et al. (2005) also used AFLP markers to characterize the Mexican germplasm collection, mostly composed of *A. comosus* accessions, but reported a low level of diversity.

To explain the apparent conflict between taxonomical and molecular data, it has been suggested that the main phenotypic traits that characterize the different commercial cultivar groups are due to similar mutations that appeared on different genetic backgrounds when the cultivars were selected (Duval et al. 2001; Kato et al. 2004). Therefore, even though there is considerable genetic variation as detected by AFLP markers, this variation does not necessarily lead to the same traditional groupings. A good example is the smooth leaves that characterize the Smooth Cayenne cultivar. Presence of leaf spines is controlled by a single genetic locus with three possible alleles (Kinjo 1993; Cabral et al. 1997; Kato et al. 2004); therefore, the presence or absence of leaf spines (spininess) can arise in very genetically different plants by the mutation of a single gene.

Isozyme and RAPD markers have been used to study the genotypic fidelity of micropropagated pineapple plantlets. Two micropropagation systems, stationary and temporary immersion, were evaluated and even though neither of the two markers was successful in identifying significant differences individually, a combination of the two was able to determine that micropropagation by temporary immersion resulted in the lower frequency of somaclonal variants (Feuser et al. 2003).

The first and only pineapple genetic map available to this date was published by Carlier et al. (2004). The authors used the two-way pseudo-testcross approach to construct two individual maps of *A. comosus* and *A. bracteatus* using a segregating population of 46 F1 individuals from fully fertile crosses between the two species. To construct the map, a combination of three different types of markers, RAPDs, AFLPs, and inter simple sequence repeats (ISSRs), were used. The *A. comosus* map contained 157 markers (33 RAPD, 115 AFLP, eight ISSR, 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 mapped population. This map covered approximately 31% of the *A. 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. 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 was approximately 57.2% of the *A. bracteatus* genome calculated as 3693 cM with a ratio of 120 kb/cM.

Since the publication of the first *A. comosus* linkage map, Dr. Leitao's group has greatly improved the quality and resolution of the map and a new version has been kindly provided for this chapter (Fig. 18.1). The linkage groups shown in this new map gather a total of 651 markers, with 505 AFLP, 124 RAPD, 20 SSRs, one expressed sequence tag (EST) and one morphological trait (piping).

Despite the economic importance of the crop, very little sequence information is still available. In fact, only 51 pineapple sequences had been deposited in the GenBank nucleotide sequence database as of 2004. Twenty-four of those sequences were reported by Neuteboom et al. (2002), who used differential screening to isolate genes preferentially expressed in root tissues. Northern analysis using RNA isolated from roots, fruits, and aerial tissues revealed that eight of the clones were predominantly expressed in roots with the rest being present in two or more tissues. The most important contribution of pineapple sequences has been provided by Moyle et al. (2005a), who reported the cloning and sequencing of 1,548 EST clones isolated from cDNA libraries constructed from green, mature fruits (408 clones) and yellow, fully ripe fruits (1,140 clones). Relative EST clone abundance in green and yellow libraries correlated well with mRNA abundance in their respective tissues as shown by northern analysis. A number of genes strongly upregulated during fruit ripening were identified; among the most interesting were two metallothionein genes and a MADS box gene. One of the metallothionein clones was extremely abundant with over 40% of all library colonies hybridizing to a radio-labeled probe. The metallothionein expression level was calculated by quantitative real time PCR to be over 50 fold higher than the β-actin control in ripening fruit tissues. The MADS box gene was highly upregulated during fruit ripening and was not detected in any other tissue. MADS box proteins are transcription factors involved in regulating various aspects of plant development (Parenicova et al. 2003). Interestingly, the recessive ripening-inhibitor (*rin*) mutation in tomato that inhibits ripening even in the presence of exogenous ethylene has been identified as a MADS box gene (LeMADS-RIN) (Vrebalov et al. 2002). It has been suggested that LeMADS-RIN acts upstream of ethylene during ripening and could provide a common mechanistic link between climacteric and non-climateric fruit ripening. It is possible that the pineapple MADS box gene is the orthologue of the tomato LeMADS-RIN and complementation studies in *rin* tomato mutants are underway.

In a further EST project devised to study gene expression in roots after nematode infestation, 4,102 EST sequences were obtained, including 1,298 early infection clones, 2,461 late infection clones, and 343 non-infected root tip clones (Moyle and Botella unpublished results). Northern analysis and quantitative real time PCR have identified a variety of genes differentially expressed during gall formation. Analysis of clone distribution by functional classification reveals that the late infection library contains a named "high proportion of clones associated with oxidative

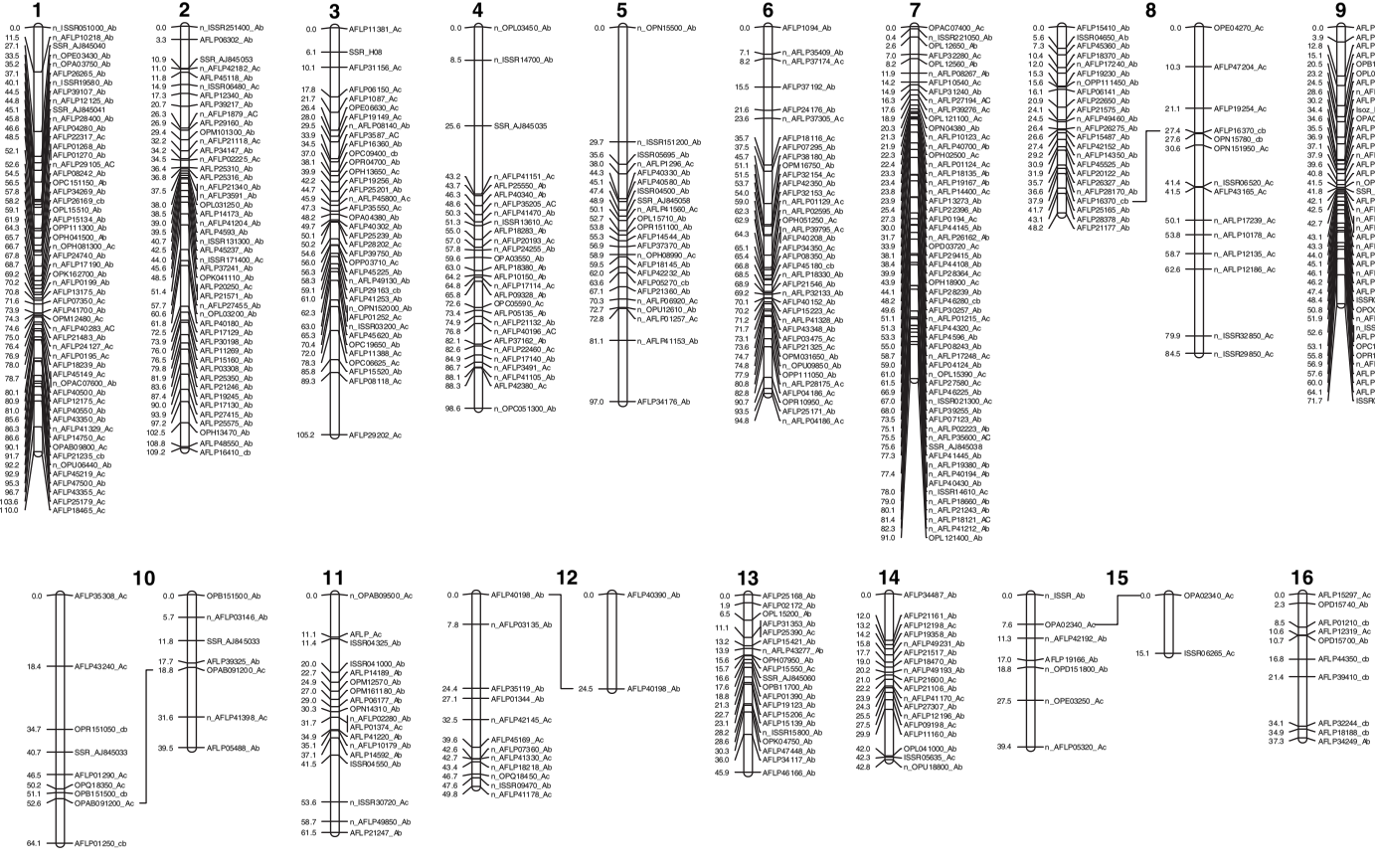


**Fig. 18.1** Integrated genetic map of *Ananas comosus* (pineapple). (1–16) Linkage groups that integrate molecular markers of var. *bracteatus* and var. *comosus*; (17–25) Linkage groups that integrate markers only of var. *bracteatus*; (26–32) Linkage groups that integrate markers only of var. *comosus*. Forty-seven very small linkage groups are not shown

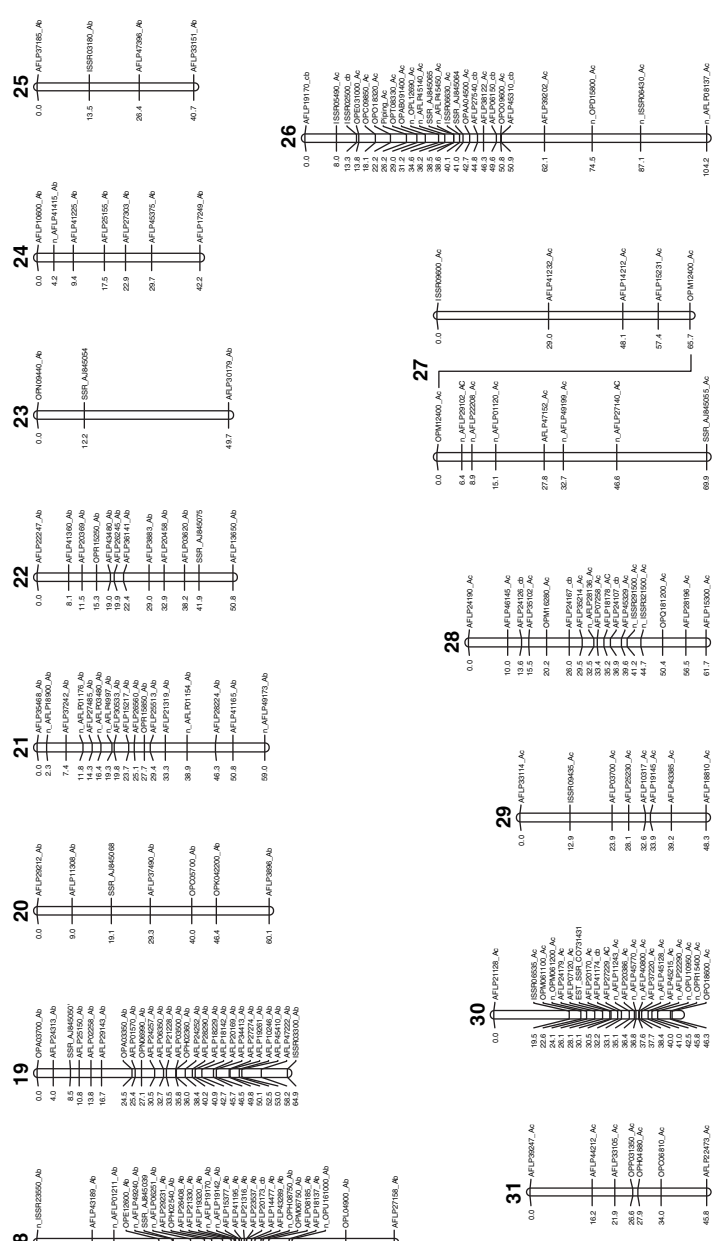

**Fig. 18.1** (continued)

Single search | Text search | Multiple search | Homology search | Multiple Homology search

## PineappleDB search results

### Full Search for 146

**All clones in Contig 146**
JBW019C06, JBW019C07, JBW019C08, JBW020A12, JBW020E01, JBW020E04, JBW020H04, JBW022A10, JBW023B12, JBW023F05, JBW023F06, JBW023H04, JBW023H05, JBW024A11, JBW024D05, JBW024D07, JBW024E03, JBW024E11, JBW025A07, JBW025D04, JBW026D03, JBW026D04, JBW026F04, JBW027F05, JBW027H07, JBW028B10, JBW028D05, JBW028D07, JBW028F09, JBW028H11, JBW029C10, JBW029F12, JBW029G05, JBW030A12, JBW030B03, JBW030B11, JBW030E10, JBW030H01, JBW031F04, JBW031G11, JBW032A08, JBW032D03, JBW033D02, JBW033E06, JBW033H03

| | |
|---|---|
| Contig sequence | CGTCCGATATCCTCTCTACCTCGAAGCTCCTCCTCTCCATAATTATATTCCCCTCCCCCGCTACCCACTTCCTCCGAGA<br>AGTTGAAGAGTAACACAACTACTCTACTCGGCACCACTTGAAGAGTGTGTGTTTGTGTGTGTGTGAGAGAGAGAGAGAG<br>AGAGAGAGCTCGATAAATTTGAGGGATCTTAATTGGAGGAGGAGGATCTGAGTAGAGGTAGTAGAGATGGGGAGAGGGA<br>GAGTTGAGCTGAAGAGGATCGAGAACAAAATCAACCGGCAAGTGACCTTCTCGAAGCGCCGCAACGGCGTCCTCAAGAA<br>GGCCTACGAGCTCTCCGTCCTCTGCGACGCCGAGGTCGCCCTCATCATCTTCTCCAGCCGCGGCAAGCTCTACGAGTTC<br>GGAAGCGTTGGCACAAGCAAGACATTAGAACGATATCAACGCTGCTGTTACAATTCTCAGGATTCAAGTGCTGGTGATC<br>GTGAGACTCAGAGCTGGTACCAGGAAATGTCCAAGTTGAAGGCAAAATTTGAGTCTCTGCAGCGCTCTCAGAGGCATTT<br>GCTCGGGGAGGATCTTGGACCGCTGAGTGTGAAAGAATTGCAGCAACTGGAGCGACAGCTTGAGTCTGCTCTTTCACAA<br>GCCAGACAGAGAAAGACTCAAATAATGATGGATCAGATGGAAGAACTTCGCAAAAAAGAACGTCAACTGGGAGAAATAA<br>ATAAGCAGCTTAGAAACAAGCTTGAGGCCGAAGGCGGCGCCTTTAGGGCCATTCAAGGATCATGGGCTTCTGATGCTAT<br>TGTGAGTGGAAATGCATTCAATATGCAGCACGCCCAATCGAGCAGCATGGAATGCGAACCCACTCTGCAAATAGGGTAT<br>CACCAATTTGTTCCTCCTGAGGCAACCATTCCAAGAACCGCCGGTGGGGGAGAACAATTTCATGCTTGGTTGGGTTCTGT<br>GAACCATTCTGGAACCTACCAGAAGCCCATATTATCGGTAATGTTTGTACTGAAATTATTATTATTATCATTAATG<br>TTATTATTATTATGTTCTGCTATTGATCGTGCCTCTGAGAACTTATGCTGTTTAATCCGTTTTGCGGACCAARTGTTGC<br>TGCTTTCTAGTGTGTGTTATCCTTTTGAACAATGTAAATTCTGTATGGCAATGCTACTAGCTTCTTGTGGGAACAATAT<br>TTACTTAATATAAATGCTATTAAGTGTTGTCATTTAAC |
| Name | MADS box protein |
| Contig length | 1241 |
| Full match description | gb\|AAQ83835.1\| MADS box protein [Asparagus officinalis] |
| Matching accession number | AAQ83835 |
| Length of match | 234 |
| Match homology | 92% |
| Functional class | TRANSCRIPTION |
| Functional class number | 04.05.01.04 |
| Alternative Functional subclass | transcriptional control |
| Functional subclass number | 0 |
| Arabidopsis homolog | At2g45650 |
| Gene Ontology<br>Based on Arabidopsis homolog | Molecular function:<br>GO:0003677 - DNA binding (ISS)<br>GO:0003700 - transcription factor activity (ISS)<br>Cellular component:<br>GO:0005634 - nucleus (IEA)<br>Biological process:<br>GO:0006355 - regulation of transcription, DNA-dependent (IEA) |
| Splice variants | None |

**Distribution of cDNAs**

| | Total | Fruit | Green mature fruit | Yellow ripe fruit | Root | Root tips | Gall VC 1-4 weeks | Gall VC 5-10 weeks |
|---|---|---|---|---|---|---|---|---|
| **Number of cDNAs** | 45 | 45 | 0 | 45 | 0 | 0 | 0 | 0 |
| **% for that tissue** | 0.797 | 2.907 | 0.000 | 3.947 | 0.000 | 0.000 | 0.000 | 0.000 |

Return to main search page

Please send comments etc to Mark Crowe

**Fig. 18.2** PineappleDB, the online pineapple bioinformatics resource (www.pgel.com.au). View of a typical entry containing sequence, functional, homology and database information

stress responses and detoxification of free radicals (Moyle and Botella unpublished results).

The entire collection of ESTs generated in the Botella lab has been made publicly available by an online pineapple bioinformatics resource PineappleDB" (www.pgel.com.au) (Moyle et al. 2005b). PineappleDB is a curated database providing integrated access to annotated EST data for cDNA clones isolated from pineapple fruit, root, and nematode-infected root gall vascular cylinder tissues. The database contains in excess of 5,600 EST sequences and 3,383 contig consensus sequences. All entries contain associated bioinformatic data including splice variants, *Arabidopsis* homologues, clone distributions, MIPS (Munich Information Center for Protein Sequence) based and gene ontology functional classifications (Fig. 18.2). In addition, the online resource provides extensive search capabilities by text or sequence homology (BLAST).

Finally, microarrays have been produced with 9,312 pineapple cDNAs printed in duplicate including the entire EST collection generated by the Botella group plus a number of unknown clones. These microarrays have been used in two independent studies to (a) study gene expression in roots and gall tissues at different times after nematode infestation, and (b) perform gene expression profiling of pineapple fruits during ripening, and the results will soon be available (Moyle and Botella unpublished results). These microarrays are available to the research community.

## 18.3 Prospects

It is clear that pineapple genomics is at its infancy and that many more resources need to be developed. Although some molecular markers are now available and a basic genetic map has been constructed, more polymorphic markers and a denser map are needed. In this respect, Leitao's group is improving the existing pineapple map and complementing it with additional molecular markers (J.M. Leitao personal communication).

Expanded EST projects are also needed to enrich the pool of pineapple cDNAs available to the research community and the development of bioinformatic based analyses to identify interesting candidate genes for the genetic improvement of this crop. A full genome sequencing initiative has not been explored yet, but it could prove invaluable for the advancement of classical breeding as well as the development of biotechnological solutions to the most important agronomic problems. Consumer oriented fruit quality improvement, such as sugar, vitamin and acid content, can also be targeted if more genomic resources are developed.

Biotechnology will undoubtedly play an important role in pineapple improvement in the not too distant future and could take advantage of new developments in genomics. Herbicide-tolerant transgenic varieties have already been produced (Sripaoraya et al. 2001a). Another important agronomic problem such as natural flowering has also been tackled and transgenic pineapples have been produced with delayed natural flowering (Trusov and Botella 2006). Fruit quality issues have also

been addressed with Ko et al. (2006) introducing transgenes to control blackheart, an internal browning of fruit flesh as a result of chilling injury.

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