Pineapple

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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 in order 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 new markers. Medium scale 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.

20.1 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 fibre properties which are not commonly found in wild types (Collins, 1951; d'Eeckenbrugge, Leal and Duval, 1997). The pineapple was used not only as a fresh fruit, but also for wine making and 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 that would lead to fewer species within the genus *Ananas*. A new system proposed by Coppens 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, Palaci and Luther, 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, d'Eeckenbrugge and de Matos, 2000), which may be temperature dependant. 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, Leal and d'Eeckenbrugge, 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 pine-

apple. 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, Ko, Sanewski and Botella, 2005).

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 was 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, Lanaud, d'Eeckenbrugge and Duval, 1995; Terry, Brown and Olmstead, 1997; Duval, Noyer, Perrier, d'Eeckenbrugge and Hamon, 2001; Ruas, Ruas and Cabral, 2001; Duval, Buso, Ferreira, Noyer, d'Eeckenbrugge, Hamon and Ferreira, 2003). Duval *et al*. (2001) studied molecular diversity in a set of 301 *Ananas* and *Pseudoananas* accessions using 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 RFLP markers (148) but less accessions (a total of 16 from 4 *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 PCR-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, Moore and Sherman, 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, Blackhall, Marchant, Power, Lowe and Davey, (Sripaoraya, Blackhall, Marchant, Power, Lowe and Davey, 2001) used RAPDs 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, 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, Nagai, Moore, Zee, Kim, Steiger and Ming, 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, Gil, Rebolledo, Rebolledo, Uriza, Martinez, Isidron and Simpson, (Paz, Gil, Rebolledo, Rebolledo, Uriza, Martinez, Isidron and Simpson, 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. Spininess is controlled by a single genetic locus with three possible alleles (Kinjo, 1993; Cabral, de Matos and d'Eeckenbrugge, 1997; Kato *et al.*, 2004), therefore the presence or absence of spiny leaves 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 none of the two markers were successful in identifying significant differences individually, a combination of both was able to determine that micropropagation by temporary immersion resulted in the lowest proportion of somaclonal variants (Feuser, Meler, Daquinta, Guerra and Nodari, 2003).

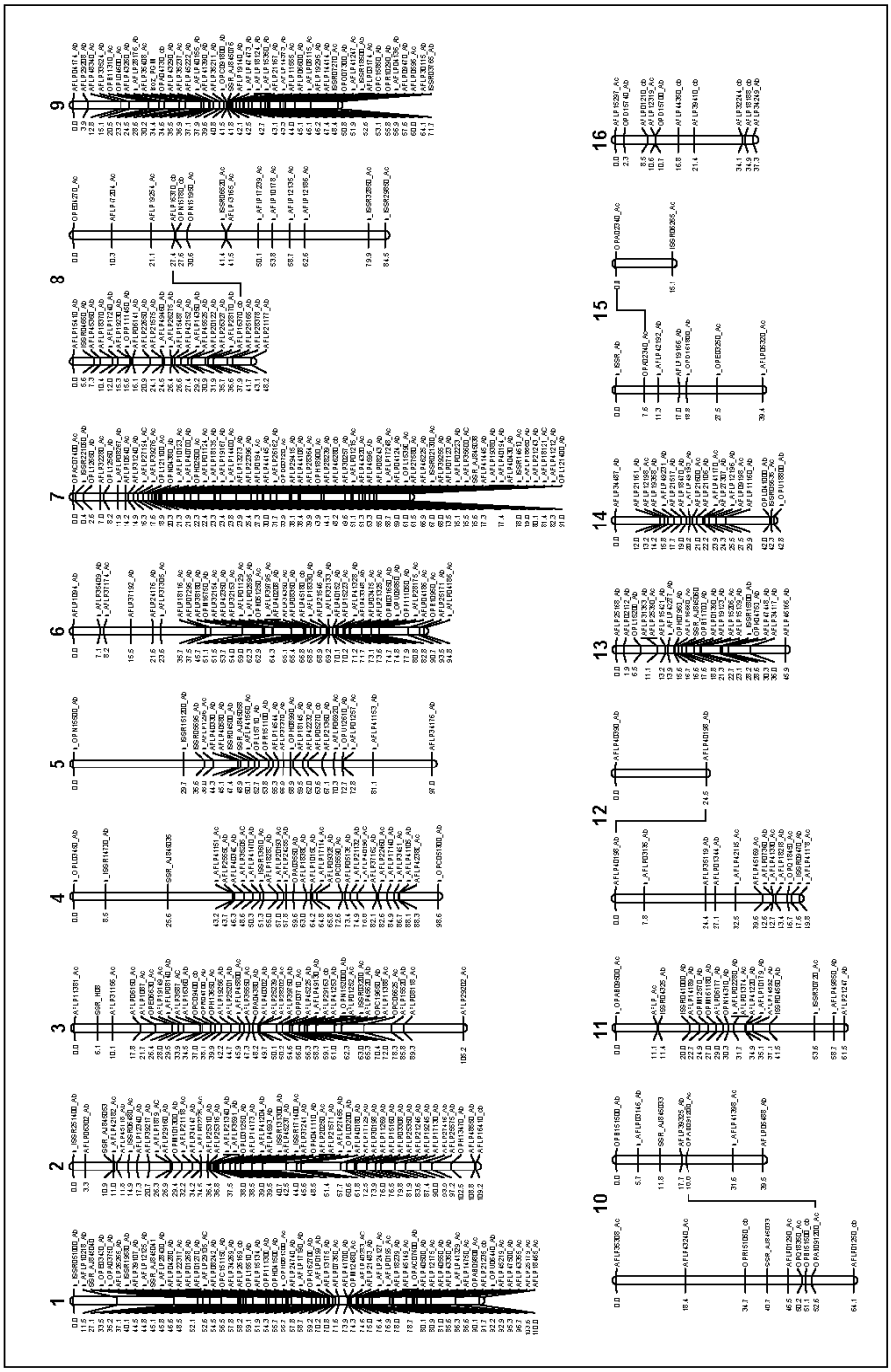
The first and only pineapple genetic map available to this date was published by Carlier, Reis, Duval, d'Eeckenbrugge and Leitao in 2004. The authors used the two-way pseudo-testcross approach to construct two individual maps of *A. comosus* and *A. bracteatus* using a segregating population of 46 F1 individuals from fully fertile crosses between the two species. To construct the map, a combination of three different types of markers were used, RAPDs, AFLPs and ISSRs. 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, Reis, Duval, d'Eeckenbrugge and Leitao, 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 4146 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 is 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 report (Fig. 1). The linkage groups shown in this new map gather a total of 651 markers, with 505 AFLP, 124 RAPD, 20 SSRs, 1 EST and 1 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, Kunimitsu, Webb and Christopher, (Neuteboom, Kunimitsu, Webb and Christopher, 2002) who used differential screening in order to isolate genes preferentially expressed in root tissues. Northern analysis using RNA isolated from roots, fruits and aerial tissues revealed that 8 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, Fairbairn, Ripi, Crowe and Botella, (Moyle, Fairbairn, Ripi, Crowe and Botella, 2005) who reported the cloning and sequencing of 1548 EST clones isolated from cDNA libraries constructed from mature green fruits (408 clones) and yellow fully ripe fruits (1140 clones). Relative EST clone abundance in green and yellow libraries correlated well with mRNA abundance in their respective tissues as proved 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 to be over 50 fold higher than the β-actin control in ripening fruit tissues by quantitative real time PCR. 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, de Folter, Kieffer, Horner, Favalli, Busscher, Cook, Ingram, Kater, Davies, Angenent and Colombo, 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, Ruezinsky, Padmanabhan, White, Medrano, Drake, Schuch and Giovannoni, 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, a total of 4,102 EST sequences have been 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



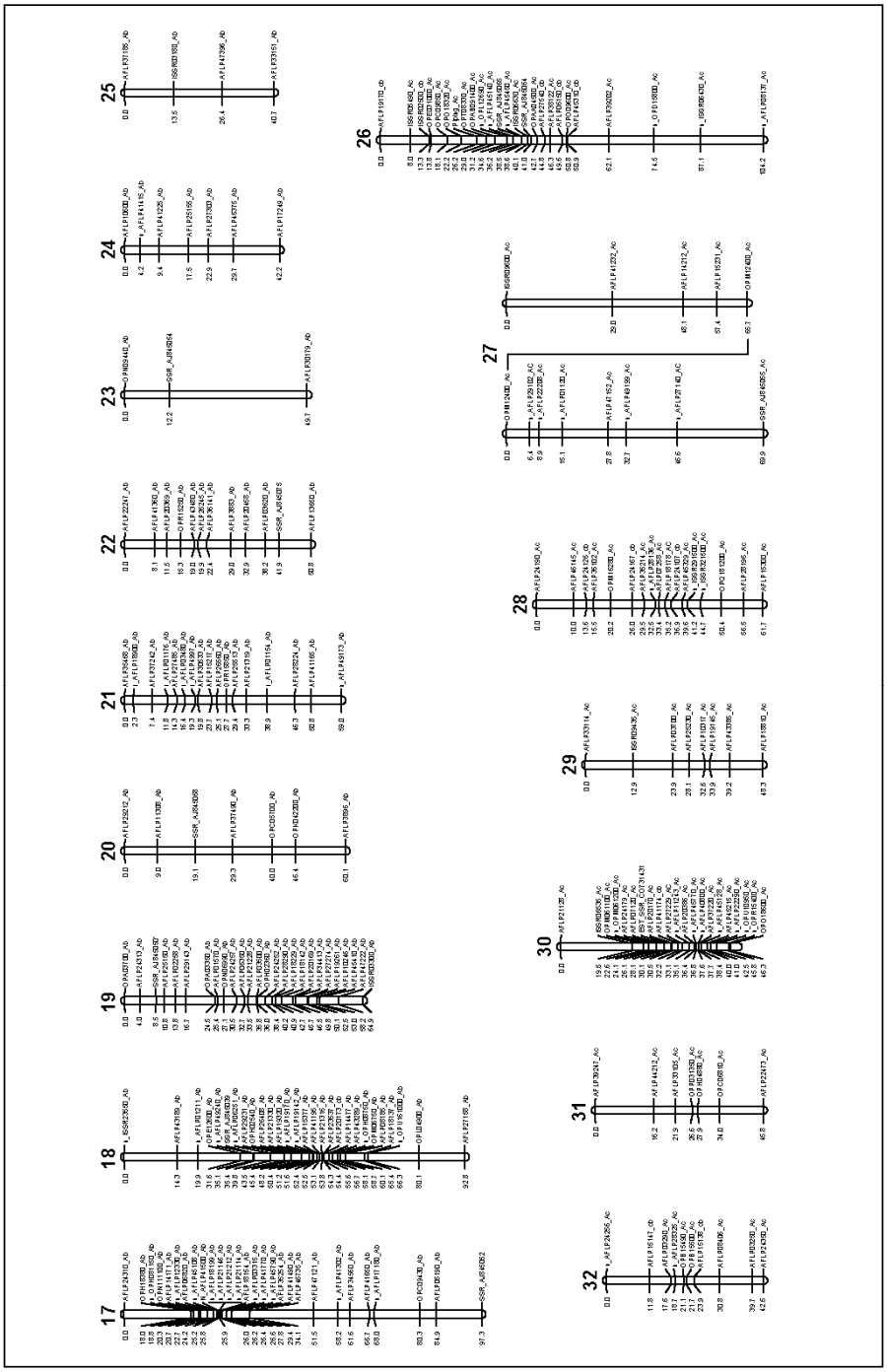


Fig. 1. (Previous pages) Integrated genetic map of *Ananas comosus* (pineapple). (1-16) Linkage groups that integrate molecular markers of var. *bracteatus* and var. *comosus*; (17-25) Linkage groups that integrate markers only of var. *bracteatus*; (26-32) Linkage groups that integrate markers only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

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 high proportion of clones associated with oxidative 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 named 'PineappleDB' (www.pgel.com.au) (Moyle, Crowe, Ripi-Koja, Fairbairn and Botella, 2005). 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 based and Gene Ontology functional classifications (Fig. 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. This microarray 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.

20.3 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, 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 in order 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.

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 AGTTGAAGAGTAACACAACTACTCTACTCGGCACCACTTGAAGAGTGTGTGTTTGTGTGTGTGAGAGAGAGAGAGAG AGAGAGAGCTCGATAAATTTGAGGGATCTTAATTGGAGGAGGAGGATCTGAGTAGAGGTAGTAGAGATGGGGAGAGGGA GAGTTGAGCTGAAGAGGATCGAGAACAAAATCAACCGGCAAGTGACGTTCTCGAAGCGCCGCAACGGGCTCCTCAAGAA GGCCTACGAGCTCTCCGTCCTCTGCGACGCCGAGGTCGCCCTCATCATCTTCTCCAGCCGCGGCAAGCTCTACGAGTTC GGAAGCGTTGGCACAAGCAAGACATTAGAACGATATCAACGCTGCTGTTACAATTCTCAGGATTCAAGTGCTGGTGATC GTGAGACTCAGAGCTGGTACCAGGAAATGTCCAAGTTGAAGGCAAAATTTGAGTCTCTGCAGCGCTCTCAGAGGCATTT GCTCGGGGAGGATCTTGGACCGCTGAGTGTGAAAGAATTGCAGCAACTGGAGCGACAGCTTGAGTCTGCTCTTTCACAA GCCAGACAGAGAAAGACTCAAATAATGATGGATCAGATGGAAGAACTTCGCAAAAAAGAACGTCAACTGGGAGAAATAA ATAAGCAGCTTAGAAACAAGCTTGAGGCCGAAGGCGGCGCCTTTAGGGCCATTCAAGGATCATGGGCTTCTGATGCTAT TGTGAGTGGAAATGCATTCAATATGCAGCACGCCCAATCGAGCAGCATGGAATGCGAACCCACTCTGCAAATAGGGTAT CACCAATTTGTTCCTCCTGAGGCAACCATTCCAAGAACCGCCGGTGGGGAGAACAATTTCATGCTTGGTTGGGTTCTGT GAACCATTCTGGAACCTACCAGAAGCCATATTATCGGTAATGTTTGTACTGAAATTATTATTATTATCATTAATG TTATTATTATTATGTTCTGCTATTGATCGTGCCTCTGAGAACTTATGCTGTTTAATCCGTTTTGCGGACCAARTGTTGC TGCTTTCTAGTGTGTGTTATCCTTTTGAACAATGTAAATTCTGTATGGCAATGCTACTAGCTTCTTGTGGGAACAATAT 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 Based on Arabidopsis homolog	Molecular function: GO:0003677 - DNA binding (ISS) GO:0003700 - transcription factor activity (ISS) Cellular component: GO:0005634 - nucleus (IEA) Biological process: 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

Fig. 2. PineappleDB, the online pineapple bioinformatics resource (www.pgel.com.au). View of a typical entry containing sequence, functional, homology and database information.

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, Marchant, Power and Davey, 2001). 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, Campbell, Jobin-Décor, Eccleston, Graham and Smith, (Ko, Campbell, Jobin-Décor, Eccleston, Graham and Smith, 2006) introducing transgenes to control blackheart, an internal browning of fruit flesh as a result of chilling injury.

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References

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

Brown, G.K., and Gilmartin, A.J. (1986). Chromosomes of the *Bromeliaceae*. Selbyanna 9, 85-93.

Brown, G.K., Palaci, C.A., and Luther, H.E. (1997). Chromosome numbers in *Bromeliaceae*. Selbyanna 18, 85-88.

Cabral, J.R.S., de Matos, A.P., and d'Eeckenbrugge, G.C. (1997). Segregation for resistance to fusariose, leaf margin type and leaf colour from the EMBRAPA Pineapple Hybridization Programme. Acta Hort. 425, 193-200.

Cabral, J.R.S., d'Eeckenbrugge, G.C., and de Matos, A.P. (2000). Introduction of selfing in pineapple breeding. Acta Hort. 529, 165-168.

Carlier, J.D., Reis, A., Duval, M.F., d'Eeckenbrugge, G.C., and Leitao, J.M. (2004). Genetic maps of RAPD, AFLP and ISSR markers in *Ananas bracteatus* and *A. comosus* using the pseudo-testcross strategy. Plant Breeding 123, 186-192.

Collins, J.L. (1951). Antiquity of the pineapple in America. Southwestern J. Anthrop. 7, 145-155.

Collins, J.L. (1960). *The pineapple*. (New York: Interscience Publishers).

Collins, J.L., and Kerns, K.R. (1931). Genetic studies of the pineapple. I. A preliminary report on the chromosome number and meiosis in seven pineapple varieties (*Ananas sativus* Lindl.) and in *Bromelia pinguin* L. J. Hered. 22, 139-142.

Collins, J.L., and Kerns, K.R. (1938). Mutations in pineapples. A study of thirty inherited abnormalities in the Cayenne variety. J. Hered. 29, 169-173.

d'Eeckenbrugge, C.G., and Leal, F. (2003). Morphology, anatomy and taxonomy. In: D.P. Bartholomew, R.E. Paull, and K.G. Rohrbach, (Eds.), *The Pineapple: Botany, Production and Uses*. CAB International, Wallingford, UK, pp. 13-32.

d'Eeckenbrugge, C.G., Leal, F., and Duval, M.F. (1997). Germplasm resources of pineapple. Hort. Reviews 21, 133-175.

Dewald, M.G., Moore, G.A., and Sherman, W.B. (1988). Identification of Pineapple Cultivars by Isozyme Genotypes. J. Am. Soc. Hort. Sci. 113, 935-938.

Duval, M.F., and d'Eeckenbrugge, G.C. (1993). Genetic variability in the genus *Ananas*. Acta Hort., 27-32.

Duval, M.F., Noyer, J.L., Perrier, X., d'Eeckenbrugge, G.C., and Hamon, P. (2001). Molecular diversity in pineapple assessed by RFLP markers. Theor. Appl. Genet. 102, 83-90.

Duval, M.F., Buso, G.S.C., Ferreira, F.R., Noyer, J.L., d'Eeckenbrugge, G.C., Hamon, P., and Ferreira, M.E. (2003). Relationships in *Ananas* and other related genera using chloroplast DNA restriction site variation. Genome 46, 990-1004.

FAOSTAT. (2005). http://apps.fao.org.

Feuser, S., Meler, K., Daquinta, M., Guerra, M.P., and Nodari, R.O. (2003). Genotypic fidelity of micropropagated pineapple (*Ananas comosus*) plantlets assessed by isozyme and RAPD markers. Plant Cell Tissue and Organ Culture 72, 221-227.

Kato, C.Y., Nagai, C., Moore, P.H., Zee, F., Kim, M.S., Steiger, D.L., and Ming, R. (2004). Intra-specific DNA polymorphism in pineapple (*Ananas comosus* (L.) Merr.) assessed by AFLP markers. Gen. Res. & Crop Evol. 51, 815-825.

Kerns, K.R. (1932). Concerning the growth of pollen tubes in pistils of Cayenne flowers. Pineapple Quarterly 1, 133-137.

Kinjo, K. (1993). Inheritance of leaf margin spine in pineapple. Acta Hort. 334, 59-66.

Ko, H.L., Campbell, P.R., Jobin-Décor, M.P., Eccleston, K.L., Graham, M.W., and Smith, M.K. (2006). The introduction of transgenes to control blackheart in pineapple (*Ananas comosus* L.) cv. Smooth Cayenne by microprojectile bombardment. Euphytica, (in press).

Leal, F., and Amaya, L. (1991). The curaga (*Ananas lucidus*, Bromeliaceae) crop in Venzuela. Econ. Bot. 45, 216-217.

Leal, F.J., and Soule, J. (1977). Maipure, a New Spineless Group of Pineapple Cultivars. HortScience 12, 301-305.

Leal, F.J., and d'Eeckenbrugge, G.C. (1996). Pineapple. In: J. Janick and J.N. Moore, (Eds.), *Fruit Breeding, Vol.I. Tree and Tropical Fruits*. John Wiley & Sons, New York, pp. 565-606.

Marchant, C.J. (1967). Chromosome evolution in the Bromeliaceae. Kew Bulletin 21, 161-168.

Moyle, R., Fairbairn, D.J., Ripi, J., Crowe, M.L., and Botella, J.R. (2005). Developing pineapple fruit has a small transcriptome dominated by metallothionein. J. Exp. Bot. 56, 101-112.

Moyle, R., Crowe, M.L., Ripi-Koja, J., Fairbairn, D.J., and Botella, J.R. (2005). PineappleDB: An online pineapple bioinformatics resource. BMC Plant Biology 5, 21.

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

Noyer, J.L. (1991). Etude preliminaire de la diversite genetique du genre *Ananas* par les RFLPs. Fruits (numero especial *Ananas*), 372–375.

Noyer, J.L., Lanaud, C., d'Eeckenbrugge, G.C., and Duval, M.F. (1995). RFLP study on rDNA variability in *Ananas* genus. Acta Hort. 425, 153–159.

Parenicova, L., de Folter, S., Kieffer, M., Horner, D.S., Favalli, C., Busscher, J., Cook, H.E., Ingram, R.M., Kater, M.M., Davies, B., Angenent, G.C., and Colombo, L. (2003). Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis: New openings to the MADS world. Plant Cell 15, 1538-1551.

Paz, E.Y., Gil, K., Rebolledo, L., Rebolledo, A., Uriza, D., Martinez, O., Isidron, M., and Simpson, J. (2005). AFLP characterization of the Mexican pineapple germplasm collection. J. Am. Soc. Hort. Sci. 130, 575-579.

Rohrbach, K.G., Leal, F., and d'Eeckenbrugge, C.G. (2003). History, distribution and world production. In: D.P. Bartholomew, R.E. Paull, and K.G. Rohrbach, (Eds.), *The Pineapple: Botany, Production and Uses*. CAB International, Wallingford, UK, pp. 1-12.

Ruas, C.D., Ruas, P.M., and Cabral, J.R.S. (2001). Assessment of genetic relatedness of the genera *Ananas* and *Pseudananas* confirmed by RAPD markers. Euphytica 119, 245-252.

Samuels, G. (1970). Pineapple cultivars. Am. Soc. Hort. Sci. Proc., 13-24.

Smith, L.B., and Downs, R.J. (1979). Bromelioides (*Bromeliaceae*). Flora Neotropica Mono. 14, 1493-2142.

Smith, M.K., Ko, H.L., Sanewski, G.M., and Botella, J.R. (2005). *Ananas comosus,* Pineapple. In: R.E. Litz, (Ed.), *Biotechnology of Fruit and Nut Crops*. CAB International, Wallingford, UK, pp. 157-172.

Sripaoraya, S., Marchant, R., Power, J.B., and Davey, M.R. (2001). Herbicide-tolerant transgenic pineapple (*Ananas comosus*) produced by microprojectile bombardment. Annals of Botany 88, 597-603.

Sripaoraya, S., Blackhall, N.W., Marchant, R., Power, J.B., Lowe, K.C., and Davey, M.R. (2001). Relationships in pineapple by random amplified polymorphic DNA (RAPD) analysis. Plant Breeding 120, 265-267.

Terry, R.G., Brown, G.K., and Olmstead, R.G. (1997). Examination of subfamilial phylogeny in *bromeliaceae* using comparative sequencing of the plastid locus ndhF. Am. J. Bot. 84, 664-670.

Trusov, Y., and Botella, J.R. (2006). Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple (*Ananas comosus* (L.) Merr.). J. Exp. Bot., (In press).

Vrebalov, J., Ruezinsky, D., Padmanabhan, V., White, R., Medrano, D., Drake, R., Schuch, W., and Giovannoni, J. (2002). A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (Rin) locus. Science 296, 343-346.