# I.1 Banana

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## 1 Introduction

Bananas (*Musa* spp.), belonging to the family Musaceae, are the perennial monocotyledons commonly grown in the tropics situated at latitude 20° above and below the equator, where there is a wide seasonal variation in rainfall and temperature. According to the FAO in 2004, 98% of world banana production was derived from developing countries, amongst which India, Brazil, China and Ecuador accounted for 50% of the total production (http://faostat.fao.org/default.aspx). It has been estimated that world production of bananas in 2004 was 70.6 million tons and the export value amounted to more than US $4 billion annually. In international trade, bananas account for ~22% of world fresh fruit production and are ranked as the second most important fruit crop after citrus. Bananas can also be socio-politically important as they are the staple food of millions of people in the developing world.

Bananas comprise both wild and cultivated genotypes with considerable genetic diversity and a wide range of morphological characteristics. In general, bananas can be classified into four genome groups – A, B, S and T – represented by *M. acuminata* ($2n = 2x = 22$), *M. balbisiana* ($2n = 2x = 22$), *M. schizocarpa* ($2n = 2x = 22$) and *Australimusa* ($2n = 2x = 20$), respectively. The haploid genome size of bananas is relatively small at 500–600 Mbp (Lysák et al. 1999). Analysis of gene content and density in the genome of *M. acuminate* reveals that it is similar to the Graminae, as genes are usually clustered in the gene-rich regions, separated by the gene-poor domains that are rich in transposons (Aert et al. 2004).

Most commercial bananas are triploids ($2n = 3x = 33$), with the genome ofAAA, AAB and ABB originating from polyploidization and interspecific hybridization of the two diploid species *M. acuminata* and *M. balbisiana*. However, not all triploids are important commercially. Most cultivars involved in international trade belong to the Cavendish sub-group (*Musa* AAA), although they account for only 12% of total banana production (Robinson 1995).

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## 2 Banana Breeding and Molecular Markers

Major efforts in different countries, including Australia, India, Brazil, Honduras and France, have been devoted to improving banana performance through breeding programs. The FAO/IAEA have supported a mutation-assisted breeding program for banana since 1994 (Jain 2005). Breeders aim to generate new banana hybrids with improved resistance to *Fusarium* wilt and Sigatoka disease,increased tolerance to nematodes and physical stress of the fruit, dwarfism, increased fruit yield and improved fruit flavour and morphology (Ortiz et al. 1995). In general, the progress of banana breeding has been relatively slow. This is due mainly to the narrow genetic variability resulting from the limited number of landraces selected from the natural germplasm, and low female fertility. Mutation treatment has been employed by subjecting cultured tissues to gamma irradiation to increase genetic variability and maintain fertility. This approach has resulted in the production of aluminium-tolerant (Matsumoto and Yamaguchi 1990) and early-flowering (Novak et al. 1990) variants. However, there has been limited success in producing novel banana variants with commercial importance by induced mutation.

As part of the breeding programs, banana research has focused on genome structure analysis and the development of biochemical and molecular markers. It has been reported that genomic in situ hybridization can be used to differentiate the chromosomes of four genome groups and interspecific cultivars of banana (D'Hont et al. 2000), while the distribution of repeated sequences in the genome can be determined by fluorescence in situ hybridization (D'Hont 2005). These techniques are useful for the development of DNA markers, as reviewed by Jarret and Gawel (1995) and Kaemmer et al. (1997). Several DNA markers for bananas have been reported. These include restriction fragment length polymorphisms (RFLP) (Gawel and Jarret 1991; Jarret et al. 1992; Ge et al. 2005), variable number of tandem repeats (VNTR) polymorphisms (Kaemmer et al. 1992; Bhat et al. 1995), microsatellite DNA (Creste et al. 2003; Buhariwalla et al. 2005), random amplified polymorphic DNA (RAPD) (Onguso et al. 2004; Martin et al. 2006; Ray et al. 2006), amplified fragment length polymorphisms (AFLP) (Wong et al. 2001; Ude et al. 2002) and inter-simple sequence repeats (Ray et al. 2006). In general, hybridization-based techniques, e.g. RFLP, are straightforward but relatively time-consuming, especially when dealing with a large number of genotypes. In order to overcome this drawback, sequence-tagged microsatellite site (STMS) markers based on VNTR polymorphism of microsatellites, which are simple sequence repeats, have been developed. This technique is based on the polymerase chain reaction (PCR) and non-radioactive methods (Kaemmer et al. 1997). Using the mapping approach to compare the efficiency of different techniques, STMS markers have been shown to be more efficient than isozyme, RFLP and RAPD markers. Mansoor et al. (2005) reported that PCR-based methods can be used to detect banana bunchy top virus (BBTV) in banana, which will be useful for screening micropropagated banana plants for BBTV. On the other hand, a modified version of AFLP,

selective amplification of microsatellite polymorphic loci, has been adopted for fingerprinting somaclonal variants in banana (Giménez et al. 2005). Because of the diverse genetic background of the banana genome, it has been suggested that characterization of somaclonal variants may require more than one molecular marker (Asif and Othman 2005). Nevertheless, it is anticipated that these molecular markers may serve as important tools for analysis of genetic makeup, detection of desirable traits, somaclonal variants and germplasm preservation in breeding programs.

## 3 Identification of Banana Genes

The availability of genes responsible for useful traits is important for the elucidation of mechanisms that regulate plant growth and development, plant responses to biotic and abiotic stress at the molecular level, and for banana improvement using genetic engineering. During recent years, several attempts have been made to clone and to characterize genes from banana (Clendennen and May 1997; Medina-Suárez R et al. 1997; Drury et al. 1999; Gupta et al. 2006; Manrique-Trujillo et al. 2006). To isolate genes expressed in response to temperature stress, Santos and co-workers (2005) constructed two cDNA libraries from leaves of *M. accuminata* ssp. *burmannicoides* subject to low (5–25 °C) and high (25–45 °C) temperatures. Analysis of expressed sequence tags (ESTs) from the two libraries revealed that 10% of ESTs were commonly present in both tissues, while 42 and 48% were present specifically in tissue under low and high temperature stress, respectively (Santos et al. 2005). However, the identity of these genes and their functions are not known. In addition, several genes have been isolated from banana fruit and their identity has been determined putatively by sequence homology with published gene sequences deposited in the Genbank database. In this chapter, these genes are classified as ripening related, pathogenesis and stress related, and unknown function.

### 3.1 Fruit Ripening

Bananas are climacteric fruits, which are characterized by low rates of respiration and ethylene production at the pre-climacteric stage during ripening, followed by a sudden upsurge at the climacteric and a shape decline at the post-climacteric stage. This pattern of ethylene production during ripening has been attributed to autocatalytic production and inhibition (Yang and Hoffman 1984). Ripening is a complex process, which involves drastic changes in various biochemical events in the fruit. These include softening, loss of chlorophyll and increased yellowing of the peel, increased conversion of starch to sugars, and alteration of flavour and aroma. A large volume of evidence shows that ethylene plays a pivotal regulatory role in ripening (Lelièvre et al. 1997; Giovannoni 2001).

Bananas are highly perishable, with a short shelf life after harvest. Several common practices have been employed in attempts to prolong the shelf life of the fruit. These include refrigeration and controlled atmosphere storage, the use of ethylene absorbant, and surface coating and moist sawdust treatments (Abdullah et al. 1990), but the practices are either too costly or ineffective. It is important to understand the regulatory mechanism of ripening in order to manipulate and control the process. The current knowledge regarding the molecular biology of ripening has been derived primarily from work with tomato (Alexander and Grierson 2002). In recent years, major efforts have been devoted to elucidating the underlying mechanism of ripening at the molecular level (Giovannoni 2001). Genes associated with ripening have been isolated and characterized from several plant species, including peach (Trainotti et al. 2003), pear (Fonseca et al. 2004), melon (Hadfield et al. 2000), strawberry (Nam et al. 1999), citrus (Moriguchi et al. 1998), kiwifruit (Ledge and Gardner 1994) and tomato (Fei et al. 2004). In banana, several ripening-related genes have also been identified by differential expression of cDNA libraries in ripening fruits (Hill and ap Rees 1995; Clendennen and May 1997; Medina-Suárez et al. 1997; Drury et al. 1999; Manrique-Trujillo et al. 2007).

In our laboratory, we isolated 80 ESTs from a cDNA library constructed from the ripening fruit of *M. acuminata* cv. Williams (Liu 2000). Sequence analysis revealed a high homology of 30 ESTs with the known genes in the Genbank database. The distribution of these ESTs and their putative functions include four for $\beta$-1,3-glucanase, three each for pectate lyase and metallothionein (MT)-like protein (MLP) 2, and one each for UDPglucose pyrophosphorylase (UGPase), MLP 1, MLP 3, actin, isoflavone reductase, 1-aminocyclopropane-1-carboxylase (ACC) oxidase, *O*-methyltransferase, *S*-adenosylmethionine (SAM) synthetase, cytochrome P450 monooxygenase, 3-hydroxy-isobutryl-1-co-enzyme A hydrolase, malate synthase, glucosidase, vesicle transport protein, ferripyochelin-binding protein, ring-$H_2$-binding protein, zinc finger protein, blue copper protein, putative cytidine 5′-triphosphate synthase, NAD-dependent isocitrate dehydrogenase and polyadenylate-binding protein 2 (Liu 2000). These enzymes/proteins are associated mainly with ethylene biosynthesis, carbohydrate metabolism, cell wall degradation, pathogenesis, senescence and stress. Some of these genes were further characterized and their roles were elucidated in banana by expression in fruit at different stages of ripening (see below).

### *3.1.1 Genes Associated with Ethylene Biosynthesis*

Ethylene is a gaseous hormone that has been shown to play an important regulatory role in a wide range of plant physiological and developmental processes, including fruit ripening. The key enzymes involved in ethylene biosynthesis are SAM synthetase, ACC synthase and ACC oxidase. While SAM synthase catalyzes the conversion of methionine to SAM, the other two enzymes are re-

sponsible for the conversion of SAM to ACC and ACC to ethylene, respectively (Yang and Hoffman 1984).

Genes encoding ACC synthase and oxidase have been cloned in banana and a range of other plant species. Expression of these genes has also been characterized in banana fruit during ripening (López-Gómez et al. 1997; Liu et al. 1999; Pathak et al. 2003). Both enzymes are encoded by multigene families, and ACC synthase has been shown to be encoded by at least nine genes (Huang et al. 2006). In banana cv. Grand Naine, the level of ACC synthase transcripts was low or undetectable in flesh tissue of pre-climacteric fruit, but it surged transiently in climacteric fruit, followed by a rapid decline in post-climacteric fruit (Liu et al. 1999). This was similar to the pattern of endogenous ACC accumulation, ACC oxidase activity and ethylene production in fruit during ripening. However, the pattern of ACC oxidase transcript accumulation differed, as the transcript level increased markedly when fruit began to ripen and it remained high at post-climacteric stages. This differential pattern of accumulation between transcripts and enzyme activity was thought to be due partly to lower concentrations of cofactors such as ascorbate and iron (Liu et al. 1999). These findings are generally in line with the results of our study, in which both ACC synthase and oxidase genes were expressed temporally in peel and pulp of fruit of cv. Williams at different stages of ripening (Liu 2000). However, differences were also observed. We detected abundant ACC synthase transcripts and increased ACC accumulation in peel of climacteric and post-climacteric fruits. These fruits also showed high levels of the ACC oxidase activity, indicating that ACC oxidase did not play a major role in autocatalytic inhibition of ethylene during ripening.

### *3.1.2 Genes Associated with Carbohydrate Metabolism*

Unripe banana fruit usually possesses large fractions of starch that constitute 20–25% of the pulp fresh weight. As ripening progresses, starch is rapidly converted to sugars, during which various genes are activated and their translation products are responsible for starch degradation and sugar synthesis. Although the physiological aspect of ripening in relation to carbohydrate metabolism is relatively well understood, knowledge regarding its molecular mechanism is virtually lacking.

To date, only a few ripening-related enzymes such as acid phosphatase (Turner and Plaxton 2001) and starch phosphorylase (Mota et al. 2002) and genes related to carbohydrate metabolism have been characterized in banana. One partial gene sequence for isoamylase-type starch-debranching enzyme (SDE) from banana fruit has been reported recently (Bierhals et al. 2004). SDE is involved in starch degradation by hydrolyzing $\alpha$-1,6-branches of amylopectin that, together with amylose, forms the starch. Interestingly, results showed little change in the levels of SDE transcript and protein during ripening. This has prompted the suggestion that pre-existing enzyme might be responsible