Regulation of Ripening and Aroma Development in Mango Fruit

Abstract

Mango, belonging to the *Anacardiaceae* family, is a significant tropical fruit crop in India. As the world's largest mango producer, India boasts a vast diversity of mango varieties, with an estimated 1,000 cultivars. Each variety is uniquely distinguished by specific characteristics, including plant architecture, fruit size, colour, taste, and aroma. These quality attributes are key determinants of fruit acceptance in the global market. The ripening process in mango involves a complex interplay of molecular and biochemical pathways, leading to significant changes in fruit characteristics. This study reviews the physiological and biochemical transformations associated with mango fruit ripening, including alterations in colour, texture, softening, sugar content, and aroma production. These changes are orchestrated through the coordinated regulation of multiple genes, highlighting the intricate mechanisms underlying mango ripening.

Keywords: Mango, fruit, ripening, aroma, genes, biochemical pathway.

Introduction

Mango (*Mangifera indica*) is one of the most popular fruits of tropical/subtropical region belonging to family *Anacardiaceae*. Mango is grown commercially in more than 87 countries ranking fifth in total production among major fruit crops worldwide. The world production of mangoes is estimated to be over 34 million tonnes per annum. India is the largest producer of mango, with an area of 23 million acres and an annual production of 18 million tonnes, which accounts for 50% of the total world production (Source: Press Information Bureau Government of India Ministry of Commerce and Industry 2018-2019). The principal mango producing states in India are Andhra Pradesh, Uttar Pradesh, Bihar, Gujarat, Karnataka, and Maharashtra (Figure 1).

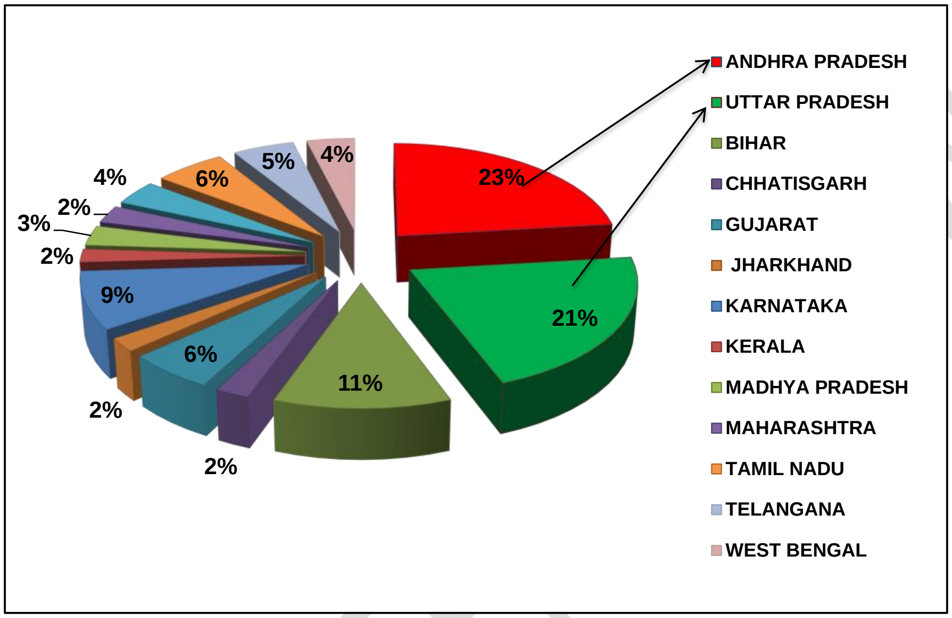

Figure 1: **Major mango producing states in India**. (Press Information Bureau Government of India Ministry of Commerce and Industry 2018-2019)

Mango is one of the most popular fruit due to its excellent flavour, richness in variety, and high nutritive value. Mango is a good source of vitamin A and C and contains about 20% total soluble sugar. The acid content of the ripe fruit varies from 0.2 to 0.5%, and protein content is about 1%. *Kent, Tomy Atkins, Alphonso,* and *Kesar* varieties are preferred in the international market. India produces and exports varieties such as *Alphonso, Dashehari, Kesar, Banganapalli,* and several others. Different Indian states grow about 30 varieties on a commercial scale. Most of the Indian varieties differ in size, shape, colour, fibre content, and flavour. These possess strong aroma and intense peel coloration. This diversity in mango provides excellent material to study different ripening associated characters.

Studies related to ripening in mango

Mango is a climacteric fruit characterized by a series of physiological and biochemical changes that occur during ripening initiated by autocatalytic production of ethylene and increase in respiration. Mango is an excellent system to study ripening related changes in climacteric fruits as many varieties exhibit significant changes in various characters that are attributed to climacteric fruit ripening such as softening, acidity, colour, and accumulation of aroma and other secondary metabolites. Gluconeogenesis is mainly active in ripening mango, which is reflected in decreased citrate and succinate levels but increased maleic acid level (Lazan *et al.*, 1986). Most of the gluconeogenic enzymes such as Fructose-1-6-diphosphatase (FDPase), Phosphoenolpyruvate carboxykinase (PEPCK), Phosphoenolpyruvate carboxylase (PEPC), Succinate dehydrogenase (SDH), Glucose-6-phosphate (G-6-Pase) and Glucokinase increase significantly during ripening, exhibiting highest activity at the ripe stage, except for malic enzyme, which remains constant throughout (Yashoda *et al.*,2006).

The activity of several carbohydrate degrading enzymes correlated with progression of fruit ripening. Different cell wall hydrolases such as PG, PL, Cellulase, Expansins, etc. have been studied at the biochemical and molecular level during mango fruit ripening. Cell wall hydrolases such as *MiExpA1* and *MiPel1* coding for expansin and pectate lyase respectively have been recognized from *Dashehari* mango, which demonstrates ripening linked expression and associated with mango softening. A rise in Ca^{+2}-dependent PL activity and pectin solubilization correlated with the pectate lyase transcript accumulation during ripening. (Sane *et al.,* 2005; Chourasia *et al.,* 2006).Cellulase activity increased in several Indian mango cultivars like *Alphonso*, *Badami*, *Dashehari*, *and, Totapuri* etc. during ripening (Selvaraj and Kumar, 1989, Chourasia *et al.*, 2008). *MiCel1,* an endo-β-1, 4-glucanase homologue from mango showed fruit specific and ripening related expression. Its expression was positively correlated with an increase in EGase activity, particularly during the later stages of ripening (Chourasia *et al.*, 2008). The β-Galactosidase enzyme has been purified from *Harumanis* mango (Ali *et al.*, 1995). It was reported that this enzyme increases during developmental stages of mango fruit (Rahman *et al.*, 2000). It was reported that Arabinanase, Galactanase, and Mannanase are very prominent enzymes in mango fruit and their activity peaks at the climacteric stage of ripening (Bhagyalakshmi *et al.*, 2002; Prabha *et al.*, 2000).

Genes involved in ethylene biosynthesis, namely ACC synthase and ACC oxidase have been identified in mango (Gomez- Lim, 1993; Zainal *et al.*, 1999; Lycett *et al.*, 1997). The transcripts of these two genes are undetectable in unripe fruit and levels are high in ripe fruit. The expression of ACC oxidase has been shown to precede the transcript of ACC synthase (Cruz-Hernandez and Gomez- Lim, 1995). Five ripening related cDNAs from two mango cultivars viz., *Alphonso* and *Totapuri* were isolated by Saiprasad and group (2004) using RT- PCR technique. The predicted polypeptides of these five clones were showing similarity to database protein sequences of PRl-1 protein, transcription initiation factor, CCR-4 protein, 18S ribosomal RNA gene, and 23S ribosomal RNA gene.

The information about mango ripening and aroma formation at the molecular level is still in infancy due to unavailability of genomic sequence. The first report about mango transcriptome came in 2014 using *Zill* variety of mango. Later in 2015, Dautt-Castro *et al.* reported transcriptomic study from *Kent* variety of mango using Illumina platform along with trinity software for the *de novo* assembly. Many aroma related genes were found from these transcriptomic studies. Dautt-Castro *et al.,* (2015) have reported six sucrose phosphate synthase genes, two sucrose phosphate phosphatase genes while seven sucrose synthase (SuSy) genes.

Besides these, 29 terpenoid backbone biosynthesis genes and Six LOX genes were identified in the transcriptome. Later on, transcriptomic data for *Dashehari* and *Alphonso* (Srivastava *et al.*, 2016; Deshpande *et al.*, 2017) was established. Fifty-four differentially expressed contigs related to aroma were detected in *Dashehari*. In *Alphonso* transcriptome data, six contigs encoding Monoterpenes synthases, five contigs encoding Sesquiterpene synthase, two furanones were found whereas multiple contigs coding for quinone oxidoreductase and O-methyltransferases were detected. A single contig encoding the hydroperoxide lyase and six contigs encoding 13-lipoxygenase were identified. The group also reported three contigs coding for epoxide hydrolase 2. Mango still faces challenges in tissue culture area, in comparison to other horticultural crops. The difficulties in getting tissue culture raised plant or transgenic are due to associated problems, viz., latent microbial infection, excessive polyphenol exudation, early explants necrosis, etc. In the early nineties, some preliminary reports about genetically transformed mango embryogenic cultures using *Agrobacterium tumefaciens* were published (Litz *et al.,* 1990; Mathews *et al.*, 1992, 1993; Cruz- Hernandez *et al.,* (1997), however, genetic transformation of mango is still a big challenge.

Studies related to the aroma and volatile in mango

In most mango growing countries, the bulk of mango produced is generally consumed as fresh fruit, harvested mostly in the ripened form. Flavour is an important attribute critical to consumer's acceptability. The changes in aroma components found in unripe and ripe fruits indicate that the increase in volatile components is linked to ripening (MacLeod and Snyder, 1985; Gomez-Lim, 1997). There have been several efforts to characterize ripe fruit flavour (Bartley and Schwede, 1987; Engel and Tressl, 1983; Koulibaly *et al., 1992*; MacLeod and Snyder, 1985). Engel and Tressl (1983) recognized monoterpenes as an essential class of volatiles contributing to mango flavour in *Haden, Keitt, Kent, Tommy Atkins*, etc. (Malundo *et al.*,1996, 1997). The only oxygenated volatile compounds found in those varieties were ethanol, acetaldehyde, and hexanal (MacLeod and Snyder, 1985; Malundo *et al.,* 1997). Indian varieties such as *Alphonso, Amrapali, Dashehari, Bombay, Mallika*, etc. have more oxygenated volatile compounds like esters, furanones, and lactones. The terpene hydrocarbons are considered to be essential contributors to the flavour of mango varieties such as *Keitt*, *Kent*, and *Tommy Atkins.* The aroma and volatile composition of mango are affected by various factors including mango species and cultivars difference, fruit maturity at harvest, post-harvest ripening and storage temperature, ripening methods, *etc.*

Kaswija *et al.* performed a comparative study on the effect on sensory attributes and aromatic composition of mango ripened by four different methods *(2006*). The authors kept one cluster of mangoes as a control, i.e., room temperature ripening (25-30ºC). The remaining three groups were given following treatments: (i) Smoked Pit Ripening (SPR), (ii) Untreated Pit Ripening (UPR) and (iii) Ethylene (fruit generated) Pit Ripening (EPR). They showed that the mangoes ripened by EPR and SPR accumulated a more significant number of aromatic compounds. The variation in aromatic volatiles among ripe fruits suggested that it might be due to the changes in the concentration of different aromatic compounds at differing stages of ripening. Lakshminarayana (1980) determined that mangoes harvested at a mature green stage and then matured at temperatures above 15 °C had better flavour than those stored below 15 °C. Controlled atmospheric storage at 13 °C and softening at 21° C + 1 °C expanded the shelf life of the mango *Kensington pride* without compromising the development of aroma compounds (Nair *et al.,* 2003; Lalel *et al.,* 2004).

Fruit maturity at harvest is one of the critical factors which affect ripening and flavour development in the ripe fruit (Vanoli *et al.*, 1995). Four maturity stages (hard mature, sprung mature green, half-ripe and ripe) were taken and studied during post-harvest ripening for flavour. The fruits harvested at sprung mature green stage exhibited a higher amount of the aroma volatiles, suggesting that sprung green stage should be preferred stage for harvesting (Lalel *et al.*, 2003a). Levels of ethylene were also found to be correlated with the profiles of specific volatiles of the ripening fruits (Lalel *et al.,* 2003b).

Pandit *et al.* (2009) had assessed volatiles blends of 22 Indian and five non-Indian cultivars using solvent extraction and gas chromatography. They reported 84 volatiles belonging to various chemical classes, namely, alcohol, aldehyde, monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, oxygenated sesquiterpene, lactone, ketone, and non-terpene hydrocarbon. 4-Ethoxy ethyl benzoate and an unidentified compound were included in this analysis in the "miscellaneous" category. Although terpene hydrocarbons dominated mango blends, their oxygenated derivatives were also present in mango cultivars and non-Indian varieties such as *Jaffna*, *Willard*, and *Parrot* (Macleod and Pieris, 1984), *Kensington Pride* (Lalel *et al.*, 2003c) and several Colombian cultivars (Quijano *et al.,* 2007). Although many authors have attempted to explore the aroma profiles of cultivated mango, few have studied the volatile composition of its mature raw fruit; no data is available on the chemistry of fruit development and maturation.

Pandit *et al.* (2009) tried to investigate the biochemistry of volatile formation from fruit maturation to ripening in the *Alphonso* variety of mango. A total of 55 volatiles belonging to various chemical classes such as aldehydes, alcohols, mono- and sesquiterpene hydrocarbons, lactones and furanones were identified. Different stages of the *Alphonso* fruit were defined by distinct volatile composition during the transformation from flower to ripe fruit. Ripe fruit was characterized by the *de novo* appearance of lactones and furanones in the blend of monoterpenes. Preethi *et al.* (2014) investigated the aroma blend of *Neelum* and *Bangnapalli*. The aroma components identified from *Neelum* and *Banganapalli* were 24 and 31, respectively. *Neelam* aroma blend constituted esterase propenal, alkanes, ketones, alcohols, lactone, and acid as associates. In *Banganapalli*, alkanes were the prominent constituent followed by esters, alcohols, ethers, fatty acid, amino acid, triterpene, and sulfur. Sulfur and nitrogen mineral components also contribute to the aroma of mango fruits. Li *et al.,* (2017) explored the aroma profiling of 25 mango cultivars from China, America, Thailand, India, Cuba, Indonesia, and the Philippines. They used headspace solid-phase micro-extraction tandem gas chromatography-mass spectrometer methods to detect the volatile compositions, their related contents, and the inter-varietal differences. A total of 127 volatiles were found in all the cultivars, belonging to different chemical classes. The highest qualitative abundance of volatiles was detected in *Zihua* and lowest in *Mallika* cultivars. Terpene hydrocarbons were the major volatiles. Among them, terpinolene, 3-carene, caryophyllene, and α-Pinene were the dominant components depending on the cultivars. Monoterpenes were primary and most abundant volatile components, whereas aldehydes were least in mango pulp.

Conclusion and future prospect

Various cell wall hydrolases such as PG, PL, Cellulase, Expansins etc. have been studied at biochemical and molecular level during mango fruit ripening. Genes involved in ethylene biosynthesis namely ACC synthase and ACC oxidase have been identified. These studies suggest that several enzymes responsible for softening of fruits during ripening are regulated directly or indirectly by ethylene, but their precise role in fruit softening is not clearly understood. In mango, 51- 95% aroma volatiles belong to terpenes. There are 578 volatile compounds identified from mango fruit in various cultivars. Chemical composition of aroma metabolites is known in different mango varieties such as *Alphonso*, *Dashehari*, and *Kensington Pride*. Mango ripening, in general, has been well studied at physiological and biochemical levels. However, molecular studies are minimal. All the work carried out in the field of formation of mango fruit aroma, and volatile is restricted to identification and estimation of compounds. No information regarding genes involved in these processes, their regulation and specific role in the production of a particular class or group of compounds is available.

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