GENETIC ANALYSIS OF ALPHONSO MANGO FLAVOR BIOGENESIS

A thesis submitted to the University of Pune

For the degree of

DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY

SAGAR S. PANDIT

Plant Molecular Biology Unit
National Chemical Laboratory
Pune 411 008 (INDIA)

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CERTIFICATE

Certified that the work in the Ph.D. thesis entitled **'Genetic analysis of Alphonso mango flavor biogenesis'** submitted by **Mr. Sagar Pandit** was carried out by the candidate under my supervision. The material obtained from other sources has been duly acknowledged in the thesis.

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DECLARATION

I hereby declare that the thesis entitled **'Genetic analysis of Alphonso mango flavor biogenesis',** submitted for Ph.D. degree to the **University of Pune** has not been submitted by me for a degree at any other university.

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CONTENTS

Title	Page no.
Acknowledgements	1
List of figures	2
List of tables	4
Chapter 1 General introduction and review of literature	7
Chapter 2 Genetic diversity in Indian and non Indian mango cultivars assessed using inter simple sequence repeat (ISSR) markers	24
Chapter 3 Gas chromatography- mass spectrometry based qualitative and quantitative diversity analysis of flavorants in mango cultivars	42
Chapter 4 Profiling of major flavorants through the development and ripening of Alphonso fruit	73
Chapter 5 Isolation of various flavor and non flavor genes from Alphonso mango and profiling their expression through the development and ripening of fruit	100
Chapter 6 Summary, conclusions and future prospective	135
Bibliography	142
Annexure I	168
Biodata	178

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LIST OF FIGURES

Chapter 1

Fig 1. Constitution and perception of flavor.

Fig 2. Key metabolic routes in the biosynthesis of volatile flavor compounds.

Fig 3. Kokan, the 700 km coastal belt of concentrated Alphonso cultivation.

Chapter 2

Fig 1. Principle coordinate analysis based on the ISSR marker data, for 70 mango cultivars (Indian as well as foreign) along with *Nothopegia colebrookiana*, the outgroup.

Fig 2a. UPGMA dendrogram for 70 mango cultivars and the distant outgroup, drawn using Winboot software and Dice similarity coefficient, showing 16 conserved clusters.

Fig 2b. UPGMA dendrogram for 70 mango cultivars and the distant outgroup, drawn using Winboot software and Jaccard similarity coefficient, showing 16 conserved clusters.

Chapter 3

Fig 1. Bar graph showing the comparison among 27 cultivars for total amount of volatiles.

Fig 2a. Pie diagrams showing relative abundance of different chemical classes in 20 monoterpene dominant cultivars.

Fig 2b. Pie diagrams showing relative abundance of different chemical classes in seven sesquiterpene dominant cultivars.

Fig 3a. Scatter plot of principle coordinate analysis (PCO) performed using Euclidean distance measure over the quantitative GC data, for 27 mango cultivars.

Fig 3b. Scatter plot of standardized principle components analysis (PCA), based on the quantitative GC data, for 27 mango cultivars.

Chapter 4

Fig 1. Development and ripening of Alphonso mango.

Fig 2a. Total volatiles in different mango tissues.

Fig 2b. Percent contribution of monoterpens to different mango tissues.

Fig 2c. Percent contribution of sesquiterpens to different mango tissues.

Fig 3. Quantitative variation within different classes of compounds through leaf, flower, development and ripening of Alphonso and Sabja fruit.

Chapter 5

Fig 1. Sequence based phylogenetic analysis of 19 genes.

Fig 2. Expression profiles of 20 genes including the internal control.

LIST OF TABLES

Chapter 2

Table 1. Mango cultivars used in the ISSR analysis with their region of cultivation.

Table 2. Performance of various ISSR primers in the genetic diversity analysis of mango.

Table 3. Cultivar specific bands obtained with various ISSR primers.

Table 4. Conserved clusters of mango cultivars extracted from two different UPGMA clustering analyses performed using Dice and Jaccard similarity coefficients, respectively.

Table 5. Similarity estimates between Alphonso and Neelum, their F_1 progeny cultivar Ratna and Sindhu, the progeny of backcross between Ratna and Alphonso.

Chapter 3

Table 1. List of aroma compounds detected from the set of 27 mango cultivars.

Table 2a. Volatile compounds and their quantities in the ripe fruits of first 14 from the current set of mango cultivars.

Table 2b. Volatile compounds and their quantities in the ripe fruits of next 13 from the current set of mango cultivars.

Table 3. Principle aroma components of different cultivars with their relative abundance.

Chapter 4

Table 1. Volatile compounds and their quantities in 14 different mango tissues.

Chapter 5

Table 1. Details of primers, annotation, and BLAST analysis for 20 different cDNA fragments that were profiled for expression through 14 mango tissues.

Table 2. Different genes from terpene biosynthesis pathway regressed with various terpene volatiles that were detected by gas chromatography.

CHAPTER 1

General Introduction and Review of Literature

Flavor and its perception

Flavor is an important attribute of the fruit that attracts humans along with other frugivores. It was one of the chief concerns that initiated domestication of fruit plants (Tanksley 2004; Aharoni et al, 2004), which in ecological terms could be seen as a plant adaptation to human preferences. From human evolution point of view, perception of flavor evolved as a complex process in which taste, mouth feel, vision, olfaction, the trigeminal system, and even auditory signals contribute to the complete appreciation of the food (Visschers et al, 2006). One may relate such a complexity to the multifarious nature of flavor. Indeed, flavor is a complex commodity and it is evident from the definition and perception that, it is a function of myriad chemical and physical entities. However, it can be defined in the simplest way as 'a combination of three characters, aroma, taste and texture' (Fig 1).

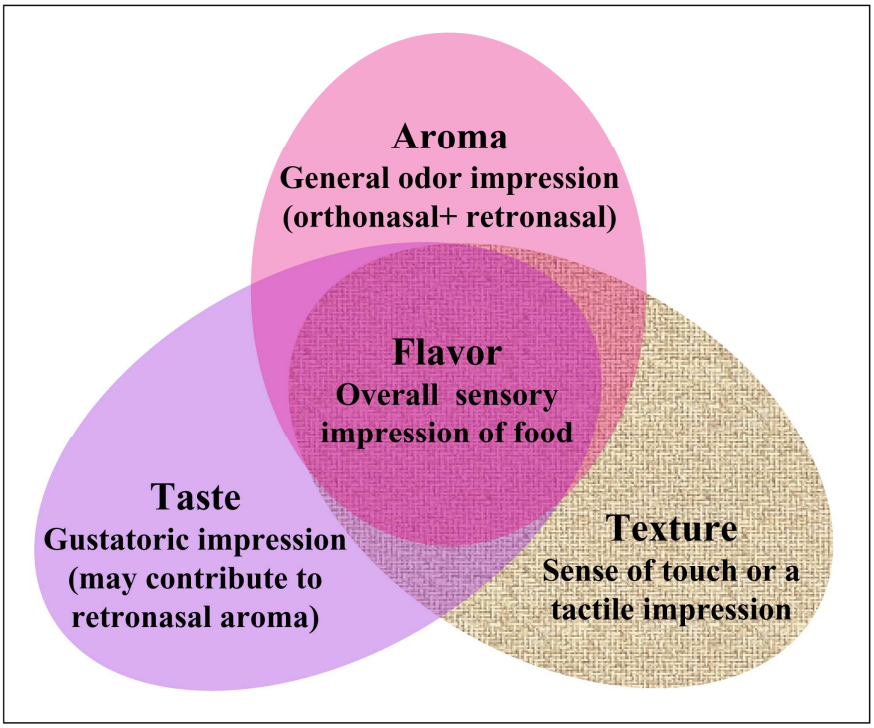


Fig 1. Constitution and perception of flavor (partly taken from Stephan et al, 2000).

Sensory impressions of the three flavor characters aroma, taste and texture is often studied by making humans, a part of the experimentation system. Such a group of people is called a sensory panel (of tasters or judges). Their perceptions of the type, intensity, amenability and detection threshold of the flavor characters are critically recorded, and are tested for statistical significance. These panelists can be trained or untrained depending upon the availability, necessity and relevance (O'Mahony, 1995; Noble, 2001; Frank, 2002; Delwiche, 2003). Although it is a conventional method, it remains the most accepted one, till date, obviously because the end user itself is the model system for the experimentation that ensures the candid application.

In most of the fruits, aroma is the most influential character. This is mainly by the virtue of human olfactory system that is extremely sensitive and even detects parts per trillion (ppt) quantities as compared to the taste that our taste buds can detect least, at parts per hundred concentrations (Christensen et al, 2007). Secondly, aroma has a dual activity; the orthonasal aroma constituted by volatile emissions, forms pre-eating, sheer olfactory impression, whereas the retronasal aroma composed of certain taste components themselves or in conjugation with cleavable volatiles, also makes post-chewing olfactory impact from the oral cavity (Robin, 1982). In addition, the orthonasal components if fed retronasally create different impression, as both these channels have different processing systems (Heilmann and Hummel, 2004).

All these features of aroma especially, of the retronasal one often alter the perception of taste (Stevenson et al, 1999). Abegaz et al (2004) partitioned the taste and aroma of tomato using nose clips for the sensory panel members to demonstrate such an effect. Their regression models were more effective at predicting sensory descriptors when taste descriptors were partitioned than when they were not partitioned. Vis-à-vis, they found the aroma descriptors more pronounced when

following taste perception than when provided simultaneously with taste descriptors. These types of effects were found most prominent for sweet taste and relevent flavors mainly those of fruits. However, this property was exploited to reduce the bitterness of the nutritional solutions containing branched-chain amino acids (Mukai et al, 2007)

Similar to that of taste, the feel of texture is also known to be altered by aroma. In yogurt, Saint-Eve et al (2004) found that fatty aroma enhanced the feel of thickness whereas the green one reduced it and created a feel of smoothness. In addition, mixture of aroma components made the yogurt to be perceived as thinner but more persistent and mouthcoating. Such influence of aroma on the texture was also demonstrated by Elmore et al (1999) and Kora et al (2003). Hollowood et al (2002) and Visschers et al (2006) further studied the cross-modality of texture and aroma perception to reveal that aroma intensity decreases with the increase in firmness of the food matrix. Visschers et al (2006) also found that these effects were independent of the ortho- or retronasal mode of aroma administration.

Obviously, aroma being such an influential character remains decisive in the market success of any fruit. It has been therefore, a subject of extensive research; especially, for its composition and biosynthesis.

Flavor detection and measurement

Sensory descriptions indicated that the composition of aroma must be complex and this task requires technological assistance. As a consequence of the development in this field, the techniques such as gas chromatography (GC) and mass spectrometry (MS) really proved to be a boon for the aroma analysis. These two techniques, in combination enabled the efficient separation, detection and identification of volatile constituents (Maarse and Visscher, 1996). These techniques, improved with high resolution, vast and ‘real time- accessed’ compound libraries generated unprecedented

pace in the flavor research. Sniff detectors empowered this research to elucidate the perceptions of different aromas (Guadagni, et al, 1966; Dravnieks and O'Donnell, 1971). Furthermore, the discrimination of compounds as odor active and odor non-active was made possible by the techniques like olfactory GC (GCO) (Rothe and Thomas, 1963; Acree, 1993; Varming et al, 2004). In GCO, the effluent of the GC column is split, with one portion of the eluted volatiles flowing to the instrument detector and the remaining to a sniff port where the odour-active compounds are identified and described. Most important information GCO revealed was that not all the volatiles are flavour-active and thus the most abundant volatiles are not necessarily the most important contributors to flavour. Note that although this concept of odor-active volatiles has gripped up rapidly, purely mechanistic qualitative and quantitative profiling of complex flavors is still a very much a state of the art, unless the perception of flavor is completely revealed and the synergistic as well as antagonistic interactions between various constituents are indisputably epitomized.

Parallel advancement occurred in aroma isolation and sampling procedures. Steam distillation and/or solvent extraction (Teranishi and Kint, 1993), the classical techniques, were extensively employed; however, they were prone to qualitative and quantitative modification of the aroma profile (Schamp and Dirinck, 1982). Addition of internal standards minimized the demerits of this method and enhanced the scope; nevertheless, these methods remained labor-intense and therefore, inapplicable for high-throughput use. For better identification and quantification of aroma ingradients purge and trap headspace sampling method was used (Schamp and Dirinck, 1982; Teranishi and Kint, 1993). In this method, volatile components were trapped and concentrated on a solid phase and were later freed from the trap by heating for the analysis by GC-MS. The only drawback this method was the low relative sensitivity

due to which the trace compounds were missed out from the complex profiles. This problem was further solved by cryofocusing (concentration at ultra low temperatures) of static headspace volatiles (Teranishsi and Kint, 1993). Solid phase micro extraction (SPME) is another sampling technique in which, volatiles get adsorbed on a fiber-coated probe that is inserted in headspace of the sample. Later this fiber is injected in to the GC, where these volatiles are released to the column by means of heating (Arthur et al, 1990 and 1992; Louch et al, 1992; Gardner et al, 1995).

Flavor constituents

As a result of this technological advance, volatile compositions of a plethora of flavors were revealed within a span of few years. Among these, the flavors like mint and vanilla turned out to be simple. Mint principally contained menthol (Lawrence, 1981; Croteau et al, 2005; Wildung et al, 2005), whereas vanilla contained vanillin (Walton et al, 2003). Nonetheless, the fruit flavors like those of apple, mango and strawberry found to be extremely complex, containing hundreds of volatile compounds. Apple aroma was found to be a mixture of esters, aldehydes, ketones, alcohols that ranged near 200 in few cultivars (Dimick and Hoskin, 1983; Lo Scalzo et al, 2001; Fuhrmann and Grosch 2002). Unlike apple, mango aroma turned out to be a mixture dominated by terpenoids (Idestein and Schreier 1985; MacLeod and Snyder, 1985; Olle et al, 1997 Pino et al, 2005; Lalel et al, 2003). Strawberry flavor also came out to be a mixture of about 350 compounds with prominent esters and furanones (Latrasse, 1991; Zabetakis and Holden, 1997; Bood and Zabetakis, 2002).

Simultaneously, floral fragrances and leaf aromas were also profiled, wherein the striking similarities were observed in the qualitative composition of these fragrances and fruit flavors. Principally, green leaf volatiles (Noordermeer, 2001; Feussner and Wasternack, 2002; Casey and Hughes, 2004) and terpenoids (Paré and

Tumlinson 1999; Pichersky and Gershenzon, 2002; Tholl et al, 2004; Aharoni et al, 2005; Franceschi et al, 2005; Keeling and Bohlmann, 2006; Schmidt and Gershenzon, 2008) were found to be the common constituents. Thus obviously, the curiosity was raised about the multitude ecological function of these chemicals and consequently, the research on their biosynthesis and its regulation, gained momentum. Initially, these compounds were thought to be the superfluous metabolic wastes or the secondary metabolites but once their numerous functions were revealed, their status was upgraded as specialized metabolites. Story of this promotion is built on numerous exciting discoveries about their temporal and spatial occurrence.

Does temporal and spatial emission of flavor relate to its function?

Methyleugenol emission in *Clarkia breweri* was observed to be restricted to petals and stigma tissues; it was also punctual, marking the precise receptivity of the flower for pollination (Raguso and Pichersky, 1995; Wang and Pichersky, 1998). This ascertained an essential function for these metabolites, 'the pollinator attraction'. It was further supported, as the pollination trick of *Ophrys sphegode* was discovered; flower of this orchid was found to mimick the female bee (*Andrena nigroaenea*) in appearance as well as in its pheromonal emissions containing C21- C29 streight chain alkanes and alkenes. Most stunning part of this discovery was that these pre-pollination cues were attractive to the male bees, whereas the post-pollination ones mimicked the emission of unwilling bees, farnesyl hexanoate. This deterred them to avoid the damage and also to direct them to the willing flower (Schiestl et al, 1999; Schiestl and Ayasse, 2001).

On damage, (*Z*)-3-hexenyl acetate and other aliphatic ester derivatives of (*Z*)-3-hexen-1-ol, the green-leaf volatiles emitted by tobacco were found to deter female *Heliothis virescens* moths from laying eggs specifically, on injured plants. Diurnal

fluctuation in the quantities of emission was noted which suggested that the blend of volatiles, rich in (*Z*)-3-hexen-1-ol esters, emitted at night was most repellent to the nocturnal *H. virescens*. This interesting phenomenon also served as the first example of involvement of plant volatiles in direct defense. Later on, experiments on few other systems also added to this view (Kessler and Baldwin, 2001; Vancanneyt et al, 2001).

Though the direct defense using volatile weapons was discovered recently, the smart role of these chemicals in the indirect defense was already better characterized, wherein plants attract the natural enemies of herbivores by emitting certain volatile chemicals. For example, damaged *Brassica oleracea* emits the volatiles that enable the predatory mite (*Cotesia rubecula*) to find the herbivorous spider mite (*Pieris rapae*), its host (Maeda et al, 2002). Volatiles induced upon egg laying by herbovire, attracted parasitoids of those eggs (Meiners and Hilker, 2000). Similarly, parasitoid *Oomyzus gallerucae* was attracted by the plant using terpenoids and green-leaf volatiles upon oviposition of the elm leaf beetle (*Xanthogaleruca luteola*) on the elm *Ulmus minor* (Wegener et al, 2000). These interactions are tritrophic and so broaden the scope of function of these volatiles.

In case of fruit, the smells of these chemicals are known to advertise the exhibitionist fruits since antiquity and therefore, aforementioned discoveries have just added the dimensions to their functionality. Nevertheless, it has successfully broadened human views on them. For example, insect chewing forces the plant to release volatiles; we experience the genuine aroma of fruits only after biting them. It suggests that plant is probably not investing its resources and machinery differentially in its organs, leaf, flower and fruit. Secondly, certain volatiles have been proved to have antibacterial and/ or antifungal properties; considering the nutritious nature of the fruit, these properties are far relevant for it than to any other plant organ. Finally,

as mentioned earlier, most of the chemicals used for the multiple functions by plants and their organs remain the same.

Thus, the basics of volatile production and release appear to be understood. All this research gives an impression that these chemicals have their own, multidimensional metabolomic network that is undeniably interwoven with the other essential as well as specialized networks (Fig 2). Information from any remote part of this supernetwork would be applicable for probing the other areas. It includes the information about metabolites themselves (as detailed above), the genes responsible for their sinthesis and the technical skills required to mine this information.

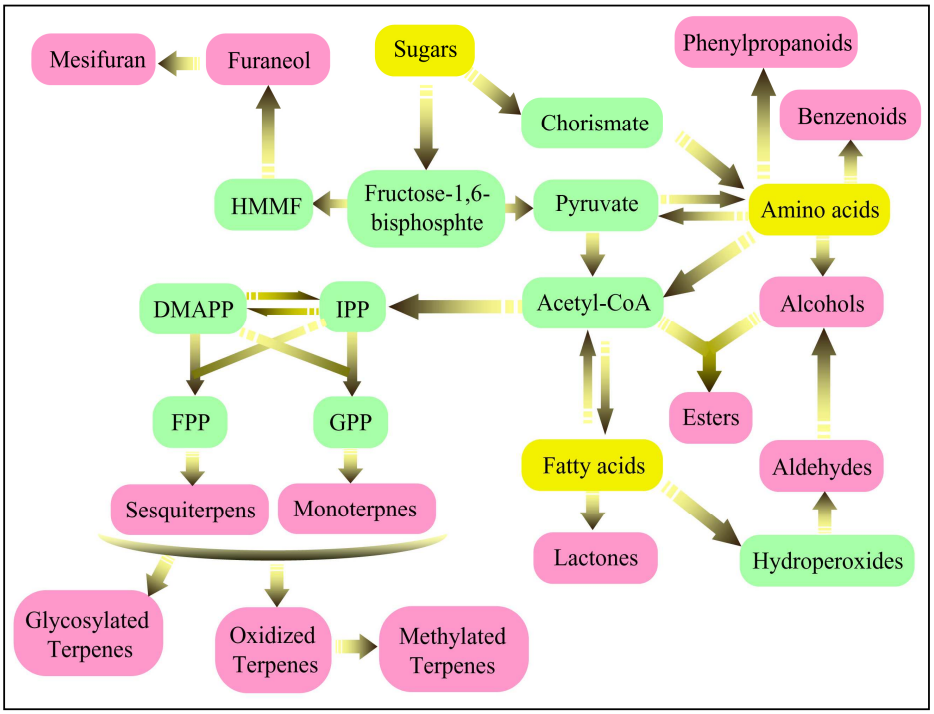


Fig 2. Key metabolic routes in the biosynthesis of volatile flavor compounds. [≡ Primary metabolites (precursors); ≡ Intermediates; ≡ Volatile products]

Molecular basis of flavor biogenesis

Fundamental and the most useful methods that are employed to understand the molecular basis of flavor biogenesis are the ones used to track the differential gene

expression (Liang and Pardee, 1992; Liang et al, 1993; Diatchenko et al, 1996; Bachem et al, 1996). In these techniques, mRNA pool from the experimental tissue can be easily compared with that of the control tissue to make out the cDNA fragments that are present in only one of the tissues. This comparison may be polymerase chain reaction (PCR) based or hybridization based depending upon the availability and suitability. Beauty of these techniques is that they can easily highlight the novel genes, for which the classical techniques used to take indefinite time and efforts. Plenty of reports can be named in the success story of these techniques (Hui et al, 2003; Voelkel and Baldwin, 2003; Eckey et al, 2004; Moyano et al, 2004).

It was realized that the sequences of aroma related genes were strickingly similar to each other, in spite of them being fished out from the different plants and organs (Bohlman et al, 1998; Gang, 2005). Considering the most useful feature of molecular biology, the complementarity of nucleotides, these gene sequences formed the excellent tools to probe the desired genes from new systems. Indeed, this attribute was thoroughly exploited, as the concept of degenerate primers was countlessly applied. Most relevant to mention, the gene responsible for the synthesis of the multifunctional volatile, linalool was isolated from *Artemisia* by the cDNA amplification with degenerate primers (Jia et al, 1999). Among the numerous, the most recent and relevant study includes the genes for the synthesis of the precursors of terpene synthesis, isoprenyl diphosphates, which were isolated from *Picea abies* using degenerate primer approach (Schmidt and Gershenzon, 2007 and 2008). The interesting outcome of this discovery was that these genes turned out to influence the production of multitude of metabolites.

The second approach that proliferated was to differentially probe the cDNA libraries of experimental and control tissues. Monoterpene synthase gene from

Arabidopsis was probed out from the cDNA library of jasmonate induced plants (Bohlmann et al, 2000); this experimentation included both, the differential expression tracking as well as its downstream persuasion. Similarly, Snapdragon monoterpene synthase gene was isolated from petal cDNA library (Dudareva et al, 2003); in this case the spatial expression was targeted. In case of other volatiles isolation of almond hydroperoxide lyase (HPL) can be mentioned, which was performed by cDNA library screening (Mita et al, 2005). In all these studies, the major advantage of using the cDNA library was that, most of the clones represented the complete reading frames of the desired genes and thus, efforts in their further characterization were negotiated.

For other approaches, the methods to obtain the complete functional sequence frames of the isolated genes were parallely developed. Rapid amplification of cDNA ends (RACE) was the most successful of these. Maize sesquiterpene synthase gene was characterized with the 'expressed sequence tag- rapid amplification of cDNA ends' (EST- RACE) approach (Schnee et al, 2000 and 2006). Similarly, banana alcohol acyl transferase (AAT) gene was characterized using cDNA RACE method (Beekwilder et al, 2004).

Although there are literally, infinite studies to narrate the success of these techniques, these basic methods have contributed far more to the transcriptome mining than the single gene discovery. Microarray made the most industrious application yielded by the long molecular biological experimentation. It provides a high throughput means for systematically studying the expression profiles of large subsets of genes from different tissues under specific physiological and environmental conditions. Initially, this technology proved its worth in the transcriptome mining of the model plant, *Arabidopsis* and was later used in innumerable systems (Schena et al,

1995; Ruan et al, 1998). Restricting to the genes for aroma biosynthesis, the most fruitful discovery was of Strawberry AAT gene (Aharoni et al, 2000). Microarrays also helped to reveal the evolution of strawberry flavor with respect to the domestication of this fruit (Aharoni et al, 2004). In fact, the other part of this discovery showed the dual substrate specificity of strawberry sesquiterpene synthase, which allowed it to produce monoterpene along with its original sesquiterpene product. This was probably the most significant finding in the scope of fruit flavor research.

Thus, today countless genes are available to portray the production dynamics of aroma if they are studied collectively. Indeed this way, researchers have been successful in elucidating the entire biosynthetic pathways for several groups of compounds (Bohlmann et al, 1998, Pichersky and Gang, 2000; Noordermeer, 2001; Chappell, 2002; Schwab, 2003; Dudareva et al, 2004). Consequently, it also brought out different modes of regulation of this synthesis and the cross-talks between different pathways (Aharoni et al, 2004; Dudareva et al, 2005). This phase seems to have significantly uncovered the basics of volatile biosynthesis and the ecological dimension of it. Eventually, the biosynthesis of chief flavor compounds of lemon (Lucker et al, 2002, Shimada et al, 2004), mint (Croteau and Martinkus, 1979; Croteau et al, 1984; Colby et al, 1993; McCaskill and Croteau, 1995; Lange et al, 2000), strawberry (Aharoni et al, 2000; Lavid et al, 2002; Beekwilder et al, 2004) and vanilla (Walton et al, 2003) was revealed and thus proficient methodological inroads were made available in the biological manufactories of flavor.

Mango: The king of fruits

At the other corner of this development, application and/or market oriented aspects gripped up, as this science was specifically relevant to the flavor and

fragrance industries. It was also supportive to the wineries and breweries. Biosynthesis of flavors of fresh fruits became one of the major thrust areas, as it promised the solution to the non-uniformity of fruit quality, which was due to the environmental impact. Apple (Flath et al, 1967; Defilippi et al, 2005; Mehinagic et al, 2006), banana (Shiota, 1993; Medina-Suarez et al, 1997; Wyllie and Fellman et al, 2000), kiwifruit (Gilbert et al, 1996), melon (Hayata et al, 2003; Albuquerque et al, 2006), Orange (Kealey and Kinsella, 1978; Nisperos-Carriedo' and Shaw, 1990), pineapple (Moyle et al, 2005; Tokitomo et al, 2005; Soler et al, 2006) and strawberry (Morton and MacLeod, 1990; Zabetakis and Holden, 1997; Bood and Zabetakis, 2002) remained few of the major interests. In this wave, mango was also analyzed for its flavor. This research remained largely unnoticed may be for being limited to the perception and analytical investigation and secondly, for being in parts.

Collective look at the data on mango flavor indicates that it contains myriad of compounds, belonging to various classes, alcohol, aldehyde, benzenoid, ester, ketone, lactone and terpenoid, the number touching 400. Indeed, the studies on African (Sakho et al, 1985), Australian (Bartley and Schwede, 1987; Bartley, 1988; Lalel et al, 2003a), Brazilian (Andrade et al, 2000), Colombian (Quijano et al, 2007), Cuban (Pino et al, 2005), Floridean (MacLeod and Snyder, 1985), Indian (Engel and Tressl, 1983; Idstein and Schreirer, 1985), Thai (Tamura et al, 2000) and Venezuelan (MacLeod and Troconis, 1982) cultivars presented mango germplasm as probably the largest and most diverse pool of free volatiles. In addition, about 150 volatiles were also found in the glycosidically bound forms by Adedeji et al (1992), Koulibaly et al (1992), Sakho et al (1997), Olle et al (1998) and Lalel et al (2003b). It is interesting to note that no other fruit has such a diversity of aroma compounds. Thus, mango has a lot to offer to biochemists, molecular biologists and the manufacturers of flavor and

fragrance, who always look for natural sources to eliminate the hazards of chemical synthesis and to improve the hygiene.

Based on the aroma profile, mango cultivars are classified as Indian and Indo-Chinese. Indian type mangos possess intense aroma whereas the Indo-Chinese have mild one. It has already been noted that the people accustomed to the later type, perceive the Indian type as medicinal or having turpentine flavor (Lizada, 1993). Indeed, some commercial Indian cultivars have high concentration of volatiles, especially terpenoids (Gholap and Bandyopadhyay, 1977; Engel and Tressl, 1983; Idstein and Schreirer, 1985), which seems to have taken its toll on the group of dispersal agents within mere 4000 years of domestication (De Candolle, 1884). Other way round, we can see the human selection influencing the domestication and ultimately the evolution of mango.

It is relevant to mention here that, India is a centre of origin as well as centre of diversity for mango (De Candolle, 1884; Gangolly et al, 1957; Subramanyam et al, 1975). Presently, India harbors a pool of more than 1000 cultivars. Till date, the studies have hardly covered a statistically significant number from this pool. Secondly, the analyses for elucidating the diversity and cultivar relationships based on the flavor profiles have not been performed (in case of Indian as well as non-Indian cultivars). In such a condition mango remains a bit unattended system and presents a healthy resource to renew our knowledge on flavor evolution.

Most of the available literature suggested that mango cultivars differ from each other by qualitative as well as quantitative variation in individual flavorants. In the similar way, these cultivars also differ in the possession of different chemical classes. Furthermore, each cultivar has been observed to have one quantitatively dominant compound (Lalel et al, 2003a). Few other studies have also reported such

basis of diversity in mango (Pino et al, 2005; Mahattanatawee et al, 2006; Pino and Mesa, 2006; Lebrun et al, 2007; Quijano et al, 2007).

A few efforts have been made to unveil the genetic diversity among the mango cultivars. Already, a range of DNA markers *viz.* AFLP, DAMD, ISSR, ITS and RAPD have been used for exploring the diversity of the global mango germplasm (Schnell et al, 1995; Bally et al, 1996; Lopez- Valenzuela et al, 1997; Eidthong et al, 1999; Chunwongse et al, 2000; Ravishankar et al, 2000; Hemanth Kumar et al, 2001; Karihaloo et al, 2003; Ravishankar et al, 2004; Srivastava et al, 2005). These analyses have put forth several facts about mango. Important to note here are 1) mango cultivars have not diverged too much on the genetic scale 2) mono- and polyembryonic mangos have different genetic bases and 3) mangos from different geographical zones differ genetically. As we have dicussed in context of flavor, here too such a divergence might be a result of selection or domestication process and more imperatively, in this selection process aroma diversity and genetic diversity must have had substantial toll on each other. Unfortunately, such conclusions remain a step away from proved, as the sampling not adequately representing the global mango pool, did not cover significant variability and a large number of cultivars pertaining to their centre of diversity, India, which probably have a better chance to uncover the history, again remain unexplored.

Alphonso: The most popular mango

Among thousands of mango cultivars, Alphonso is the most popular one (Tharanathan et al, 2006). Aroma of this cultivar is an appropriate representative of the vast diversity that we have discussed, as it contains volatile members of almost all the chemical classes (Engel and Tressl, 1983; Idstein and Schreirer, 1985). This fruit is also blessed with attractive color, ample, sweet, low fiber containing pulp and long

shelf life to suit its fresh use. Ripe Alphonso fruits are popularly used in the processed and canned foods. Similarly, the raw fruits of Alphonso are also used in the food products like pickles, tarts, curries and salads. Strikingly, such an ideal fruit is not uniform in its quality over the widespread cultivation localities in India. Therefore, its cultivation is concentrated in Kokan (or Konkan), the 700km long, narrow coastal belt of western India (Fig 3). Even within this belt, northern, central and southern Alphonso mangos taste and smell different. It is known that the post-harvest treatment to these mangos is the same throughout Kokan, wherein they are packed in hay and are immediately transported for ripening to the city of Mumbai, an export hub. Thus, what differs here is the pre-harvest environment of the cultivation zones. It is clear from this fact that the environment that reigns during the fruit development, sows for the alteration in the quality of ripe fruit. Similar phenomenon has also been reported in Australian, Kensington Pride mangos (Hofman et al, 1997) and several other fruits (Paull and Chen, 2000).

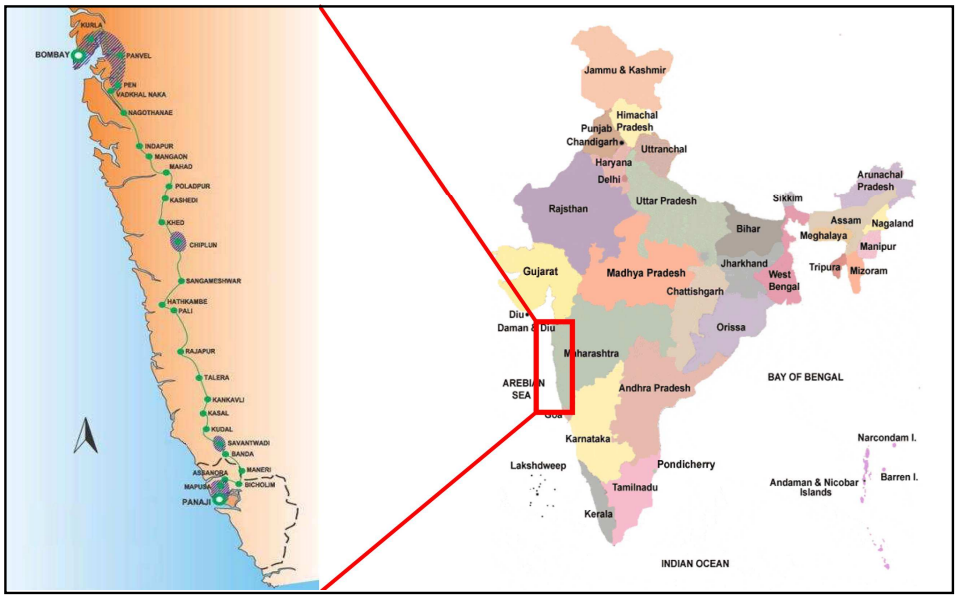


Fig 3. Kokan, the 700km narrow coastal belt, with concentrated Alphonso cultivation.

Genesis of thesis and its organization

This information suggests that for mango, especially Alphonso, there is enough commercial pressure as a driving force to conduct aforementioned studies. Secondly, it demands a comprehensive experiment, wherein the difference in the zonal microclimates and respective fruit qualities will be assessed concurrently. On the positive side, the genotype (Alphonso) being same through all the localities, complexity of the system is automatically negotiated. Such an approach would surely uncover the secrets underlying the complex biological processes and the biological interactions with environment. It will also provide new source of flavorants along with a lot of information on their biosynthesis.

Present thesis discusses many of these concerns, which have been organized in the following manner

Chapter 1: General Introduction and review of literature

Chapter 2: Genetic diversity in Indian and non-Indian mango cultivars assessed using inter simple sequence repeat (ISSR) markers

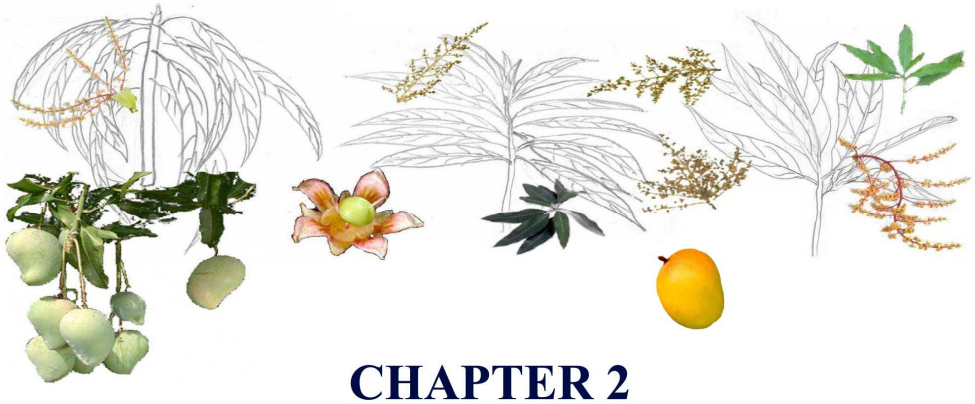
Chapter 3: Gas chromatography- mass spectrometry based qualitative and quantitative diversity analysis of flavorants in mango cultivars

Chapter 4: Profiling of major flavorants through the development and ripening of Alphonso fruit

Chapter 5: Isolation of various flavor and non flavor genes from Alphonso mango and profiling their expression through the development and ripening of fruit

Chapter 6: Summary, conclusions and future prospects

Bibliography



CHAPTER 2

Genetic Diversity in Indian and Non Indian Mango Cultivars Assessed Using Inter Simple Sequence Repeat (ISSR) Markers

Work described in this chapter has been published in *Current Science* (Pandit et al, 2007).

Part of this work has been communicated to *BMC Genetics*.

Introduction

Mango originated in the Indo-Burmese region during the earlier period of Cretaceous era (De Candolle, 1884; Gangolly et al, 1957; Subramanyam et al, 1975) and gradually spread to the tropical and subtropical regions around the world. India is thought to be the primary center of diversity along with its status as the center of origin for mango. Presently India harbors more than 1000 mango cultivars and represents the biggest mango germ pool in the world. Australia, China, Florida, Israel and Thailand are the other regions that also maintain a healthy germplasm of mango.

Over the various mango growing regions, mango breeding attempts are always on for creating better cultivars. Precise information on the genetic relationships within such germplasm diversity is always needed for carrying out efficient breeding programs. In order to assess the genetic diversity in mango, PCR based DNA markers are considered be the best tools (Joshi et al, 1999). A range of DNA markers *viz.* AFLP, DAMD, ISSR, ITS and RAPD have been used for exploring the diversity of the global mango germ pool (Schnell et al, 1995; Bally et al, 1996; Lopez- Valenzuela et al, 1997; Eiadthong et al, 1999a and b; Chunwongse et al, 2000; Ravishankar et al, 2000; Hemanth Kumar et al, 2001; Karihaloo et al, 2003; Ravishankar et al, 2004; Srivastava et al, 2005). Among these, inter simple sequence repeat (ISSR) (Zietkiewicz et al, 1994) is a reproducible semi arbitrary primed PCR method that uses simple sequence repeats as primers, combining most of the advantages of microsatellites and amplified fragment length polymorphism (AFLP), to the universality of randomly amplified polymorphic DNA (RAPD) (Gemas et al, 2004). ISSRs offer more probability than any other PCR marker system in the repeat regions of the genome, which are the most potent regions for producing cultivar specific markers. Automated PCR base makes ISSRs the markers of choice for screening the

genotypes. Here, ISSR marker system has been used to assess the diversity among 70 mango cultivars.

Materials and methods

Cultivar selection

For the present study, 60 elite Indian cultivars were selected on the basis of their consistency in behavior for the last 30 years at their growing region, their promising features for breeding and their plausibility to race in the global market. Among these 60, 38 are south Indian cultivars including 'Villai Kolumban', as the only Indian polyembryonic cultivar in the set and 16 are north Indian cultivars. Eight promising Indian cultivars with the undecided south Indian or north Indian origin (designated as Indian throughout the analysis) were also included in order to reveal their parentage or at least their alliance in the mango cultivar cladogram. Among these 60, Alphonso, Badami, Baramahi Hapus, Banarasi Hapus and Kala Hapus real are suspected to be synonymous to each other and were selected especially for confirming their status. Ratna is F_1 progeny cultivar of the Neelum $\times$ Alphonso cross, while Sindhu is the outcome of Ratna- Alphonso backcross. Ten non-Indian cultivars were included in this analysis to test the assumption that over the centuries, selection criteria for the world market have been significantly different than those for the Indian market and under such differential selection pressure the non-Indian cultivars may show isolation from the diversity pool of Indian mango. These ten include five Floridian cultivars, three Israeli and one Australian ('Kensington' as non Indian polyembryonic cultivar) and Taiwanese cultivar each. The list of cultivars along with their origins is given in Table 1. The two above-mentioned polyembryonic cultivars, Villai Kolumban and Kensington were included as the 'close outgroup' taxa in the

study. *Nothopegia colebrookiana* Blume. (Anacardiaceae), a distant relative of the genus *Mangifera* L, which is available in the local forests, was selected as the 'distant outgroup'.

Plant material

Flushing leaves from 70 mango cultivars (Table 1) were collected from the experimental orchards at the Regional Fruit Research Station (RFRS) of Dr. Balasaheb Savant Kokan Krishi Vidyapeeth [(DBSKKV) (Dr. Balasaheb Savant Kokan Agricultural University)], Vengurle, Maharashtra, India and those of *N. Colebrookiana* were collected from the forest of Amboli, Maharashtra. All leaf samples were frozen in liquid nitrogen for transportation to the laboratory and subsequently stored at –80^{o}C until processed.

DNA extraction

DNA was extracted as described by Doyle and Doyle (1990). DNA concentrations were determined by the comparison with the intensity of standard DNA (λ DNA, Bangalore Genei, India) after electrophoresis in ethidium bromide stained 0.8% agarose gel.

DNA amplification

A set of 100 ISSR primers, procured from University of British Columbia (UBC, Vancouver, USA), was used for amplification of plant DNA.

Initially all 100 UBC primers were screened with ten mango cultivars wherein at least one cultivar represented each geographic region along with one out-group. The primers that generated polymorphism were used for the final experiment with all the 70 cultivars along with one outgroup.

Polymerase chain reaction (PCR) was carried out in 25 µl volume as detailed by Deshpande et al (2001). The amplified products were separated on 2.0% agarose

gel in 0.5 X TAE buffer and bands were detected by ethidium bromide staining as suggested by Deshpande et al (2001). The size of each fragment was estimated with reference to a DNA size marker ϕX 174/ *Hae* III digest (Bangalore Genei, India).

The band pattern obtained by each ISSR primer was scored by visual inspection and the bands were recorded as present (1) or absent (0). From the band patterns obtained with each primer, the cultivar specific bands (if any) along with their sizes were recorded.

Statistical analysis

Similarity estimate 'D' was calculated as 2Nab/ Na+Nb for each primer and also collectively for all 33 primers (Wetton et al, 1987). The probability that a fragment in one cultivar is also found in another for all pairwise comparisons (Fingerprinting potential of a marker) was then calculated for each primer as $[(X_D)^n]$ where, X_D represents the average similarity index for all pairwise comparisons and n is the average number of bands amplified by the primer (Ramakrishna et al, 1995). $[(X_D)^n]$ was also calculated considering the data from all 33 primers together.

The binary score data from ISSR amplification was directly fed to the multivriate statistical package (MVSP) (Kovach, 2002) for Principal Co-Ordinate analysis (PCO).

The genetic distance matrices were generated by the 'Windist' software option from the Winboot package (Yap and Nelson, 1996) with Dice and Jaccard coefficients. Bootstrapping (Felsenstein, 1985) was done by 'Winboot' software to construct the dendrograms using unweighted pair group method with arithmetic averages (UPGMA) algorithm with 500 replicates.

Entire analysis from the tissue collection to the statistical data analysis was repeated twice.

Table 1. Mango cultivars used in the ISSR analysis with their region of cultivation.

Sr no.	Name of Cultivar	Region of cultivation	Sr. no.	Name of Cultivar	Region of cultivation
1	13-3	Israel	36	Keitt	Florida
2	Alphonso	South India	37	Kent	Florida
3	Badaigol	India	38	Kensington	Australia
4	Badami	South India	39	Kesar	North India
5	Banarasi Batli	North India	40	Kingphone	Taiwan
6	Banarasi Hapus	South India	41	Ladaio	South India
7	Bangalore Goa	South India	42	Langra	North India
8	Baramahi Hapus	India	43	Lili	Israel
9	Baramasia	South India	44	Mahalanjeo	South India
10	Bengali pairi	North India	45	Maharaja of Mysore	South India
11	Bekurad	India	46	Makaram	India
12	Bombay green	South India	47	Maya	Israel
13	Borsha Kalamshar	North India	48	Mulgoba	South India
14	Chandrama	South India	49	Musharad	South India
15	Chinna Suvarnarekha	South India	50	Naliaro	South India
16	Chittur Badami	South India	51	Neelum	South India
17	Creeping	South India	52	Osteen	Florida
18	Dadamio	North India	53	Pairi	South India
19	Dasheri	North India	54	Palmar	Florida
20	Devrukhio	South India	55	Pau	India
21	Dilpasand	South India	56	Police	India
22	Dudh peda	South India	57	Rajapuri	North India
23	Fakira	North India	58	Rangar	India
24	Fernandin	South India	59	Ratna	South India
25	Gadhemar	South India	60	Roos	South India
26	Goamankur	South India	61	Rumani	South India
27	Gopta of Navasari	North India	62	Sabja	South India
28	Hamlet	South India	63	Saleem	South India
29	Hathizool	North India	64	SB Chausa	North India
30	Jamadar	North India	65	Sindhu	South India
31	Kajalio	South India	66	Tankij- amadi	India
32	Kala Pahad	North India	67	Tomy-Atkins	Florida
33	Kalahapus real	South India	68	Totapuri	South India
34	Karanjio	South India	69	Vanraj	North India
35	Karelia	North India	70	Villai Kolumban	South India
			71	*Nothopegia colebrookiana*	South India

Results

ISSR profiles in mango cultivars

Out of 100 ISSR primers, 40 showed amplification in 11 genotypes that were used for the initial screening. Of these 40, 33 primers generated reproducible polymorphic DNA amplification patterns in all the 71 genotypes. Twenty-seven of these 33 primers belonged to the anchored di-nucleotide repeat class; remarkably, 15 of these 27 belong to either 'AG repeat' class or its complementary 'CT repeat' class (Table 2).

Table 2 explains the performance of the each of the 33 ISSR primers with 71 genotypes. These primers yielded a total of 420 scorable bands on amplification and their sizes ranged between 200 bp to 2000 bp. The number of scorable bands generated by the individual primers ranged between seven (UBC 864) and 18 (UBC 809 and UBC 852). Out of 420, 408 bands (97.14%) were polymorphic and only 12 bands (2.86%) were monomorphic. Most of the primers (24 of 33= 67%) exhibited 100% polymorphism while the least polymorphism (8 of 11 loci= 72.72%) was shown by UBC 810. The average number of bands amplified from the pool of 71 genotypes by UBC 889 was 2.28 and that by UBC 812 was 8.33; other primers produced average number of scorable bands within the range of these two values.

Cultivar specific bands

Twelve different cultivar specific bands were obtained from the amplification profiles with eight ISSR primers (Table 3). Tomy- Atkins stood out to be a unique cultivar with maximum of five specific bands produced by various primers. Primer UBC 813 produced three specific bands for Vanraj making three as the highest number of specific bands that any primer has produced. UBC 852

Table 2. Performance of various ISSR primers in the genetic diversity analysis of mango.

UBC Primer no.	Primer sequence	Bands scored	No. of polymorphic bands	Polymorphism (%)	Average no. of bands produced in 71 genotypes (n)	Average similarity $[(X)_D] \pm SD$	Probability of identical match by chance $[(X_D)^n]$
807	$(AG)_8T$	16	16	100	6.01± 1.43	0.70± 0.13	1.25×10^{-1}
808	$(AG)_8C$	18	18	100	6.21± 1.43	0.75± 0.11	1.68×10^{-1}
809	$(AG)_8G$	08	08	100	5.23± 1.12	0.81± 0.15	3.39×10^{-1}
810	$(AG)_8T$	11	08	72.72	6.15± 1.09	0.83± 0.11	3.24×10^{-1}
811	$(GA)_8C$	13	12	92.30	4.69± 1.17	0.76± 0.13	2.90×10^{-1}
812	$(GA)_8A$	15	14	93.33	8.33± 1.58	0.76± 0.11	1.09×10^{-1}
813	$(CT)_8T$	09	09	100	3.88± 0.49	0.92± 0.13	7.27×10^{-1}
815	$(CT)_8G$	10	10	100	3.38± 1.38	0.64± 0.17	2.30×10^{-1}
830	$(TG)_8G$	11	11	100	2.35± 1.26	0.75± 0.18	5.15×10^{-1}
834	$(AG)_8YT$	12	12	100	4.94± 1.47	0.71± 0.17	1.96×10^{-1}
835	$(AG)_8YC$	16	15	93.75	3.91± 1.18	0.82± 0.10	4.67×10^{-1}
836	$(AG)_8YA$	15	13	86.66	8.18± 1.22	0.83± 0.08	2.18×10^{-1}
840	$(GA)_8YT$	16	16	100	6.18± 1.27	0.77± 0.11	2.06×10^{-1}
844	$(CT)_8RC$	13	13	100	3.88± 1.59	0.64± 0.15	1.87×10^{-1}
845	$(CT)_8RG$	13	13	100	6.42± 2.04	0.62± 0.15	5.10×10^{-2}
848	$(CA)_8RG$	10	09	90.00	3.71± 0.81	0.84± 0.12	5.33×10^{-1}
852	$(TC)_8RA$	18	18	100	3.76± 1.30	0.86± 0.10	5.74×10^{-1}
855	$(AC)_8YT$	17	17	100	5.47± 1.28	0.66± 0.13	1.09×10^{-1}
856	$(AC)_8YA$	16	15	93.75	6.49± 1.26	0.79± 0.10	2.30×10^{-1}
857	$(AC)_8YG$	14	14	100	3.70± 1.91	0.72± 0.14	3.01×10^{-1}
859	$(TG)_8RC$	08	08	100	3.97± 1.24	0.70± 0.16	2.44×10^{-1}
862	$(AGC)_6$	08	08	100	5.83± 0.56	0.95± 0.15	7.58×10^{-1}
864	$(ATG)_6$	07	06	85.71	3.32± 0.95	0.77± 0.14	4.19×10^{-1}
866	$(CTC)_6$	11	11	100	4.59± 1.34	0.79± 0.13	3.52×10^{-1}
876	$(GATA)_2(GACA)_2$	14	14	100	4.39± 1.52	0.65± 0.13	1.54×10^{-1}
878	$(GGAT)_4$	11	10	90.90	5.49± 1.30	0.77± 0.12	2.40×10^{-1}
881	$GGG(TGGGG)_2TG$	09	09	100	4.73± 1.06	0.73± 0.16	2.27×10^{-1}
884	$HBH(AG)_7$	14	14	100	7.78± 1.75	0.68± 0.13	5.00×10^{-2}
886	$VDV(CT)_7$	16	16	100	7.28± 1.59	0.78± 0.12	1.79×10^{-1}
887	$DVD(TC)_7$	16	16	100	5.47± 1.1	0.80± 0.11	3.14×10^{-1}
889	$DBD(AC)_7$	08	08	100	2.28± 1.32	0.70± 0.18	4.54×10^{-1}
890	$VHV(GT)_7$	15	15	100	6.46± 1.31	0.79± 0.12	2.20×10^{-1}
891	$HVH(TG)_7$	12	12	100	3.22± 1.64	0.79± 0.12	4.70×10^{-1}
Total no. of primers: 33		**Total bands: 420**	**Total polymorphic bands: 408**	**Average % Polymorphism: 97.14%**	**Mean average no. of bands produced by 33 ISSR primers: 5.08± 1.58**	**0.76± 0.06**	**2.54×10^{-1}**

produced one specific band each in SB Chausa and Tomy- Atkins and was the only primer that could produce specific bands in two different cultivars.

Table 3. Cultivar specific bands obtained with various ISSR primers [Bands specific to the outgroup (*N. colebrookiana*) are not mentioned here.]

Name of the cultivar	**Primer producing specific band(s)**	**Size of the specific band (bp)**
Fakira	UBC 878	500
Gadhemar	UBC 836	2000
Neelum	UBC 884	500
S B Chausa	UBC 852	400
	UBC 811	300
	UBC 834	700
Tomy- Atkins	UBC 852	1000
	UBC 866	1400
	UBC 866	1500
	UBC 813	1000
Vanraj	UBC 813	400
	UBC 813	300
Total no. of cultivars: 6	**No. of primers generating cultivar specific bands: 8**	**Total no. of cultivar specific bands: 12**

Fingerprinting potential of ISSR primers

The probability of matching DNA fingerprints of any two mango cultivars $[(X_D)^n]$ was 2.54×10^{-1} when calculated for the entire 33 primer set of ISSR (420 loci) (Table 2). When calculated for each primer it ranged between 1.09×10^{-1} (UBC 812 and UBC 855) and 5.1×10^{-2} (UBC 845) (Table 2).

PCO analysis

Separation of non-Indian cultivars from the Indian cultivars was revealed in the PCO (Fig 1). Foreign cultivars were placed in the first and the fourth quadrants with the close cluster of Floridian cultivars including the Israeli cultivar Maya (cultivar no. 47; Table 1). However, Tomy-Atkins (cultivar no. 67; Table 1), the Floridian cultivar was placed distantly in the fourth quadrant from the pool of mango cultivars. 13-3 (cultivar no. 1; Table 1) was placed centrally to the clusters of Indian and foreign cultivars. *N. colebrookiana* (outgroup no. 71; Table 1) was placed completely aloof in the fourth quadrant. All the Indian cultivars were observed to be grouped together closely. However, seven Indian cultivars Badaigol (cultivar no. 3; Table 1), Badami (cultivar no. 4; Table 1), Banarasi Batli (cultivar no. 5; Table 1), Banarasi Hapus (cultivar no. 6; Table 1), Baramahi Hapus (cultivar no. 8; Table 1), Baramasia (cultivar no. 9; Table 1), Chandrama (cultivar no. 14; Table 1) and Chittur Badami (cultivar no. 16; Table 1) were not placed very closely to the cluster of Indian cultivars and 13-3 (cultivar no. 1) was placed with these cultivars.

No geographical separation was revealed between the north Indian and the south Indian cultivars by any of these statistical combinations.

Cluster analysis

Both UPGMA dendrograms drawn using Dice (Fig 2a and b) and Jaccard coefficients, showed highly similar grouping of cultivars. Both showed high bootstrap separation of outgroup (100%) from the mango clade and that of Tomy- Atkins (average 95 and 94%, respectively) from the big cluster of 69 mango cultivars. Though non-Indian cultivars grouped separately from most of the Indian cultivars, eight Indian cultivars grouped with the foreign cultivars. Congruence in the composition of small clusters [3- 5 operational taxonomic units (OTU)] or OTU pairs

was remarkable in both the dendrograms; however, for most of the clusters Dice coefficient produced higher bootstrap values than those of Jaccard coefficient (Fig 2a and b).

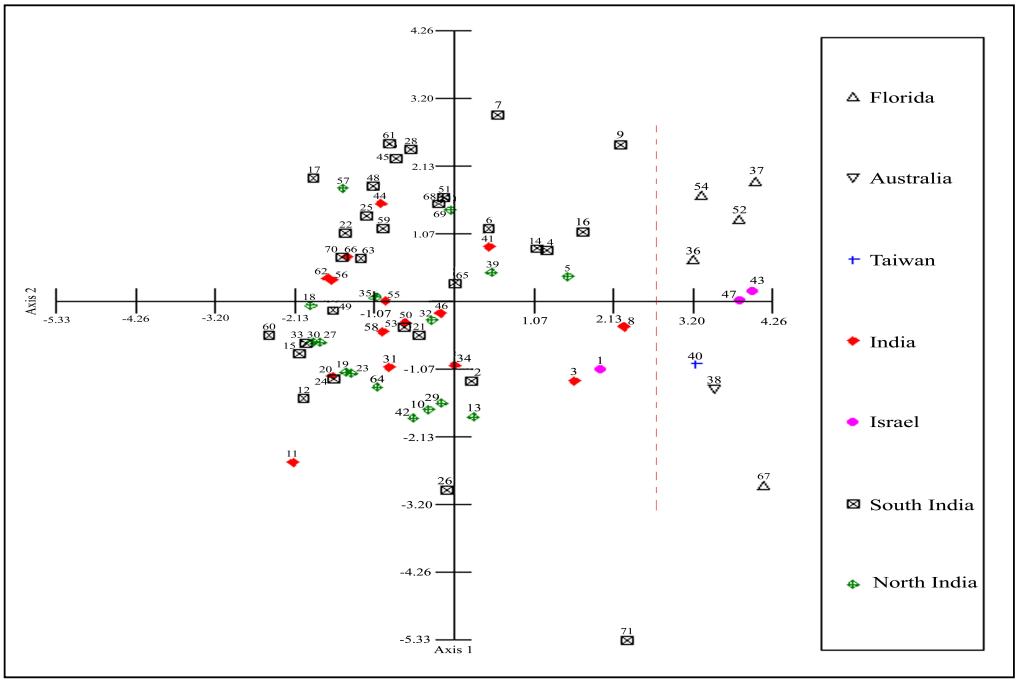


Fig 1. Principle coordinate analysis based on the ISSR marker data, for 70 mango cultivars (Indian as well as foreign) along with *Nothopegia colebrookiana*, the outgroup. Numbers denoting the plotted data points represent respective mango cultivars as listed in Table 1.

Separation among the Indian cultivars was seen with low bootstrap values; however, the cultivars those were suspected to be synonymous, opted different subgroups. Villai Kolumban subgrouped with Karelia within the group of Indian cultivars in both the trees. Similarly, Kensington grouped with the non Indian cultivars. Six cultivars with the unknown origin were found scattered over the dendrograms. Among these, Badaigol remained in the eight Indian cultivars that grouped with the foreign cultivars. Remaining five subgrouped with various south

Indian cultivar clusters; Bekurad with Bombay green and Chinnasuvarnarekha; Makaram with Mahalanjeo and Maharaja of Mysore; and lastly, Pau, Police and Rangar with Naliaro and Pairi.

Integrity of smaller clusters or OTU pairs was remarkable in both the Dendrograms. Sixteen clusters, including seven pairs were observed in both these dendrograms (Table 4). First cluster was the biggest consisting nine non Indian and eight Indian cultivars. Ratna and Sindhu clustered with Neelum in both these analyses.

Table 4. Conserved clusters of mango cultivars extracted from two different UPGMA clustering analyses performed using Dice and Jaccard similarity coefficients, respectively.

No.	Cultivars of the conserved clusters
1.	Keitt, Kent, Lili, Maya, Palmar, Osteen, Kingphone, Kensington, 13-3 , Badaigol, Banarasi Hapus, Banarasi Batli, Badami, Baramasia, Baramahi Hapus, Chittur Badami, Chandrama
2.	Dadamio, Devrukhio, Dudhpeda, Gadhemar, Gopta of Navsari, Fakira
3.	Bengali pairi , Bekurad, Bombay Green, Chinna Suvarnarekha
4.	Tankij-amadi, Totapuri, Vanraj
5.	Bangalore Goa, Creeping, Hamlet, Mulgoba
6.	Naliaro, Pairi, Pau, Rangar
7.	Kajalio, Kala Pahad, Kalahapus Real, Karanjio
8.	Roos, Sabja, Saleem
9.	Neelum, Ratna, Sindhu
10.	Alphonso, Dilpasand
11.	Langra, Musharad
12.	Rajapuri, Rumani
13.	Goamankur, Hathizool
14.	Kesar, Ladaio
15.	Karelia, Villai Kolumban
16.	Mahalanjeo, Maharaja of Mysore

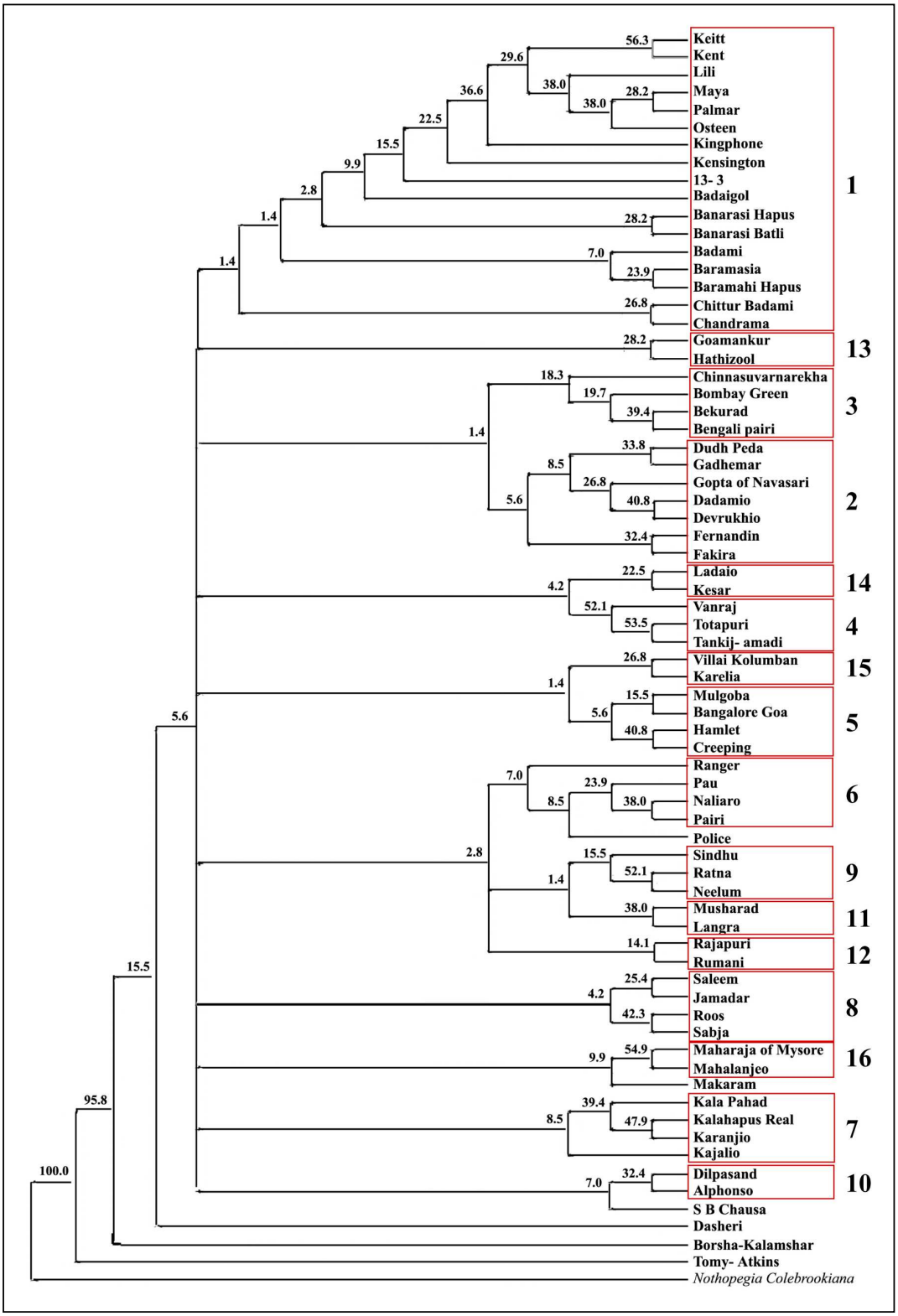


Fig 2a. UPGMA dendrogram for 70 mango cultivars and the distant outgroup, drawn using Winboot software and Dice similarity coefficient, showing 16 conserved clusters.

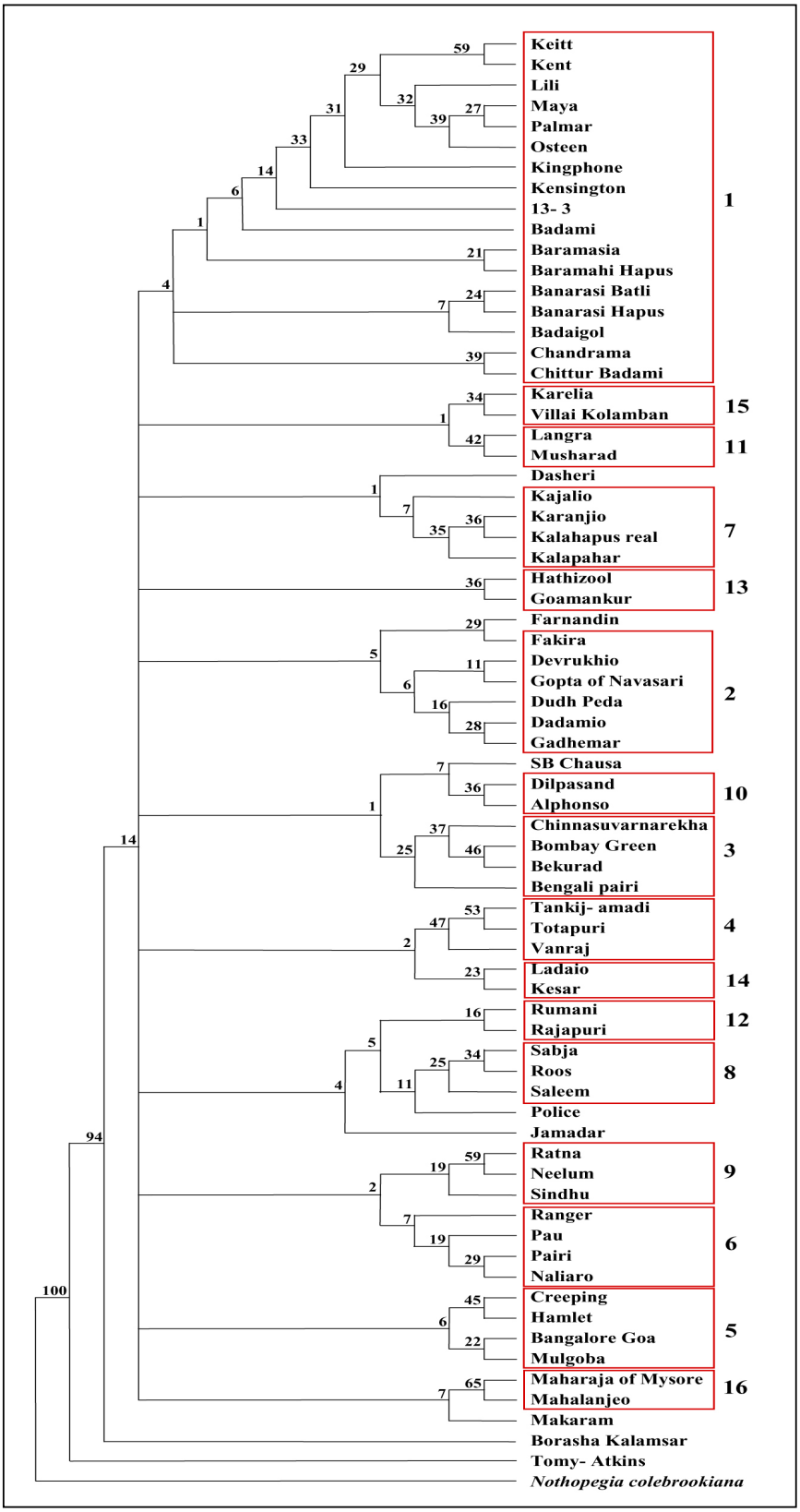


Fig 2b. UPGMA dendrogram for 70 mango cultivars and the distant outgroup, drawn using Winboot software and Jaccard similarity coefficient, showing 16 conserved clusters.

Similarity estimates of Neelum × Alphonso progeny

Similarity estimates for the group of Alphonso, Neelum, Ratna and Sindhu are given in Table 5. Similarity between Alphonso and Neelum was 0.581, which was the lowest among those values produced by these 4 cultivars. The highest similarity was noted between Neelum and Sindhu (0.678).

Table 5. Similarity estimates between Alphonso, Neelum their F_1 progeny cultivar Ratna and Sindhu, the progeny of backcross between Ratna and Alphonso.

	Alphonso	**Neelum**	**Ratna**	**Sindhu**
Alphonso	1			
Neelum	0.581	1		
Ratna	0.595	0.678	1	
Sindhu	0.587	0.614	0.628	1

Discussion

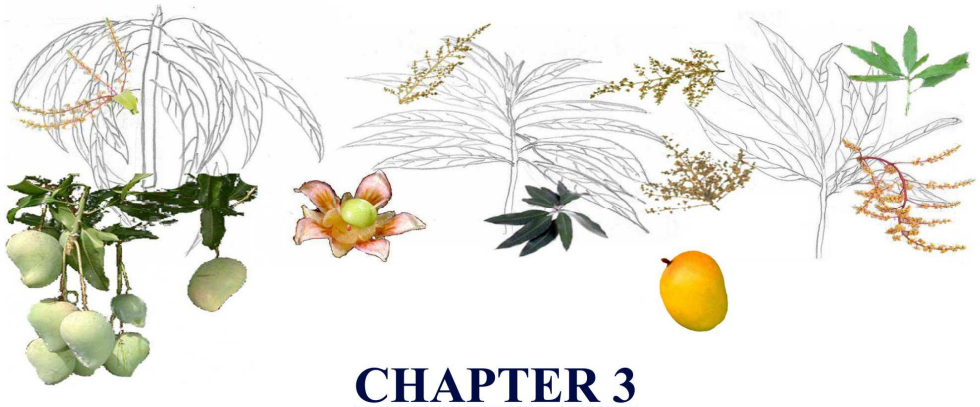
To the best of our knowledge there are only two reports (Eiadthong et al, 1999b; Srivastava et al, 2005), where the genetic diversity in mango is assessed by ISSR markers. The report by Eidthong et al (1999b) mainly dealt with the amplification in Thai cultivars by seven ISSR primers of which four were anchored dinucleotide repeat primers. Srivastava et al (2005) have also used seven ISSR primers which include two anchored dinucleotide repeat primers. Here, 100 ISSR primers have been screened to find that the majority of anchored ISSR primers (>80%) that can produce multilocus amplifications in mango, belong to the dinucleotide repeat class. It indicates that the mango genome is likely to be rich in the dinucleotide repeat regions. Eidthong et al (1999b) also reported variability in the band patterns in order to distinguish any two cultivars, which could not be obtained in the current set of cultivars. Indian cultivars showed higher homogeneity in the ISSR

targeted regions; it was also true for the selected foreign cultivars. Based on the present study including the highest number of cultivars, number of primers as well as the number of loci scored, it can be postulated that the variability observed by previous researchers could be because of small sample size used and such variability might get eliminated with the increase in the sample size and rule out the possibility that ISSRs can become a direct and comprehensive marker system in mango cultivar identification. Nonetheless, we report cultivar specific bands from the present set of cultivars (Table 3). It should be noted that these specific bands are more in case of two most diverged cultivars Tomy Atkins and Vanraj. These two probably define the either extremes of the dendrograms in the present set of cultivars as the similarity between them calculated using all Dice as well as Jaccard similarity coefficients was the lowest of all the pairwise cultivar comparisons (0.504 and 0.337 respectively). Rest of the cultivar pool is considerably homogenous for the ISSR targeted genomic regions, which is evident from the 'close' pairwise similarity values. Srivastava et al (2005) reported the RAPD and DAMD bands that were specific to cultivar Neelum and its hybrid descendents; no such bands could be found in Alphonso, Neelum, Ratna and Sindhu group with the ISSR marker system. Moreover, the present analysis revealed some interesting information about this group of cultivars, wherein Neelum emerged as a genetically close parent for Ratna. Surprisingly, Sindhu, which is a backcross between Ratna and Alphonso, was also placed closer to Neelum than Alphonso (Table 3 and 4). ISSR's property to target repeat regions might be one of the causes of such grouping.

Various markers that have been used to assess the genetic diversity in mango have proved to be successful in various dimensions. RAPD markers in case of mango could resolve the monoembryonic and polyembryonic cultivars clearly (Lopez-

Valenzuela et al, 1997; Karihaloo et al, 2003). Such a differentiation has not been reported by AFLP, ISSR or any other system. Kensington and Villai Kolumban, the two polyembryonic cultivars might have diverged as close outgroup taxa for the present set of cultivars as per the *priori* assumption if ISSRs would have had that resolution power. Present data is strong enough to state that no such differentiation has taken place in the SSR regions of mango genome. Secondly, Lopez-Valenzuela et al (1997) and Schnell et al (1995) mentioned the geographical clustering of cultivars with the RAPDs, which we have also obtained quite clearly and consistently with the ISSRs. Considering the extent of genomic regions spanned by the ISSRs and the RAPDs, this phenomenon can be quoted as an evidence for the initiation of spatial isolation in the global mango germ pool. Isolation within Indian cultivars as North Indian and South Indian has also been reported by Karihaloo et al (2003) and Ravishankar et al (2004), (Dendrogram as well as PCO results) using the RAPDs. This grouping was observed to be dissolved by increase in the sample size (Ravishankar et al, 2000) and by the change in selected cultivar set (Chunwongse et al, 2000). ISSRs in the present study completely merge both the groups. This was clearly evident from the high probability to find the shared bands among two cultivars, the scatter plot as well as from the low bootstrap values in the dendrograms. This could be because of the cultivar spreading in the recent past and higher rate of new cultivar generation in India. While clustering, small conserved groups that were observed instead of major lineages also support the postulate of recent history. In order to obtain a better picture, such studies should be carried out with the larger sample size, which will span the vast diversity of Indian cultivars and also with different types of markers, each in statistically significant number. India being the

center of origin and the primary center of diversity for mango, such a study holds worth scientific importance.



CHAPTER 3

Gas Chromatography- Mass Spectrometry Based Qualitative and Quantitative Diversity Analysis of Flavorants in Mango Cultivars

Work described in this chapter has been communicated to *Journal of Agricultural and Food Chemistry*.

Introduction

Mango is one of the oldest cultivated tropical fruits. It is popularly known as 'The King of Fruits' (Tharanathan et al, 2006) and is a delicious and widely cultivated fruit for a fresh market use. India is the largest mango producer and contributes 63.2% of total 15 million tons of global mango production. India exports 50,000 tons mango annually to different countries including Middle East, Europe and United States, and the demand is increasing all the time. Thus mango plays a vital role in foreign exchange earnings and income generation particularly in rural areas of India (FAO, 2002).

India also possesses a large diversity of mango cultivars with an estimated number exceeding one thousand. Each cultivar is identified by the characteristic combination of properties such as the plant architecture, fruit size, color, taste, flavor etc. This diversity of characters, with a continuous variation in each one, creates extreme complexity in the identification and classification of cultivars. Although, morphological and molecular diversity analyses have helped significantly in cultivar identification (Naik and Gangolly, 1950; Ravishankar et al, 2000; Hemanth Kumar et al, 2001; Karihaloo et al, 2003; Srivastava et al, 2005; Pandit et al, 2007), the use of biochemical features, the actual desired traits, is necessary to supplement this task and confer a functional dimension. The volatile component of flavor, the aroma, presents a good experimental system for such endeavor as it includes array of chemicals from various classes such as alcohol, aldehyde, ester, ketone and terpene. Secondly, this is the component that principally affects the market success of any fruit. For mango, the work in this direction has been previously done by Lebrun et al (2007), Pino et al (2005), Mahattanatawee et al (2006), Pino and Mesa (2006) and Quijano et al (2007).

Most of the cultivars considered in these studies were non-Indian; thus a large number of cultivars pertaining to their centre of diversity, India, still remain unattended.

Paucity of information in case of large number of Indian cultivars can be mainly attributed to their absence at the commercial platform. This absence was because of the technological dearth that held the dealers in negotiating the perishability of these fruits (Lizada, 1993). However, considering today's technological advance, these cultivars serve an effervescent reservoir to present new and attractive cultivars to the world. Such a study is also set to help the breeding attempts that aim towards producing a supreme cultivar for the international market. In this background, requirement to assess these cultivars for the diversity and relationships among them always persists. Therefore, in the present work, 27 mango cultivars that include Indian as well as non-Indian ones, [as stated in chapter 2] have been analyzed based on the aroma profiles of their ripe fruits. An attempt has also been made to assess the diversity and the relationship among these cultivars, based on qualitative as well as quantitative presence of compounds. In addition, this study has enabled us to peep into the aroma bank of Indian mangos and also to portray the flavor discrepancies among the cultivars.

Materials and methods

Plant material

Mature fruits of all the cultivars used in the present analysis were collected from the orchards at Vengurle (Maharashtra, India). Except Makaram, Musharad and Sabja, all the North and South-Indian cultivars were chosen based on their popularity

at international and/or local markets; similarly, non-Indian cultivars were chosen for their success in the international markets.

All the fruits were incubated at 28^{o}C for ripening. Ripe fruits were snap frozen in liquid nitrogen and were stored at -80^{o}C till further use.

Volatiles extraction

Volatile extraction procedure for the fruits of all 27 cultivars was the same. Tissue was ground to fine powder in liquid nitrogen. Volatiles were extracted from 10g tissue for 1hr at 28ºC using 40ml dichloromethane. 40µg nonyl acetate was added as an internal standard. The supernatant was dehydrated with anhydrous sodium sulphate and was concentrated to 1ml using vacuum-rotary evaporator. These extracts were incubated overnight at -20ºC and were centrifuged at 10,000 rpm at 4ºC for 15 min to pellet out high molecular weight lipids.

Gas chromatography- Flame ionization detector (GC-FID) and Gas chromatography- Mass spectrometry (GC-MS) analyses

Clarus 500 (Perkin Elmer, USA) gas chromatograph equipped with Rtx-5MS (Restek, USA) capillary column (30m x 0.32mm i.d. x 0.25µm film thickness) was used for all the analyses; column temperatures were programmed from 40ºC for 5 min, raised to 220ºC at 10ºC/min and held isothermal for 5 min. Injector and detector temperatures were 200 and 250ºC, respectively. Helium was used as carrier gas at a flow rate 1 ml/min.

Mass spectra were obtained using Clarus 500 (Perkin Elmer, USA) gas chromatograph- mass spectrometer at 70 eV with a scan time of 0.2 sec for m/z 30-300 under the GC conditions same as those applied in GC-FID analysis. Using a series of *n*-paraffins (C_5- C_{22}), retention indices were determined for all the peaks. Compounds were identified by comparing acquired mass spectra with those of

authentic external standards and those stored in NIST/ NBS mass spectral library. To confirm the annotation, the retention indices of the predicted compounds were compared with those of authentic external standards and also with those reported in NIST mass spectral library (USA) (data version NIST 05, software version, 2.0d).

Quantification was carried out by internal standard method, where concentrations of different volatiles were normalized with that of nonyl acetate.

Statistical analysis

All the data used for the present analysis were generated from triplicate experiments. All statistical analyses except Principal coordinate analysis (PCO) and Principal components analysis (PCA) were performed using Systat statistical software (version 11, Richmond, CA, USA). Significance of variation in quantity of each volatile with respect to different cultivars was assessed by ANOVA. Least significant differences (Fisher's protected LSD) were calculated at level $p \leq 0.05$ following a significant F-test. PCO and PCA were performed on the quantitative data, using multivariate statistical package 3.13 (MVSP) (Kovach, 2002). PCO was performed using Euclidean distance measure and with all the rows subjected to $\log_e$ transformation. PCA was conducted in standardized mode (using a correlation matrix). In order to investigate which attributes of the character (quantity or uniqueness) are influencing the ordination, PCO and PCA were also carried out with the transposed data.

Results

Eighty-four different volatile constituents were detected from the blends of 27 cultivars (Table 1, 2a and 2b). Aroma of cultivar Alphonso contained the highest number of volatiles (35), whereas Pairi fruits contained the lowest number (16).

Different cultivars contain different amounts of volatiles

Comparison between the based on the total amount of volatile content present per gram of tissue cultivars is given in Fig 1. Based on this comparison, Langra showed the highest amount of volatiles (2495µg g^{-1}) followed by Alphonso (1201µg g^{-1}) and Dudhpeda (1133µg g^{-1}). Lowest concentration of volatiles was found in Chandrama (15.6µg g^{-1}). Most of the cultivars (21) showed odorant concentration below 500µg g^{-1}.

Relative abundance of volatile classes in the aroma of different cultivars

These volatiles were contributed by different chemical classes namely, alcohol, aldehyde, monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, oxygenated sesquiterpene, lactone, ketone and non-terpene hydrocarbon. 4-ethoxy ethylbenzoate and an unidentified compound were placed under 'miscellaneous' category in the present analysis. Aroma blends of cultivars Makaram, Sabja and Sindhu contained volatiles from all these classes (miscellaneous compounds not considered), whereas aroma blend of Totapuri was represented by only four chemical classes.

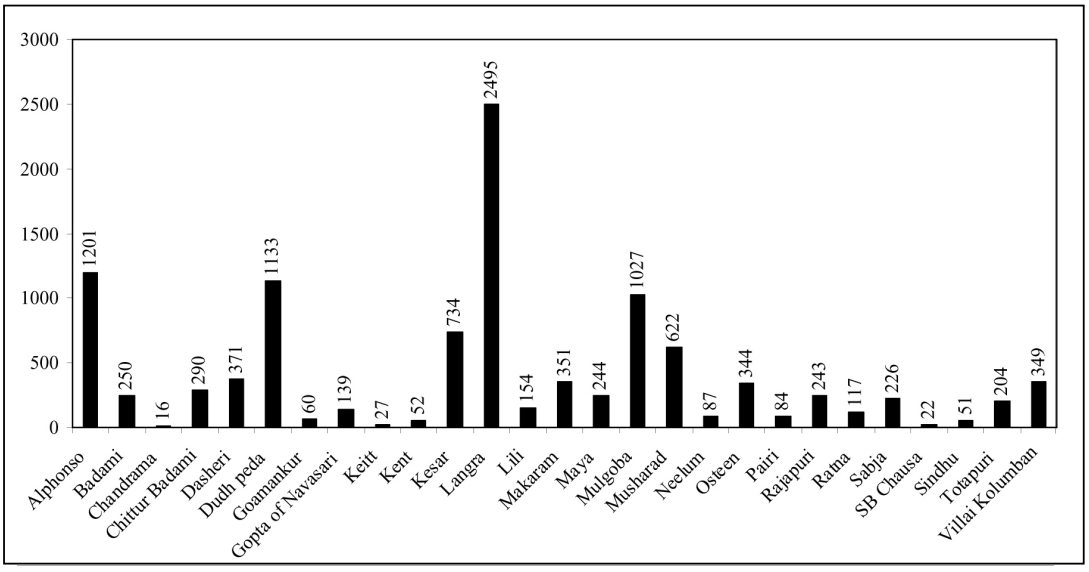


Fig 1. Bar graph showing the comparison among 27 cultivars for total amount of volatiles (µg g^{-1}).

Table 1. List of aroma compounds detected from the set of 27 mango cultivars; reported (KI_{rep}) [in NIST/ EPA/ NIH mass spectral library (USA) (data version NIST 05, software version, 2.0d)] and calculated (KI_{calc}) (RTX-5 MS) Kovat's indices as well as the odor descriptions of these compounds are also given.

No.	Compound name	KI_{rep}	KI_{calc}	Odor descriptions
1	Toluene	773	760	Pungent, caramel, ethereal, synthetic, fruity, rubbery, solvent-like
2	2-Hexanol	800	807	Mushroom, green, ripe, berry, astringent, metallic
3	4-Hydroxy-2-pentanone		826	
4	(*E*)-2-Hexenal	854	861	Apple-like, fruity, strawberry, cherry, green, almond-like, herbal, leafy
5	(*Z*)-3-Hexen-1-ol	857	864	Powerful odor of fresh green grass
6	1-Hexanol	871	876	freshly mowed grass
7	γ-Butyrolactone	915	921	Sweet, aromatic, caramel
8	α-Pinene	939	937	Harsh, terpene-like, minty ®, harsh, terpene-like, coniferous
9	Camphene	954	952	Sweet, fruity, camphoraceous, pine, oily, herbal
10	α-Methylbutyrolactone	973	959	
11	Sabinene	976	977	Fresh, citrus note, spicy, sweet, woody
12	β-Pinene	979	980	Musty, green, sweet, pine, resin, turpentine, woody
13	3-Methyl-3-cyclohexen-1-one		986	
14	β-Myrcene	991	994	Metallic, musty, geranium, sweet, fruity, ethereal, soapy, lemon, spicy, woody
15	α-Phellandrene	1003	1008	Fruity, minty, herbaceous, citrus, lime, pepper
16	δ-3-Carene	1011	1013	Floral, mango leaf like, mango peel, tropical, spicy, fresh, pepper
17	*p*-Cymene	1023	1029	
18	Limonene	1029	1034	fresh citrus, orange-like
19	(*Z*)-Ocimene	1050	1043	Citrus-like, herbaceous

No.	Compound name	KI_{rep}	KI_{calc}	Odor descriptions
20	(*E*)-Ocimene	1037	1054	Herbaceous, mild, citrus, sweet, orange, lemon
21	β-Terpinene	1071	1064	Citrus-like, terpeny, herbaceous, fruity, sweet
22	γ-Hexalactone	1056	1064	Coumarin-like, sweet odor
23	Mesifuran	1065	1067	Sweet, fruity "sherry" like odor; xeres wine-like note
24	Terpinolene	1089	1067	Sweet, citrus, pine-like odor
25	Furaneol	1060	1082	Fruity, caramelized pineapple-strawberry odor & taste
26	4-Carene	1084	1092	
27	Nonanal	1101	1104	Fatty-floral-rose, waxy odor; citrus taste in dilution
28	δ-Hexalactone		1101	Coumarinic odor; coconut, cream, chocolate notes
29	1,3,8-*p*-Menthatriene	1110	1118	Sulfury, terpeny
30	(*E*) 2,6-Dimethyl-1,3,5,7-octatetraene,	1134	1136	
31	3,4-Dimethyl-2,4,6-octatriene	1110	1135	
32	*allo*-Ocimene	1142	1135	Fresh, grassy
33	Unidentified monoterpene 1		1135	
34	*trans*-*p*-2,8-Menthadien-1-ol	1123	1142	
35	*trans*-Limonene oxide	1142	1145	Fresh, citrus-like, mild green
36	Unidentified monoterpene 2		1162	
37	*p*-Mentha-1,5-dien-8-ol	1170	1168	
38	Borneol	1169	1175	Camphoraceous, musty
39	*p*-Cymen-8-ol	1183	1195	
40	Naphthalene	1181	1193	Strong, mothball odor
41	Dodecane	1200	1201	
42	α-Terpeneol	1189	1199	Peach-like, anise, oily, fruity, floral, minty, toothpaste
43	(*Z*)-2,6-Dimethyl-3,5,7-octatriene-2-ol		1201	
44	(*E*)-2,6-Dimethyl-3,5,7-octatriene-2-ol	1209	1213	
45	*trans*-Carveol	1217	1228	Caraway-like, green, oily,
46	Tridecane	1300	1300	
47	γ-Octalactone	1261	1268	Caramel-like
48	δ-Octalactone	1268	1296	Coumarin-like, sweet odor and taste; creamy note

No.	Compound name	KI_{rep}	KI_{calc}	Odor descriptions
49	α-Longipinene	1353	1363	
50	Copaene	1377	1387	Woody, earthy
51	Longicyclin	1373	1385	
52	Tetradecane	1400	1396	
53	β-Elemene	1397	1402	Waxy, herbaceous
54	α-Gurjunene	1410	1423	Earthy, mango-like
55	Longifolin	1408	1421	
56	β-Caryophyllene	1428	1434	Musty, green, spicy, woody, terpene-like, fruity, sweet
57	Unidentified sesquiterpene 1		1442	
58	α-Guaiene	1440	1450	Sweet-woody, balsamic, peppery
59	Geranyl acetone	1453	1453	Fresh, floral, rosy-green, fruity odor
60	γ-Gurjunene	1473	1464	
61	Humelene	1454	1469	Oily, fruity, woody
62	Decalactone	1467	1485	peach like fruit aroma
63	Germacrene D	1485	1498	Oily, green, woody
64	β-Selinene	1485	1503	Herbaceous
65	δ-Decalactone		1520	Creamy, peachy, fatty odor; cream-like taste
66	Ledene	1487	1511	
67	Unidentified sesquiterpene 2		1512	
68	δ-Guaiene	1505	1521	Oily
69	δ-Cadanine	1523	1536	
70	4-Ethoxy ethylbenzoate		1543	fruit-like
71	α-Panasinsen	1518	1535	
72	Elemol	1550	1552	Sweet-woody, mild, weak floral, green
73	Germacrene D-4-ol	1576	1567	
74	1,2-Longidione		1583	
75	Hexadecane	1600	1600	
76	Caryophyllene oxide	1606	1629	Sweet, fruity, sawdust, herbaceous
77	Unidentified compound		1600	
78	Unidentified oxygenated sesquiterpene		1658	
79	τ-Muurolol	1641	1659	
80	α-Cadinol	1653	1673	Green, waxy, woody
81	δ-Cadinol	1674	1696	

No.	Compound name	KI_{rep}	KI_{calc}	Odor descriptions
82	Unidentified sesquiterpene 3		1700	
83	Octadecane	1800	1799	
84	Hexadecanal	1819	1820	

Degree of representation of various chemical classes in the aroma composition of different cultivars is depicted in Fig 2. In all the 27 cultivars, mono- and sesquiterpene hydrocarbons dominated the aroma blends. These two classes were represented in all the cultivars; however, two cultivar groups were observed based on the relative dominance of these two types of volatiles in them. Twenty cultivars formed the first group (Fig 2a) in which, monoterpene hydrocarbon concentrations were higher than those of sesquiterpene hydrocarbons. In this group (a), contribution of monoterpene hydrocarbons ranged between 51% (Keitt) and 95% (Kesar). In the second group (b) of seven cultivars (Fig 2b), is a reversed condition, sesquiterpene hydrocarbon concentrations were higher, ranging between 41% (Pairi) and 73% (Goamankur).

Aldehydes were also represented in the entire set; however their quantities were low in most of the cultivars. Relative concentrations of aldehydes ranged between 0.03% in Kesar and 14.36% in Pairi. Members of non-terpene hydrocarbon class were also detected in all the cultivars; this class contributed to the volatile blend least in Musharad (0.03%) and most in Keitt (13.33%). Alcohol was the next class on the basis of qualitative dominance; except in Chittur Badami, Keitt, Kesar, Mulgoba, Musharad, Pairi, Rajapuri, SB Chausa and Totapuri, members of this class appeared in 18 cultivars. Among these cultivars, the relative cumulative concentrations of alcohols ranged from 0.02% (Alphonso) to 1.64% (Ratna). Nineteen cultivars contained ketones, among these Sindhu showed the highest relative amount (9.95%).

Table 2a. Volatile compounds and their quantities (µg g^{-1}) in the ripe fruits of first 14 from the current set of mango cultivars (1≡ Alphonso; 2≡ Badami; 3≡ Chandrama; 4≡ Chittur Badami; 5≡ Dasheri; 6≡ Dudh peda; 7≡ Goamankur; 8≡ Gopta of Navasari; 9≡ Keitt; 10≡ Kent; 11≡ Kesar; 12≡ Langra; 13≡ Lili; 14≡ Makaram). Concentration values followed by the same alphabet are not significantly different from each other at 0.05% level of significance, when all 27 cultivars were compared with each other, separately for the concentration of each compound.

Compound name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Aldehydes														
(*E*)-2-Hexenal	0.532^{c}	0.048^{ab}	0^{a}	0^{a}	0^{a}	2.186^{e}	0.040^{ab}	0^{a}	0^{a}	0.149^{b}	0^{a}	0^{a}	0.068^{ab}	0.262b
Nonanal	0^{a}	0^{a}	0.194^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Hexadecanal	0.821^{a}	0.57^{a}	0.596^{a}	0.741^{a}	2.267^{ab}	17.76^{e}	1.22^{ab}	13.56^{cd}	0.304^{a}	0.493^{a}	0.229^{a}	4.834^{b}	0^{a}	0.515^{a}
Alcohols														
2-Hexanol	0.280^{c}	0.231^{bc}	0^{a}	0.326^{d}	0.218^{b}	0^{a}	0^{a}	0.209^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0.197^{b}	0.283^{c}
(*Z*)-3-Hexen-1-ol	0^{a}	0^{a}	0^{a}	0^{a}	0.026^{ab}	0.924^{d}	0.043^{ab}	0.165^{b}	0^{a}	0.158^{b}	0^{a}	1.224^{e}	0.059^{ab}	0.473^{c}
1-Hexanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.160^{b}
Monoterpene hydrocarbons														
α-Pinene	3.983^{b}	7.439^{b}	0.163^{ab}	4.394^{b}	1.954^{ab}	5.409^{b}	0.277^{ab}	1.921^{ab}	0.541^{ab}	0.465^{ab}	25.18^{e}	25.21^{e}	0.671^{ab}	4.791^{b}
Camphene	0.314^{bc}	0^{a}	0^{a}	1.038^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Sabinene	0^{a}	0^{a}	0.242^{a}	0.441^{ab}	0^{a}	7.841^{d}	0.208^{a}	7.855^{d}	2.602^{c}	0.475^{ab}	0^{a}	0^{a}	0.109^{a}	1.854^{bc}
β-Pinene	1.174^{c}	0.22^{ab}	0.124^{ab}	0.286^{ab}	0.084^{ab}	3.689^{g}	0.174^{ab}	3.109^{f}	0.847^{bc}	0^{a}	0.716^{bc}	0.515^{b}	0.09^{a}	1.579^{de}
β-Myrcene	8.93^{a}	0.777^{a}	2.012^{a}	140.2^{d}	4.438^{a}	852.5^{g}	0.618^{a}	1.614^{a}	0.79^{a}	1.277^{a}	12.52^{a}	67.67^{bc}	2.465^{a}	2.664^{a}
α-Phellandrene	0^{a}	0^{a}	0^{a}	0^{a}	0.957^{b}	0^{a}	0^{a}	0.178^{a}	0^{a}	0^{a}	0.155^{a}	21.42^{f}	0.642^{b}	0^{a}
δ-3-Carene	0.046^{a}	0^{a}	0^{a}	15.79^{b}	0^{a}	13.86^{b}	0^{a}	0^{a}	4.919^{ab}	17.97^{b}	0^{a}	2046^{f}	89.7^{c}	0.526^{a}
p-Cymene	1.405^{bc}	2.416^{d}	0.976^{b}	3.177^{ef}	0^{a}	3.916^{f}	1.199^{bc}	1.188^{bc}	1.167^{bc}	1.419^{bc}	1.157^{bc}	6.525^{h}	1.506^{bc}	1.568^{c}
Limonene	0.586^{ab}	0^{a}	0.281^{ab}	3.615^{cd}	311.4^{h}	5.281^{d}	0.273^{ab}	2.436^{b}	1.415^{ab}	1.246^{ab}	0.549^{ab}	74.23^{g}	3.523^{cd}	1.765^{ab}

Compound name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(*Z*)-Ocimene	1055^{e}	3.442^{a}	4.735^{a}	1.312^{a}	0^{a}	93.05^{c}	7.395^{ab}	2.269^{a}	0.858^{a}	1.581^{a}	598.3^{d}	14.85^{ab}	35.54^{b}	9.209^{ab}
(*E*)-Ocimene	36.01^{c}	203.4^{f}	0.297^{a}	1.549^{ab}	4.944^{ab}	7.268^{ab}	0.259^{a}	12.24^{b}	0.575^{a}	0^{a}	56.57^{d}	3.5^{ab}	1.744^{ab}	128.6^{e}
β-Terpinene	0^{a}	0^{a}	0^{a}	0.884^{ef}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.184^{g}	0^{a}	2.426^{i}	0.156^{b}	0.37^{c}
Terpinolene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
4-Carene	0^{a}	0^{a}	0^{a}	5.716^{ab}	0^{a}	4.507^{ab}	0^{a}	0^{a}	0^{a}	1.606^{ab}	0^{a}	102.3^{d}	6.644^{b}	1.804^{ab}
1,3,8-*p*-Menthatriene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.249^{b}	0^{a}
(*E*)-2,6-Dimethyl-1,3,5,7-octatetraene	0^{a}	0^{a}	0.276^{ab}	0^{a}	0^{a}	5.546^{e}	0.596^{ab}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.552^{ab}
3,4-Dimethyl-2,4,6-octatriene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	4.346^{b}	0^{a}	0^{a}	0^{a}
allo-Ocimene	4.954^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Unidentified monoterpene 1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.684^{d}
Unidentified monoterpene 2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Sesquiterpene hydrocarbons														
α-Longipinene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Copaene	1.065^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.174^{a}	0.977^{c}	0^{a}	0^{a}	0.994^{c}	2.219^{e}	0^{a}	0.548^{b}
Longicyclin	0^{a}	0^{a}	0^{a}	7.551^{h}	1.134^{c}	5.575^{g}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
β-Elemene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.859^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
α-Gurjunene	0^{a}	0^{a}	1.952^{c}	0^{a}	0^{a}	0^{a}	3.62^{e}	0^{a}	0^{a}	2.179^{d}	0^{a}	0^{a}	0^{a}	4.979^{f}
Longifolene	0.462^{ab}	0^{a}	0^{a}	45.23^{i}	4.644^{c}	34.45^{h}	0^{a}	0^{a}	0^{a}	0^{a}	0.373^{a}	1.734^{b}	0.802^{ab}	0^{a}
β-Caryophyllene	23.89^{g}	10.77^{cd}	1.469^{a}	19.99^{f}	15.67^{e}	21.8^{fg}	6.631^{bc}	49.44^{i}	1.78^{ab}	8.441^{c}	16.82^{e}	43.04^{h}	3.532^{ab}	5.134^{bc}
Unidentified sesquiterpene 1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
α-Guaiene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	5.178^{e}	0^{a}	0^{a}
γ-Gurjunene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.638^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2.832^{c}
Humelene	13.04^{f}	5.127^{c}	0.322^{ab}	10.1^{e}	8.07^{d}	12.32^{f}	3.33^{bc}	24.11^{h}	1.012^{ab}	4.288^{c}	8.706^{de}	21.57^{g}	1.866^{b}	0^{a}
Germacrene D	0.931^{a}	0^{a}	0^{a}	0^{a}	2.703^{a}	1.845^{a}	0^{a}	14.76^{c}	0^{a}	0^{a}	0^{a}	8.739^{b}	0^{a}	0^{a}

Compound name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
β-Selinene	0[a]	7.102[a]	0[a]	19.41[b]	0[a]	0[a]	25.64[b]	0[a]	3.536[a]	4.663[a]	0[a]	0[a]	0[a]	162.6[c]
Ledene	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Germacrene B	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	9.72[b]	0[a]	0[a]
δ-Guaiene	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	11.28[d]	0[a]	0[a]
δ-Cadanine	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	6.478[d]	0[a]	0[a]
α-Panasinsen	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	2.264[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	7.421[c]
Unidentified sesquiterpene 3	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Oxygenated monoterpenes														
trans-*p*-2,8-Menthadien-1-ol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.288[b]	0[a]
trans-Limonene oxide	0[a]	0[a]	0[a]	0[a]	0.222[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
p-Mentha-1,5-dien-8-ol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.226[b]	0[a]
Borneol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
p-Cymen-8-ol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.598[a]	0[a]
α-Terpeneol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
(*Z*) 2,6-Dimethyl-3,5,7-octatriene-2-ol	0.641[b]	0[a]	0.22[ab]	0[a]	0[a]	7.599[d]	0.356[ab]	0[a]	0[a]	0[a]	0.133[ab]	0[a]	0[a]	0.303[ab]
(*E*) 2,6-Dimethyl-3,5,7-octatriene-2-ol	0.341[d]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.083[b]	0[a]	0[a]	0.586[e]
trans-Carveol	0[a]	0[a]	0[a]	0[a]	0.227[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Oxygenated sesquiterpenes														
Elemol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Germacrene D-4-ol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
1,2-Longidione	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Caryophyllene oxide	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Unidentified oxygenated sesquiterpene	0[a]	0[a]	0[a]	1.622[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
τ-Muurolol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]

Compound name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
α-Cadinol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2.162^{c}	0^{a}	1.245^{b}
δ-Cadinol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Lactones														
γ-Butyrolactone	1.176^{b}	0.160^{a}	0.145^{a}	0^{a}	0.068^{a}	0^{a}	0.150^{a}	0^{a}	0^{a}	0^{a}	0.141^{a}	4.186^{c}	0.113^{a}	0.136^{a}
α-Methylbutyrolactone	0.185^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
γ-Hexalactone	2.017^{e}	0^{a}	0.398^{b}	0^{a}	0.398^{b}	2.298^{f}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
δ-Hexalactone	2.278^{c}	0^{a}	0^{a}	0^{a}	0^{a}	3.646^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
γ-Octalactone	2.226^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
δ-Octalactone	1.66^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.094^{b}	0^{a}	0^{a}	0^{a}
γ-Decalactone	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
δ-Decalactone	0.195^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Ketones														
4-Hydroxy-2-pentanone	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.540^{b}	0^{a}	1.592^{c}	0^{a}	0^{a}
3-Methyl-3-cyclohexen-1-one	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Mesifuran	17.83^{f}	6.383^{d}	0^{a}	0.446^{ab}	1.377^{b}	14.44^{e}	0.279^{a}	0^{a}	1.864^{b}	0^{a}	1.322^{b}	0.615^{ab}	0^{a}	0.642^{ab}
Furaneol	6.849^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Geranyl acetone	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Non-terpene hydrocarbons														
Toluene	1.456^{f}	0.293^{b}	0.234^{ab}	0.925^{de}	0.298^{b}	2.014^{h}	0.368^{bc}	0.279^{ab}	0.487^{c}	0.528^{c}	0.465^{bc}	1.025^{e}	0.257^{ab}	0.588^{c}
Naphthalene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2.649^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Dodecane	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Tridecane	0^{a}	0^{a}	0.042^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.901^{e}	0^{a}	1.039^{f}	0^{a}	0^{a}	0.232^{b}	0^{a}
Tetradecane	0.254^{ab}	0.281^{ab}	0^{a}	1.647^{e}	7.9^{h}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.414^{b}	1.23^{d}	0.499^{bc}	0.737^{c}
Hexadecane	0^{a}	0.617^{c}	0.397^{bc}	1.697^{f}	0.549^{c}	1.352^{e}	0.589^{c}	0.395^{bc}	0.229^{ab}	0.255^{b}	0^{a}	1.237^{e}	0.586^{c}	0.585^{c}

Compound name	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Octadecane	0.184^{ab}	0.526^{c}	0.511^{c}	0^{a}	0.503^{c}	0.978^{d}	0.547^{c}	0.312^{bc}	0.181^{ab}	0^{a}	0.644^{c}	1.554^{f}	0.542^{c}	0.554^{c}
Miscellaneous														
4-Ethoxy ethylbenzoate	0.273^{e}	0^{a}	0.086^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.535^{f}	0.198^{d}	0^{a}	0.093^{b}	0^{a}
Unidentified compound	8.497^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3.109^{b}	0^{a}	0^{a}	0^{a}

Table 2b. Volatile compounds and their quantities (µg g^{-1}) in the ripe fruits of next 13 from the current set of mango cultivars (15≡ Maya; 16≡ Mulgoba; 17≡ Musharad; 18≡ Neelum; 19≡ Osteen; 20≡ Pairi; 21≡ Rajapuri; 22≡ Ratna; 23≡ Sabja; 24≡ SB Chausa; 25≡ Sindhu; 26≡ Totapuri; 27≡ Villai Kolumban). Concentration values followed by the same alphabet are not significantly different from each other at 0.05% level of significance, when all 27 cultivars were compared with each other, separately for the concentration of each compound.

Compound name	15	16	17	18	19	20	21	22	23	24	25	26	27
Aldehydes													
(*E*)-2-Hexenal	0.115ab	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.279^{b}	1.054^{d}	0^{a}	0.239^{b}	0^{a}	0^{a}
Nonanal	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.074^{b}	0^{a}	0^{a}	0^{a}	0^{a}
Hexadecanal	6.109^{b}	1.232^{ab}	15.1^{d}	1.992^{ab}	1.112^{a}	12.04^{c}	0.839^{a}	1.614^{ab}	4.501^{b}	0.671^{a}	2.536^{ab}	0.261^{a}	3.76^{b}
Alcohols													
2-Hexanol	0^{a}	0^{a}	0^{a}	0.267^{c}	0.262^{c}	0^{a}	0^{a}	0^{a}	0.399^{e}	0^{a}	0^{a}	0^{a}	0^{a}
(*Z*)-3-Hexen-1-ol	0.913^{d}	0^{a}	0^{a}	0^{a}	0.803^{d}	0^{a}	0^{a}	1.681^{f}	1.106^{e}	0^{a}	0.390^{c}	0^{a}	2.298^{g}
1-Hexanol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.233^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0.366^{d}
Monoterpene hydrocarbons													
α-Pinene	7.034^{b}	136.6^{f}	0.499^{ab}	6.757^{b}	4.253^{b}	1.142^{ab}	21.49^{e}	12.18^{c}	0.129^{a}	0.1^{a}	2.02^{ab}	14.13^{d}	3.214^{ab}
Camphene	0^{a}	2.331^{e}	0^{a}	0.369^{c}	0^{a}	0^{a}	0.913^{d}	0.277^{bc}	0^{a}	0^{a}	0^{a}	0.205^{b}	0^{a}

Compound name	15	16	17	18	19	20	21	22	23	24	25	26	27
Sabinene	0^{a}	0^{a}	1.238^{b}	0^{a}	8.079^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.438^{ab}	0^{a}	0^{a}
β-Pinene	0.754^{bc}	13.15^{h}	0.54^{b}	0.565^{b}	3.416^{fg}	0^{a}	1.849^{e}	1.378^{de}	0^{a}	0^{a}	0.479^{b}	1.644^{e}	0.268^{ab}
β-Myrcene	5.102^{a}	680.3^{f}	180.5^{e}	30.35^{ab}	8.281^{a}	14.96^{ab}	121.2^{c}	3.156^{a}	50.19^{b}	0.109^{a}	0.688^{a}	102.9^{c}	8.61^{a}
α-Phellandrene	1.554^{c}	0^{a}	0^{a}	0.133^{a}	3.016^{e}	0^{a}	0^{a}	0^{a}	0^{a}	0.069^{a}	0^{a}	0^{a}	2.332^{d}
δ-3-Carene	163.9^{d}	4.179^{ab}	0^{a}	5.128^{ab}	239.7^{e}	4.857^{ab}	13.6^{b}	2.293^{a}	0.272^{a}	1.299^{a}	0.15^{a}	0^{a}	23.67^{b}
p-Cymene	2.247^{cd}	2.708^{de}	0^{a}	1.833^{cd}	3.067^{e}	1.685^{c}	2.312^{d}	2.33^{d}	1.257^{bc}	1.607^{c}	1.176^{bc}	1.307^{bc}	5.507^{g}
Limonene	6.561^{d}	4.845^{d}	0^{a}	1.385^{ab}	15.39^{f}	1.038^{ab}	3.413^{cd}	1.493^{ab}	0.38^{ab}	0.242^{a}	0.517^{ab}	0.669^{ab}	11.87^{e}
(*Z*)-Ocimene	1.568^{a}	89.17^{c}	27.75^{ab}	0.27^{a}	1.723^{a}	6.926^{ab}	13.97^{ab}	52.61^{b}	1.939^{a}	0.07^{a}	19.55^{ab}	58.93^{bc}	1.334^{a}
(*E*)-Ocimene	0.931^{a}	10.49^{ab}	4.746^{ab}	0.258^{a}	1.328^{a}	0.482^{a}	1.619^{ab}	2.404^{ab}	0.904^{a}	0^{a}	0.684^{a}	3.737^{ab}	0^{a}
β-Terpinene	0.101^{ab}	1.787^{h}	0^{a}	0.101^{ab}	0.978^{f}	0^{a}	0.83^{e}	0.331^{c}	0^{a}	0.019^{ab}	0^{a}	0.129^{b}	0.713^{d}
Terpinolene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.181^{b}	0^{a}	0^{a}	0^{a}
4-Carene	11.53^{b}	3.504^{ab}	0^{a}	3.001^{ab}	28.77^{c}	2.684^{ab}	5.097^{ab}	1.712^{ab}	0^{a}	3.351^{ab}	0^{a}	0^{a}	238.8^{e}
1,3,8-*p*-Menthatriene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3.959^{c}
(*E*)-2,6-Dimethyl-1,3,5,7-octatetraene	0^{a}	0^{a}	0.928^{b}	0^{a}	0^{a}	0^{a}	0^{a}	2.772^{d}	0^{a}	0^{a}	2.058^{c}	0^{a}	0.819^{b}
3,4-Dimethyl-2,4,6-octatriene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
allo-Ocimene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Unidentified monoterpene 1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.311^{c}	0^{a}	0^{a}	0.875^{b}	0^{a}	0^{a}
Unidentified monoterpene 2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.606^{b}
Sesquiterpene hydrocarbons													
α-Longipinene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.172^{b}	0^{a}	0^{a}	0^{a}
Copaene	0^{a}	0^{a}	2.763^{f}	0^{a}	0.648^{b}	0^{a}	0^{a}	0^{a}	1.513^{d}	0^{a}	0^{a}	1.007^{c}	0^{a}
Longicyclin	0^{a}	0^{a}	0^{a}	2.649^{e}	0^{a}	1.894^{d}	4.697^{f}	0.926^{b}	0^{a}	1.042^{bc}	0^{a}	0^{a}	0^{a}
β-Elemene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
α-Gurjunene	0^{a}	0^{a}	0^{a}	0^{a}	1.524^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Compound name	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
Longifolene	0^{a}	29.29^{g}	0^{a}	14.68^{f}	0^{a}	10.89^{e}	34.85^{h}	8.516^{d}	2.006^{b}	5.147^{c}	0^{a}	0^{a}	0^{a}
β-Caryophyllene	4.643^{b}	23.43^{g}	12.62^{d}	3.099^{ab}	9.677^{cd}	14.2^{de}	7.946^{c}	4.75^{b}	70.8^{j}	2.036^{ab}	4.424^{ab}	6.365^{bc}	2.715^{ab}
Unidentified sesquiterpene 1	0^{a}	0^{a}	6.742^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
α-Guaiene	0^{a}	0^{a}	0^{a}	0.57^{b}	0^{a}	0^{a}	0^{a}	0^{a}	2.693^{d}	0^{a}	0^{a}	0.843^{b}	0^{a}
γ-Gurjunene	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Humelene	2.007^{b}	11.61^{ef}	0^{a}	1.674^{ab}	4.554^{c}	8.114^{d}	3.871^{c}	2.291^{bc}	34.06^{i}	1.332^{ab}	2.304^{bc}	3.016^{bc}	1.216^{ab}
Germacrene D	0^{a}	0^{a}	88.94^{e}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	22.54^{d}	0.22^{a}	0^{a}	0^{a}	0^{a}
β-Selinene	25.82^{b}	0^{a}	237.6^{d}	0^{a}	3.896^{a}	0^{a}	0.123^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.568^{a}	0^{a}
Ledene	0^{a}	2.185^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.069^{b}	0^{a}
Germacrene B	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	9.397^{b}	0^{a}	0^{a}	0^{a}	0^{a}
δ-Guaiene	0^{a}	0^{a}	0^{a}	1.764^{b}	0^{a}	0^{a}	0^{a}	0^{a}	5.375^{c}	0^{a}	0^{a}	1.979^{b}	0^{a}
δ-Cadanine	0^{a}	0^{a}	31.65^{e}	1.256^{b}	0^{a}	0^{a}	0^{a}	0^{a}	5.701^{c}	0^{a}	0.379^{a}	1.502^{b}	0^{a}
α-Panasinsen	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Unidentified sesquiterpene 3	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.545^{b}	0^{a}	0^{a}	0^{a}
Oxygenated monoterpenes													
trans-p-2,8-Menthadien-1-ol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
trans-Limonene oxide	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
p-Mentha-1,5-dien-8-ol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Borneol	0^{a}	0^{a}	0^{a}	0.387^{b}	0^{a}	0^{a}	0.919^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
p-Cymen-8-ol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	32.82^{b}
α-Terpeneol	0^{a}	0^{a}	0^{a}	0.274^{c}	0^{a}	0^{a}	0.734^{d}	0^{a}	0.218^{b}	0^{a}	0^{a}	0^{a}	0^{a}
(*Z*) 2,6-Dimethyl-3,5,7-octatriene-2-ol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.945^{c}	0^{a}	0^{a}	2.388^{c}	0^{a}	0^{a}
(*E*) 2,6-Dimethyl-3,5,7-octatriene-2-ol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.258^{c}	0^{a}	0^{a}
trans-Carveol	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Compound name	15	16	17	18	19	20	21	22	23	24	25	26	27
Oxygenated sesquiterpenes													
Elemol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.604[b]	0[a]	0[a]	0[a]	0[a]
Germacrene D-4-ol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.633[b]	0[a]	0[a]	0[a]	0[a]
1,2-Longidione	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.142b	0[a]	0[a]	0[a]
Caryophyllene oxide	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.918[d]	0.072[b]	0.149[c]	0[a]	0[a]
Unidentified oxygenated sesquiterpene	0[a]	0[a]	0[a]	2.363[b]	0[a]	0[a]	0.232[a]	0[a]	0[a]	0.156[a]	0[a]	0[a]	0[a]
τ-Muurolol	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	2.295[b]	0[a]	0[a]	0[a]	0[a]
α-Cadinol	0[a]	0[a]	4.719[d]	0[a]	0[a]	0[a]	0[a]	0[a]	2.224[c]	0[a]	0[a]	0[a]	0[a]
δ-Cadinol	0[a]	0.51[c]	0[a]	0.372[b]	0[a]	0[a]	0.525[c]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Lactones													
γ-Butyrolactone	0[a]	0.326[a]	0[a]	0[a]	0.144[a]	0[a]	0.192[a]	0.233[a]	0.299[a]	0[a]	0.090[a]	0[a]	0.167[a]
α-Methylbutyrolactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
γ-Hexalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	1.085[d]	0.559[c]	0[a]	0[a]
δ-Hexalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.701[b]	0[a]	0[a]
γ-Octalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
δ-Octalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.266[c]	0[a]	0[a]	0[a]
γ-Decalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
δ-Decalactone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Ketones													
4-Hydroxy-2-pentanone	0[a]	0[a]	5.102[e]	2.514[d]	0.513[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0.450[ab]	0[a]	0[a]
3-Methyl-3-cyclohexen-1-one	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.086[b]
Mesifuran	0[a]	0[a]	0[a]	1.514[b]	0.232[a]	0[a]	0[a]	0[a]	0[a]	0.642[ab]	3.81[c]	0[a]	0[a]
Furaneol	0.468[b]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.134[a]	0[a]	0[a]	0[a]	0[a]
Geranyl acetone	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0.813[b]	0[a]	0[a]

Compound name	15	16	17	18	19	20	21	22	23	24	25	26	27
Non-terpene hydrocarbons													
Toluene	0.471^{bc}	1.877^{g}	0.172^{ab}	0.343^{bc}	0.465^{bc}	0.419^{bc}	0.449^{bc}	0.785^{d}	1.729^{g}	0.109^{a}	0.263^{ab}	0.349^{bc}	0.308^{bc}
Dodecane	0^{a}	0.663^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.268^{d}	0^{a}	0.511^{b}	0^{a}	0.126^{a}	0^{a}
Tridecane	0^{a}	0.616^{d}	0^{a}	0^{a}	0.41^{c}	0^{a}	0^{a}	1.415^{h}	0.168^{b}	0^{a}	1.193^{g}	0^{a}	0^{a}
Tetradecane	0^{a}	2.251^{f}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3.072^{g}	0^{a}	0.265^{ab}	0.874^{c}	0.8^{c}	0.627^{bc}
Hexadecane	0^{a}	1.616^{f}	0^{a}	0.63^{cd}	0.217^{ab}	0.866^{d}	0.697^{cd}	1.437^{ef}	0^{a}	0.077^{ab}	0.172^{ab}	0.93^{d}	0.442^{bc}
Octadecane	1.324^{e}	1.255^{e}	0^{a}	0.509^{c}	0^{a}	0.69^{c}	0^{a}	0^{a}	0.268^{b}	0^{a}	0^{a}	0^{a}	0.699^{c}
Miscellaneous													
4-Ethoxy ethylbenzoate	0.143^{c}	0^{a}	0^{a}	0^{a}	0.626^{g}	0^{a}	0.167^{cd}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.082^{b}
Unidentified compound	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

The highest relative concentration of lactones was noted in SB Chausa (6.38%); these compounds were present only in 18 cultivars, where they occupied less than 1% of the blend in 15 of them. Oxygenated mono- and sesquiterpenes were detected in 14 and 10 cultivars, respectively. Their highest relative concentrations were measured from Villai Kolumban (10.38%) and Neelum (3.27%), respectively.

Principle aroma components

We found that in the aroma blends of many cultivars (18), only one or two compounds occur in major quantities (Table 3); we termed these volatiles as principle aroma components. Furthermore, these compounds were at least three folds higher than any other constituents. These principle compounds were chiefly monoterpene hydrocarbons. We found that non-Indian cultivars invariably had δ-3-carene as a major constituent, whereas Indian cultivars had (*Z*)-ocimene and β-myrcene. Two group b cultivars, Goamankur and Makaram had β-selinene as a major component; five of the group b cultivars did not show clear prominence of any compound(s).

Ordination and cultivar relationships

In the PCO drawn using Euclidean distances, first five axes covered more than 70% variation, whereas in PCA this much variation was covered by the first four axes. Grouping seen in PCO was obscure as all the cultivars were almost evenly spaced through the scatter plot (Fig 3a); while, in PCA the grouping was clear (Fig 3b). PCA pointed that five non-Indian cultivars form a separate group from the scattered Indian cultivars. These cultivars were placed in the second quadrant with high positive scores on both the PCs. North-Indian cultivar Langra was also placed in this group of non-Indian cultivars. Though, such a compact arrangement was not observed in PCO, all non-Indian cultivars showed uniform negative scores on the first axis. However, with

PCO, another North-Indian cultivar SB Chausa was placed within this scatter instead of Langra.

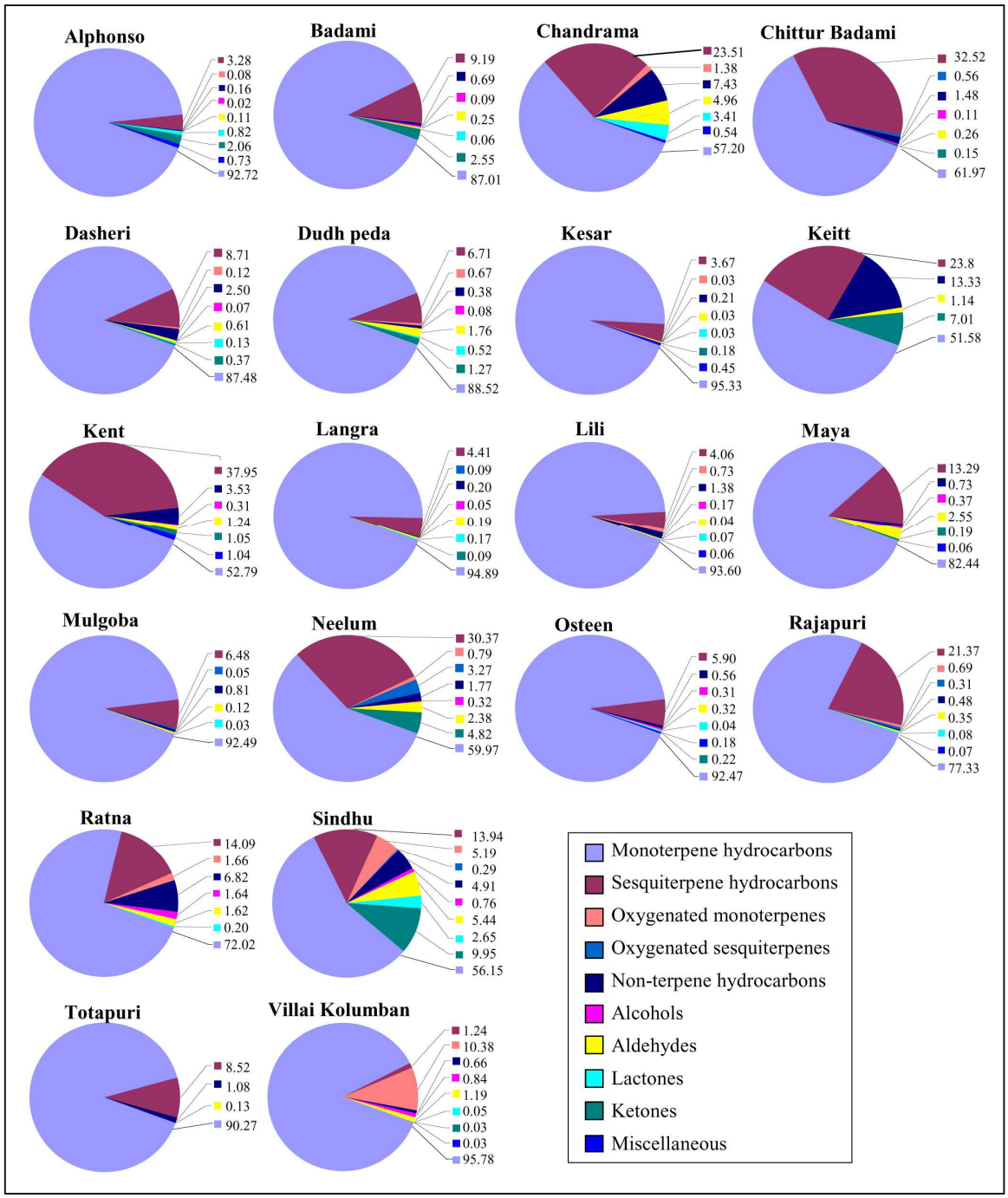


Fig 2a. Pie diagrams showing relative abundance of different chemical classes in 20 monoterpene dominant cultivars.

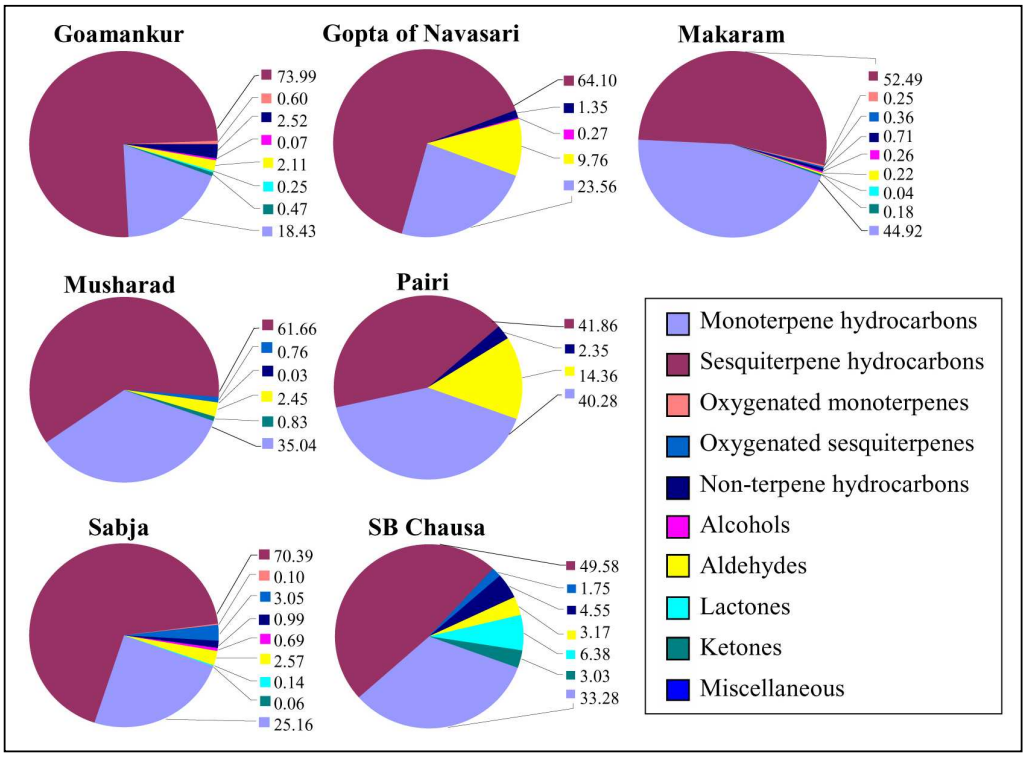


Fig 2b. Pie diagrams showing relative abundance of different chemical classes in seven sesquiterpene dominant cultivars.

PCA also showed polarization among the Indian cultivars (Fig 3b). South-Indian cultivars were scattered in a linear fashion. Except Langra, all North Indian cultivars were placed near one pole of this arrangement. Makaram, the cultivar of unknown Indian origin was also placed among these North-Indian cultivars. All north-Indian cultivars but Kesar, made positive contributions to the first PC. Thirteen of total 16 South-Indian cultivars negatively contributed to this PC.

Four cultivar pairs were created in both the multivariate analyses. These were Alphonso- Kesar, Chittur Badami- Rajapuri, Dudh peda- Mulgoba and Maya- Osteen. In both these analyses, the constituents of each of these pairs made equivalent contributions to the respective axes. Notably, except Alphonso- Kesar, other three pairs are constituted by the members of same respective geographical background.

Table 3. Principle aroma components of different cultivars with their relative abundance (%).

No.	Cultivar	Principle compound(s)	Percent abundance
1	Chittur Badami	β-Myrcene	48
2	Dudh peda	β-Myrcene	75
3	Mulgoba	β-Myrcene	66
4	Rajapuri	β-Myrcene	50
5	Totapuri	β-Myrcene	51
		(*Z*)-Ocimene	29
6	Langra	δ-3-Carene	82
7	Lili	δ-3-Carene	58
		(*Z*)-Ocimene	23
8	Maya	δ-3-Carene	67
9	Osteen	δ-3-Carene	70
10	Dasheri	Limonene	84
11	Alphonso	(*Z*)-Ocimene	88
12	Kesar	(*Z*)-Ocimene	81
13	Ratna	(*Z*)-Ocimene	45
14	Sindhu	(*Z*)-Ocimene	38
15	Badami	(*E*)-Ocimene	81
16	Villai Kolumban	4-Carene	68
17	Goamankur	β-Selinene	43
18	Makaram	β-Selinene	46
		(*E*)-Ocimene	37

Group of seven cultivars, each having more proportion of sesquiterpene hydrocarbons than monoterpene hydrocarbons in the aroma (Fig 2b) could also be distinguished in the PCA. From this group, Goamankur, Makaram and Musharad contributed to fourth PC with negative values that were notably high than the contributions by any other cultivar. In the similar situation, Gopta of Navasari, Pairi, Sabja and SB Chausa contributed to the fifth PC with high positive loading. In the PCO, except Pairi, all these cultivars showed positive contributions to the second axis.

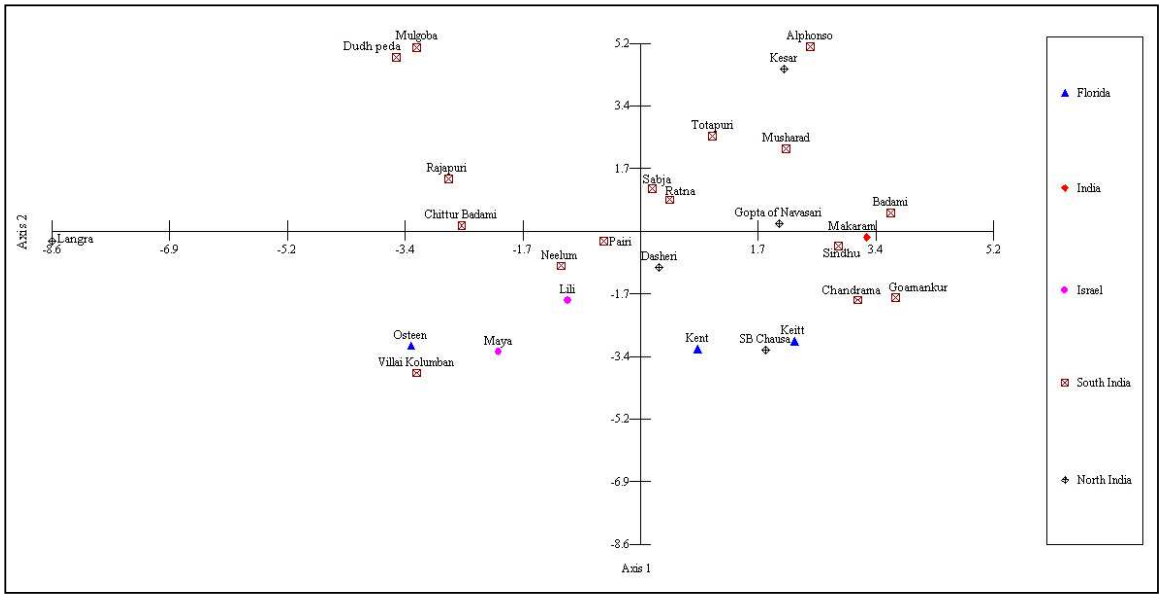


Fig 3a. Scatter plot of principle coordinate analysis (PCO) performed using Euclidean distance measure over the quantitative GC data, for 27 mango cultivars.

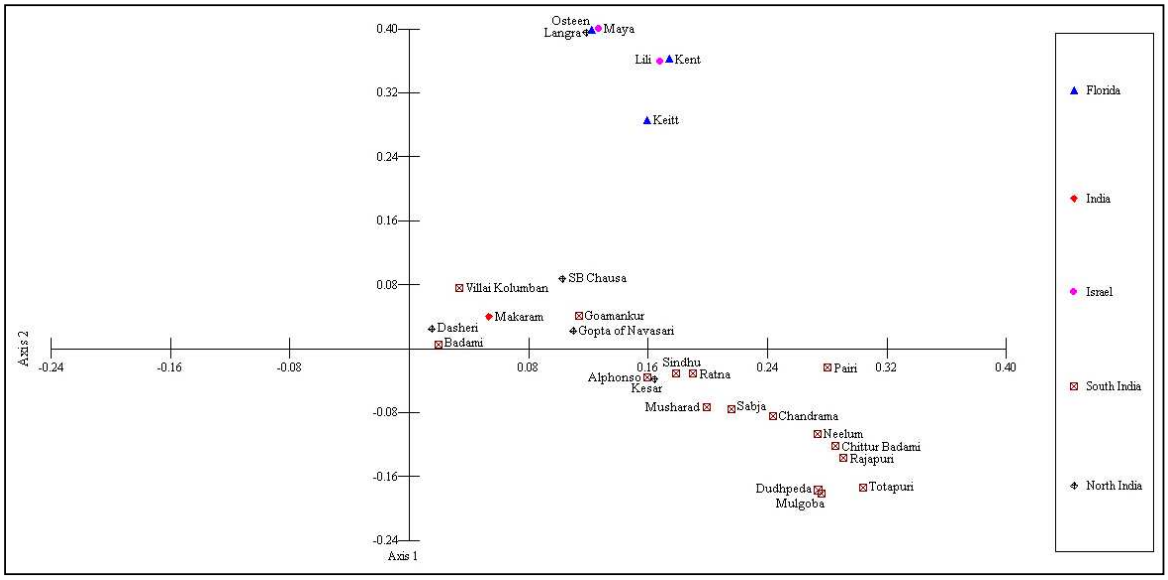


Fig 3b. Scatter plot of standardized principle components analysis (PCA), based on the quantitative GC data, for 27 mango cultivars.

In the PCO based on transposed data, more than 70% variation was represented by first three axes; however, these three axes were strongly influenced by the compounds that had high quantity in one or more cultivars. These compounds

were δ-3-carene, (*Z*)-ocimene, (*E*)-ocimene, β-myrcene, limonene and β-caryophyllene. Cultivar specific or less common compounds, even if they were at lower quantities, contributed to the first axis with low values as compared to the first mentioned category of compounds. Rest of the compounds did not contribute significantly to any of the axes. On the other hand, in the PCA of transposed data, it took nine PCs to cover 70% variation. Here, cultivar specific or less common compounds such as furaneol, *allo*-ocimene, (*Z*)-2, 6-dimethyl-3, 5, 7-octatriene-2-ol and all lactones made prominent contribution to the first PC. The second and third PC covered the variation by high concentration volatiles. Rest of the compounds, irrespective of being less common and/ or scanty, contributed evenly to the remaining axes.

Discussion

Researchers have pointed that there can hardly be a typical chemical formulation to be known as mango flavor (Wilson et al, 1990; Pino and Mesa, 2006). Indeed, the information on this has revealed that combinations in over 300 different volatiles pertaining to various chemical classes characterize the aroma of various mango cultivars (Pino and Mesa, 2006). Thus, mango flavor is a complex commodity, where the constituents vary qualitatively as well as quantitatively by cultivar.

Gross quantitative variations

The variations in the aroma profiles of different mango cultivars start with the concentration of volatiles per unit fresh weight of the fruit. In Australian mangos this concentration varied from 1.4 to 10.9µg g^{-1} (Bartley, 1988). Pino et al (2005) reported quantitative variation in Cuban mangos in the range of 18 and 123µg g^{-1}. In Columbian mangos this amount was between 17 and 78µg g^{-1}. In the present set of

cultivars, the both noted for the lowest and the highest concentration of volatiles were Indian (Fig 1). Among non-Indian cultivars, we found Keitt having the lowest and Osteen having the highest concentration; however, both were intermediates in the broad range displayed by Indian cultivars. It is known that the aroma of Indian mangos is perceived as strong medicinal or turpentine aroma (Lizada, 1993); such perception is probably true in case of only few commercialized cultivars, as the extent of quantitative variation found in current set of Indian cultivars is the broadest among all the reported estimates. It should also be noted that such estimates are often a function of the subtleties of different extraction methods.

Dominance of various volatile classes in different cultivars

Variations among the cultivars are better known by their principle aroma components. For example, Alphonso mango aroma is known to constitute about 90% of terpene hydrocarbons, especially, (*Z*)-ocimene (Idstein and Schreirer, 1985); however, Alphonso is also known for qualitative contribution from a series of lactones, the odorants characteristic for this cultivar (Idstein and Schreirer, 1985; Wilson et al, 1990). Aromas of Australian mango, Kensington Pride and Sri Lankan mangos Parrot and Willard were abundant with α-terpinolene (MacLeod and Pieris, 1984; Lalel et al, 2003). African cultivar Smith and Floridian cultivars, Keitt, Kent and Tommy Atkins were known to have high concentration of δ-3-carene (MacLeod and Snyder, 1985; Olle et al, 1997; Lalel et al, 2003; Pino et al, 2005). Large number of Colombian and Cuban mangos also showed dominant presence of δ-3-carene, α-pinene, α-phellandrene and terpinolene (Pino et al, 2005; Quijano et al, 2007). Our analysis ensured such a trend among mango cultivars, as the principle aroma components were detected in 18 cultivars. Distinction between the principle

compounds of Indian and non-Indian cultivars points towards the selection criteria that the cultivars might have faced during their domestication.

Overall, monoterpene hydrocarbons have been reported to be the principle and abundant odorants in numerous mango cultivars. As a blend, volatiles from this class of compounds produce green, herbaceous, lemon, orange, musty and sweet character (Table 1) and many times are the sole representatives of mango aroma.

Present study adds to the scenario of monoterpene dominance in mango (Fig 2a) to suggest that, the diversity of mango cultivars can be used as a monoterpene bank that would be useful for flavor and fragrance industry. Though in fruit, these compounds are known as aroma constituents, by their broad spatial occurrence in plants, they are better acknowledged as defense chemicals against insect herbivores (Chapter 1). Under such circumstances, their diversity can also be exploited for understanding their biogenesis for the possible application in plant-herbivore interactions.

To the best of our knowledge, this work is the first, to report sesquiterpene dominant mango cultivars (Fig 2b). All seven such shared Indian origin wherein, four were South-Indian (Goamankur, Musharad, Pairi and Sabja), two North-Indian (Gopta of Navasari and SB Chausa) and one of unknown Indian origin (Makaram). In other 20 cultivars, though sesquiterpenes were relatively less abundant, their absolute concentrations and diversity were high. These compounds have important role in mango aroma as they add woody, spicy, oily and waxy characters (Table 1). Because they are the second big group of aroma volatiles in mango, based on their relative dominance observed in the present set of cultivars, we propose to classify mango cultivars as per the two chemotypes that are mentioned above.

Though terpene hydrocarbons dominated mango aromas, their derivatives (oxygenated) were qualitatively as well as quantitatively less common among mango cultivars. Previous studies also revealed this fact in case of Alphonso, Baladi (Engel and Tressl, 1983), Jaffna, Willard, Parrot (Macleod and Pieris, 1984), Kensington Pride (Lalel et al, 2003) and several Colombian (Quijano et al, 2007) cultivars. Thus, these compounds remain the minor flavorants in mango.

As compared to mono- and sesquiterpene hydrocarbons, other odorants are known to occur at lower quantities in different mango cultivars. Aldehyde is the class that follows terpenes in concentration. These are produced by lipid peroxidation and similar to mono- and sesquiterpenes, aldehydes also contribute to the chemical defense of the plants. In Sri Lankan Jaffna, aldehydes constituted for ~4% of the aroma blend and in Venezuelan mango (McLeod and Troconis, 1982) they constituted for ~17%. In the present set of cultivars we have reported similar range. Pino et al, (2005) discovered aldehydes as common components of Cuban mangos and stated that they impart fresh, grassy and fatty-green odor to the mangos.

Instantly after production, aldehydes are often derivatized to alcohols and esters (Schreirer, 1984; Matsui, 2006). Both these are the important classes of aroma volatiles in mango. Owing to the extraction method, we did not detect any aldehyde-derived esters in the current experiment; however several alcohols were found in the present array of cultivars. Most of the reports suggest that alcohols contribute with quite lower number and quantities to the mango aroma; present study supports such observation.

Lactones constitute another important class of flavorants. These compounds are known from apricot, peach, nectarine and coconut. They are responsible for the peach like, sweet and fruity character of the blend. In fact, these are the compounds

that impart the characteristic fruity smell in some mango cultivars like Alphonso. Wilson et al (1990) stated that lactones can be detected at their extremely low concentrations; therefore even on being quantitatively minor contributors, their impact on the overall flavor character is high. Idstein and Schreirer (1985) detected 14 lactones from Alphonso mango which is the highest number of these compounds known from any single fruit (Wilson et al, 1990). Pino et al (2005) and Quijano et al (2007) have also reported few lactones from Cuban and Colombian cultivars. In the present study, we could detect lactones in 18 of 27 cultivars; thus, it appeared that they were uncommon among mango cultivars and could probably help in classifying the large diversity of mangos based on their presence and absence. Furthermore, presence and stability of lactones has been evaluated in canned mango products (Hunter et al, 1974); thus, their utility has been proved for the mango flavor industry.

Ordination and cultivar relationships

PCA scatter plot showed better resolved structure in the scatter plot as compared to the one showed by PCO. This might be because, the results by linear measures of distance such as Euclidean, tend to be overshadowed by the outliers or extremes. Though quantitative dominants are the indispensable characters, in such cases they mask the otherwise reasonable effect of other characters and polarize the plot. In the alternative way, such data can be transformed to a binary form to emphasize only the presence and absence of character states; however, this flattening of data will be as drastic as the polarization by Euclidean methods. PCA provides a solution where one can use correlation matrix instead of a distance matrix; this brings ‘normalization or standardization’ effect in the data. We utilized this attribute to process the current data which was quantitative, but was immoderately variable to be handled by linearity based algorithms. Indeed, this featured in PCA (Fig 3b), where it

brought a notable structure in the scatter by resolving the Indian and non-Indian cultivar groups. In all, this group formation could be attributed to the attempt of data mining by refinement in the procedure of ordination.

Geography based grouping among the mango cultivars has already been proved using the DNA marker data (Chapter 2; Schnell et al, 1995; Lopez-Valenzuela et al, 1997; Pandit et al, 2007a). Current analysis imparts the functional attribute to this finding and suggests that such grouping might be a result of long selection process. It can be inferred that the selection criteria for the aroma of non-Indian and Indian cultivars had been different over the years. Secondly, as the germplasm transfer among these pools was probably not efficient and experienced inbreeding. High flavorant diversity that was shared by the cultivars from both the pools, assortment of odorants at the quantitative levels and extensive data mining efforts that were required consequently to ascertain such grouping indicate that the separation events have recent history and might be still underway.

Karihaloo et al (2003) and Ravishankar et al (2004) reported the isolation within Indian cultivars as North- Indian and South- Indian using the RAPD markers; however, ISSR markers did not reveal such grouping (Chapter 2; Pandit et al, 2007). Though, not as prominent as that between Indian and non-Indian cultivars, present analysis portrays such grouping and suggests that the selection criteria for the volatile blend of mangos might also be different in these two parts of India.

Emergence of conserved but small operational taxonomic units (OTUs) from this analysis is similar to that reported by Pandit et al (2007), using ISSR markers; this supports the postulate of recent domestication of mango. Incongruence about the member cultivars of OTUs in these two analyses poses the need for further analysis in this direction. However, it also points that the cultivar selection was mostly based on

chance and, the genetic and functional attributes were not considered simultaneously during this selection.

Analyses on transposed datasets revealed that the grouping among the cultivars is a function of the qualitative as well as quantitative differences between the characters. Compounds that influenced quantitatively were δ-3-carene, (*Z*)-ocimene, (*E*)-ocimene, β-myrcene, limonene and β-caryophyllene, whereas those had a qualitative contribution were furaneol, *allo*-ocimene, (*Z*)-2, 6-dimethyl-3, 5, 7-octatriene-2-ol and all lactones. Most of these have been principle compounds in variety of fruits including mango. Thus, this analysis also highlights the substantial contribution of these compounds to the mango aroma as well as to its selection process. The compounds not having significant contribution must be studied for their role and their detection limits.

In summary, mango cultivars differ in the total concentration of volatiles that they produce, in the qualitative and quantitative composition of these volatiles and the principle volatile components. Mono- and/ or sesquiterpene hydrocarbons are the major constituents of Indian as well as non-Indian cultivars and these cultivars can be classified in mono- or sesquiterpene dominating groups. On the quantitative basis, δ-3-carene, (*Z*)-ocimene, (*E*)-ocimene, β-myrcene, limonene and β-caryophyllene are the major aroma compounds in mango, whereas furaneol, *allo*-ocimene, (*Z*)-2, 6-dimethyl-3, 5, 7-octatriene-2-ol and all lactones have qualitative importance. Indian and non-Indian cultivars differ in their volatile profiles and indicate that they might have faced different selection criteria (for their aroma) in the different geographical regions.

CHAPTER 4

Profiling of Major Flavorants through the Development and Ripening of Alphonso Fruit

Work described in this chapter has been communicated to *Food Chemistry*.

Introduction

Alphonso is the most popular and most exported mango [*Mangifera indica* L. (Anacardiaceae)] cultivar of India (Tharanathan et al, 2006; Vasanthaiah et al, 2006). This fruit is blessed with attractive color, ample, sweet, low fiber containing pulp and long shelf life. Ripe Alphonso fruits are popularly used in the processed and canned foods. Similarly, the raw fruits of Alphonso are also used in the food products like pickles, tarts, curries and salads. However, the market success of this cultivar can be principally attributed to its flavor. Cut as well as uncut fruit of this cultivar emits an alluring blend of volatiles. Therefore, it is the flavor of choice for the mango lovers all over the world.

Many studies have tried to reveal composition of ripe Alphonso aroma (Bandyopadhyay and Gholap, 1973a, b; Engel and Tressl, 1983; Idestein and Schreier, 1985; Gholap et al, 1986; Wilson et al, 1990); whereas, a couple of attempts have been made to know the composition of raw mango (Bandyopadhyay and Gholap, 1973a; Gholap and Bandyopadhyay, 1977; Gholap et al, 1986). On the basis of these studies as well as by the general organoleptic perception it is understood that, aroma of ripe Alphonso retains the odor character of raw fruit with the dominant addition of sweet and fruity flavor. However, the exact nature of this aroma composition change during development and ripening is yet to be systematically studied. Such information would be helpful for mango growers as the temporal and spatial occurrence profiles of volatiles may indicate the right time points of harvesting maturity and ripeness (Almora et al, 2004).

This type of information will also help in solving the other problems related to this cultivar. These problems mainly include, occurrence of spongy tissue, a physiological disorder, which is a result of preharvest climatic perturbations and

cultivation locality dependent variation in fruit quality, especially, flavor (Om Prakash, 2004; Ravindra and Shivashankar, 2004; Vasanthaiah et al, 2006). Due to the later, such a near-ideal flavor is not uniform over the widespread cultivation localities in India so, its cultivation is concentrated in Kokan (or Konkan), the 700 km long, narrow coastal belt of western India. Even within this belt, northern, central and southern Alphonso mangos taste and smell different. Such a variation caused by the differences in the pre-harvest environments, is commonly observed in several fruits including mangos (Romani et al, 1983; Wright and Harris, 1985; Hofman et al, 1997; Paull and Chen, 2000). To negotiate with such situation, a comprehensive experiment must be designed, wherein the difference in the zonal microclimates and respective fruit qualities must be assessed concurrently. Such an approach would uncover the secrets underlying these complex phenomena and also, the biological interactions with environment. However, in case of Alphonso, the reference information regarding the biochemistry of development and ripening, which is a prerequisite for such an experiment, is barely available. Therefore, current work aims at discovering the baseline chemistry of Alphonso development and ripening. For this work, from numerous available features, we have selected aroma of this fruit as a parameter because, it can be precisely characterized and most of its constituents are characterized for their biosynthesis, therefore any information regarding these constituents will glean the metabolomics of the fruit.

For the present work we have chosen Alphonso mango, grown at Deogad (South Kokan, Maharashtra, India) as, it is known to be the best flavored and most demanded mango (Wikipedia, 2008). In future, we intend to extend this study for central as well as north Kokan grown Alphonso mangos in order to negotiate with the microclimate related problems. In this analysis, along with those from the developing

and ripening fruits, volatiles from leaf and flower tissues of cultivar Alphonso were also analyzed in order to understand plant's dynamics of volatile production. Fruits of cultivar Sabja, which are described as mild and unpleasant smelling, were used for comparison with Alphonso fruits. Sabja is a local, chance-selected seedling that is rarely cultivated and is not commercialized.

In addition to the metabolomics view, to broaden the scope and understanding of this work, we have also described the aroma variations in terms of aroma character of different compounds and their chemical classes. The contribution of each odorant to the blend is usually measured using the ratio of its quantity to its odor detection threshold (Chen et al, 2007); in this regard, the impact of different aroma components in the flavors of selected tissues is also discussed.

Materials and methods

Plant material

All the tissues of cultivar Alphonso used in the present analysis were collected from the orchards at Deogad (Maharashtra, India) and those of cultivar Sabja were collected from the orchards at Vengurle (Maharashtra, India). Alphonso fruit takes about 90 days to mature after the fruit-set and further 15 to 20 days to ripe at 28ºC. Inflorescences were tagged in the respective orchards to ensure the pollination date and the fruits of 5, 15, 30, 60 and 90 days after pollination (DAP) and of 2, 5, 10, 15 and 20 days after harvesting (DAH) (five intervals each from the developing and ripening mangos) were collected and used for the present analysis. Along with the fruit tissues, leaf and flower tissues were also included in the analysis making total 12 samples from cultivar Alphonso. As, Sabja fruit ripens within two days after harvesting, only two stages, mature unripe and ripe were selected for the analysis.

Volatiles extraction

Extraction procedure for all 14 tissues was the same. 10g tissue was ground to fine powder in liquid nitrogen and extracted for 1hr at 28ºC with 40ml dichloromethane. While mixing the crushed tissue to the solvent, α-terpinene (100µg), tolualdehyde (60µg) and methyl phenyl acetate (50µg) were added as internal standards. The supernatant was washed with anhydrous sodium sulphate and concentrated to 1ml using vacuum-rotary evaporator. After overnight incubation at -20ºC the extracts were centrifuged at 10,000 rpm at 4ºC for 15 min to pellet out high molecular weight lipids.

Gas chromatography- Flame ionization detector (GC-FID) and Gas chromatography- Mass spectrometry (GC-MS) analyses

Analyses were carried out using Clarus 500 (Perkin Elmer, USA) gas chromatograph equipped with Rtx-5MS (Restek, USA) capillary column (30m x 0.32mm i.d. x 0.25µm film thickness); column temperatures were programmed from 40ºC for 5 min, raised to 220ºC at 10ºC/min and held isothermal for 5 min. Injector and detector temperatures were 200 and 250ºC, respectively. Helium was used as a carrier gas at a flow rate 1 ml/min.

Mass spectra were obtained using Clarus 500 (Perkin Elmer, USA) gas chromatograph- mass spectrometer at 70 eV with a scan time of 0.2 sec for m/z 30-300 under the GC conditions same as those applied in GC-FID analysis. The retention indices for all the peaks were determined using a series of *n*-paraffins (C_5- C_{22}) (Table 1). Compound identification was carried out by comparing acquired mass spectra with those of authentic external standards and those stored in NIST/ NBS mass spectral library. In addition, to confirm the identification, the retention indices of the predicted compounds were compared with those of authentic external standards and also with

those reported earlier in NIST/ EPA/ NIH mass spectral library (USA) (data version NIST 05, software version, 2.0d).

Quantification was carried out by internal standard method, where concentrations of different volatiles were normalized with those of respective internal standards.

Statistical analysis

All the statistical analyses were carried out using Systat statistical software (version 11, Richmond, CA, USA). Changes in quantity of each volatile through the selected tissue set, were assessed by ANOVA in different combinations of tissues consisting: 1) all 14 tissues 2) ripening Alphonso fruits (90DAP to 20DAH) and 3) raw and ripe Sabja fruits; such batch wise processing was necessary for characterizing the variations within the entire set as well as within each of these subsets, independent of the others. Cumulative concentration was calculated for each chemical class, for each tissue and ANOVA was also performed to compare all 14 tissues on the basis of quantitative changes in these chemical classes. Least significant differences (Fisher's protected LSD) were calculated at level $p \leq 0.05$, following a significant F-test. Any compounds detected below the quantitation limits were not considered in the present statistical analysis; however their quantities were denoted as trace (T) in table 1.

Results

Fifty-six different volatile components were identified and profiled from the above mentioned set of 14 tissues (Fig 1) (Table 1). Alphonso leaf contained 32, flowers 29 and fruit (inclusive of all developing and ripening stages) contained 45 volatile flavorants; Sabja fruits (raw as well as ripe) showed presence of 32 volatile compounds. Thirty-two volatiles produced each, by leaf, ripe (15DAH) and over-ripe

(20DAH) stages of Alphonso fruit, was the maximum number produced by any single tissue. This analysis also revealed that, 45 compounds were produced only by the fruit (developing and/or ripening Alphonso or Sabja); whereas, two compounds each were specifically synthesized by flower and leaf of Alphonso. When Alphonso and Sabja were compared for the presence of these compounds in any of their considered tissues, 44% (25) of the compounds found common to both these cultivars; of the remaining 56% (31) compounds 43% (24) exclusively belonged to Alphonso, whereas 13% (7) specifically occurred in Sabja.

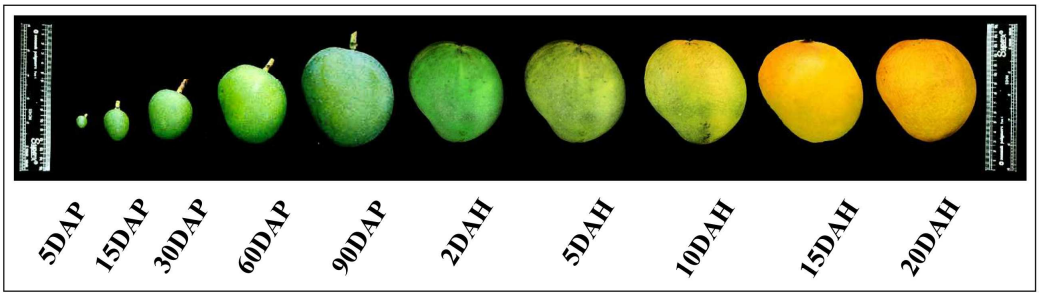


Fig 1. Development and ripening of Alphonso mango. 5DAP to 90DAP is the period of development where, DAP≡ days after pollination and 90DAP≡ harvesting maturity. 2DAH to 20DAH is the ripening period, where DAH≡ days after harvesting, 15DAH≡ exact ripe stage and 20DAH≡ overripe fruit.

Among the sampled Alphonso fruit tissues, the highest concentration of volatiles was detected in 5DAP (15665µg g^{-1}) and the lowest in 2DAH (62µg g^{-1}) (Fig 2a); ripe fruits (15DAH) (966µg g^{-1}) had more than ten folds higher content of volatile odorants than the raw fruits (90DAP) (94µg g^{-1}). Volatile concentration in flowers (1850µg g^{-1}) was more than that in leaf (842µg g^{-1}). Raw Sabja fruits showed more content of aroma compounds (212µg g^{-1}) than raw Alphonso fruits. Ripe Sabja retained only 60% (130µg g^{-1}) concentration of volatiles from its raw form; this concentration was also about seven fold lower than that in ripe Alphonso.

Aldehydes

Aldehydes detected in this analysis were, (*E*)-2-hexenal, nonanal and hexadecanal. These three compounds showed entirely different trends of their quantitative presence during the maturation and ripening of mango (Table 1). (*E*)-2-hexenal, the C6 green leaf volatile (GLV), was found high in early developmental stages (highest in 15DAP fruits); it could not be detected in the late maturation and early ripening stages. However, it reappeared in the ripe and over-ripe mango in small amounts. (*E*)-2-hexenal was also present in leaf and flowers in small quantities. Although, nonanal was present in all sampled tissues, it was more in the ripening fruits as compared to the developing ones. Alphonso flowers showed the highest abundance of this compound. Hexadecanal was not detected in the leaves, flowers and in developing fruits; however, it appeared in highest amount in the mature fruit and gradually decreased while ripening. In Sabja, all three aldehydes decreased during the process of ripening. The highest cumulative amount of aldehyde was noted in raw Sabja fruit (13.7µg g^{-1}), followed by that in 15DAP (8.0µg g^{-1}) and (7.4µg g^{-1}) 90DAP Alphonso fruits, respectively (Fig 3).

Alcohols

All the alcohols detected from the present array of tissues belonged to the class of C6 GLVs. 1-Hexanol and (*E*)-2-hexen-1-ol both, were exclusively produced by the flowers. 2-hexanol was found in all the analyzed tissues; in flowers, its amount was considerably high followed by that in 5DAP, 15DAP and 30DAP fruits. The lowest amount of 2-hexanol was noted in ripe Alphonso fruits (15DAH). Like other alcohols, maximum quantity of (*Z*)-3-hexen-1-ol was marked in flowers; in Alphonso fruits it was detected only in 5 and 15DAP stages. In raw and ripe Sabja fruits, (*Z*)-3-

hexen-1-ol concentration did not vary. Overall, flowers showed high presence of alcohols as compared to the other tissues (Fig 3).

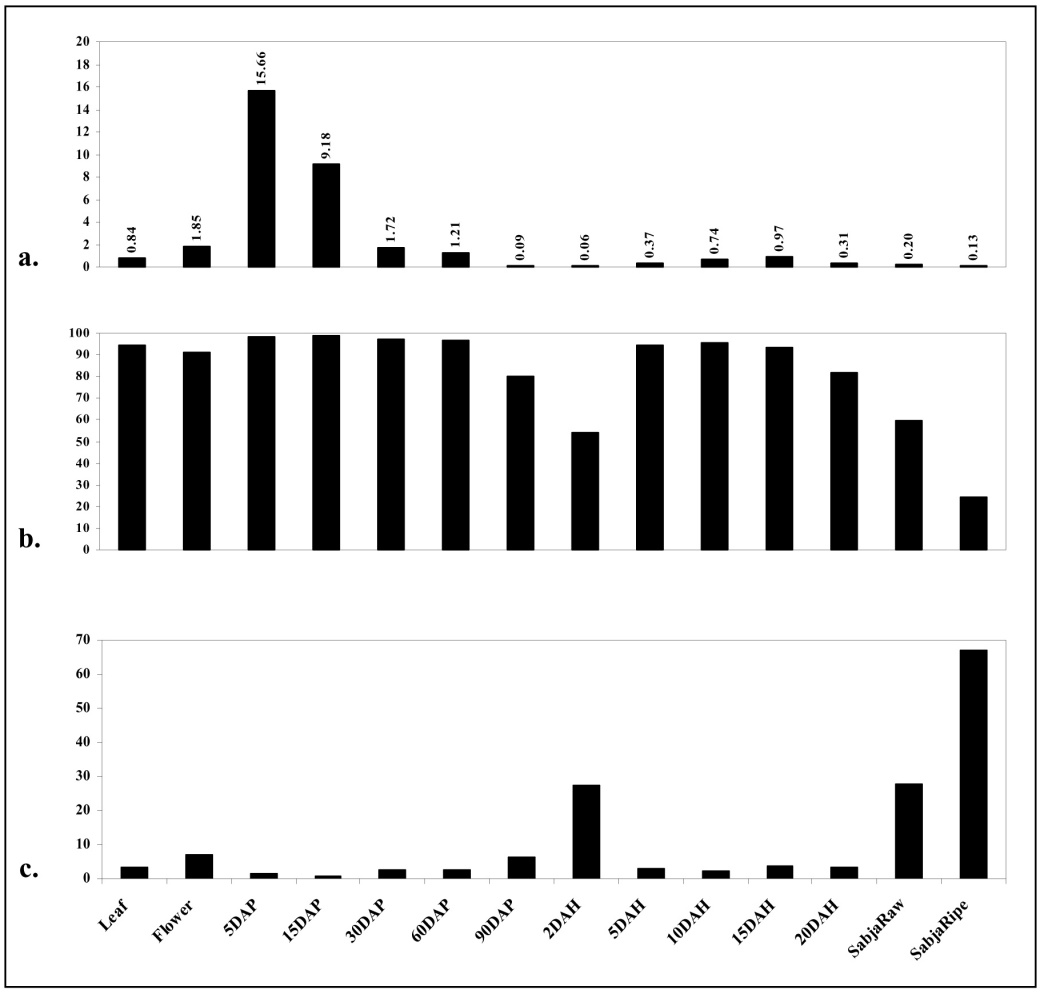


Fig 2a. Total volatiles (mg g^{-1}) in different mango tissues; **b.** percent contribution of monoterpens and **c.** percent contribution of sesquiterpens to different mango tissues. The variety of flavorants identified in the current analysis could be broadly classified as alcohols, aldehydes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, lactones, ketones, non-terpene hydrocarbons and miscellaneous (Table 1). Among these 56 compounds, monoterpene hydrocarbons were numerically dominant (14), followed by eight lactones and seven sesquiterpene hydrocarbons.

Table 1. Volatile compounds and their quantities (µg g^{-1}) in 14 different mango tissues (leaf, flower, developing and ripening fruits of cultivar Alphonso as well as mature and ripe fruits of cultivar Sabja). For each row, the values followed by the same alphabet do not differ significantly from each other, where the significance of comparison ($p \leq 0.05$) among all the 14 tissues is represented by small alphabets; that among the post-harvest stages of Alphonso is represented by the capital letters; and between raw and ripe Sabja is denoted by Greek letters.

	Compound	KI_{calc}	KI_{rep}	Leaf	Flower	5DAP	15DAP	30DAP	60DAP	90DAP	2DAH	5DAH	10DAH	15DAH	20DAH	SRw	SRp
	Aldehydes																
1.	(*E*)-2-Hexenal	861	854	0.21^{ab}	1.59^{c}	0^{a}	8.03^{f}	2.54^{e}	1.39^{d}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0.45^{bC}	0.29^{abB}	$1.44^{d\beta}$	$0.92^{c\alpha}$
2.	Nonanal	1104	1101	2.95^{d}	3.97^{e}	1.18^{b}	T	0.50^{ab}	0.22^{ab}	0.12^{abA}	0.66^{bB}	0.69^{bB}	1.84^{cC}	T	0.47^{abB}	$0.71^{b\beta}$	$0.08^{ab\alpha}$
3.	Hexadecanal	1820	1819	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	7.27^{dC}	4.03^{cB}	1.64^{bA}	1.00^{abA}	0.89^{abA}	0.22^{aA}	$11.52^{e\beta}$	$3.51^{c\alpha}$
	Alcohols																
4.	2-Hexanol	808	800	0.28^{ab}	0.71^{d}	0.59^{cd}	0.66^{cd}	0.57^{c}	0.31^{ab}	0.33^{abBC}	0.41^{bC}	0.34^{bBC}	0.27^{abA}	0.21^{aA}	0.29^{abA}	$0.31^{ab\alpha}$	$0.36^{b\alpha}$
5.	(*Z*)-3-Hexen-1-ol	865	857	0.96^{b}	3.33^{e}	1.41^{c}	2.65^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0.60^{b\alpha}$	$0.96^{b\beta}$
6.	(*E*)-2-Hexen-1-ol	874	861	0^{a}	0.61^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
7.	1-Hexanol	876	871	0^{a}	3.15^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
	Monoterpene hydrocarbons																
9.	Tricyclene	924	926	0.64^{c}	1^{d}	0.24^{b}	0.30^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
10.	α-Thujene	931	931	1.13^{c}	2.32^{d}	0.54^{b}	0.63^{b}	0^{a}	0.13^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
11.	α-Pinene	937	939	47.83^{c}	91.55^{d}	21.23^{b}	18.95^{b}	2.30^{a}	2.33^{a}	0.14^{aA}	0.35^{aB}	1.20^{aD}	1.73^{aE}	2.95^{aF}	0.79^{aC}	$0.56^{a\beta}$	$0.08^{a\alpha}$
12.	Camphene	952	954	5.90^{c}	10.01^{d}	2.04^{b}	2.34^{b}	0.27^{a}	0.31^{a}	0^{aA}	0^{aA}	0.27^{aB}	0.29^{aB}	0.32^{aB}	0^{aA}	$0^{a\alpha}$	$0^{a\alpha}$

	Compound	KI_{calc}	KI_{rep}	Leaf	Flower	5DAP	15DAP	30DAP	60DAP	90DAP	2DAH	5DAH	10DAH	15DAH	20DAH	SRw	SRp
13.	Sabinene	977	976	0.93^{b}	0^{a}	1.30^{c}	1.21^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
14.	β-Pinene	980	979	22.86^{c}	37.49^{d}	7.27^{b}	7.29^{b}	0.94^{a}	1.11^{a}	0.07^{aA}	0.05^{aA}	0.55^{aC}	0.69^{aD}	0.99^{aE}	0.26^{aB}	$0^{a\alpha}$	$0^{a\alpha}$
15.	β-Myrcene	994	991	9.56^{b}	27.18^{c}	91.05^{f}	71.52^{e}	12.26^{bc}	19.13^{c}	0.60^{aA}	0.38^{aA}	5.95^{abD}	5.14^{abC}	6.79^{abE}	1.71^{abB}	$118.6^{g\beta}$	$28.80^{d\alpha}$
16.	δ-3-Carene	1013	1011	0.08^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0.89^{e}	0^{aA}	1.28^{fD}	0.08^{bB}	0.55^{dC}	0.04^{abAB}	0.09^{bB}	$2.01^{g\beta}$	$0.16^{c\alpha}$
17.	*p*-Cymene	1029	1027	1.46^{bc}	3.18^{e}	1.77^{c}	2.96^{e}	1.79^{c}	1.50^{bc}	0.90^{abA}	1.02^{abA}	1.27^{bC}	2.14^{dD}	1.07^{abB}	1.05^{abB}	$1.16^{b\beta}$	$0.73^{a\alpha}$
18.	Limonene	1034	1029	7.37^{d}	12.15^{e}	2.95^{c}	2.67^{c}	0.52^{b}	0.50^{b}	0.14^{aA}	0.31^{abBC}	0.29^{abB}	0.48^{bC}	0.41^{abC}	0.40^{abBC}	$0.66^{b\beta}$	$0.23^{ab\alpha}$
19.	(*Z*)-Ocimene	1043	1050	659.8^{a}	1446^{a}	14818^{c}	8602^{b}	1579^{a}	1096^{a}	70.18^{aB}	27.34^{aA}	328.9^{aD}	664.9^{aE}	852.3^{aF}	240.1^{aC}	$0.48^{a\alpha}$	$1.17^{a\beta}$
20.	(*E*)-Ocimene	1054	1037	31.56^{bc}	53.59^{cd}	474.1^{f}	352.3^{e}	68.36^{d}	46.24^{c}	3.06^{aB}	1.32^{aA}	13.62^{abD}	28.29^{bE}	31.08^{bcF}	8.67^{aC}	$2.39^{a\beta}$	$0.49^{a\alpha}$
21.	β-Terpinene	1064	1071	2.14^{b}	2.07^{b}	0^{a}	0^{a}	0^{a}	T	0^{aA}	0.14^{aB}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	$0^{a\alpha}$	$0^{a\alpha}$
22.	4-Carene	1092	1084	2.57^{c}	0^{a}	T	0^{a}	0^{a}	T	0^{aA}	1.26^{bB}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	$1.14^{b\beta}$	$0^{a\alpha}$
23.	*allo*-Ocimene	1135	1142	1.75^{b}	3.17^{c}	12.11^{d}	12.46^{d}	3.25^{c}	1.93^{bc}	0.24^{aA}	0.10^{aA}	0.98^{abB}	3.38^{cC}	3.78^{cC}	1.28^{abB}	$0^{a\alpha}$	$0^{a\alpha}$
	Sesquiterpene hydrocarbons																
24.	β-Caryophyllene	1434	1428	17.70^{b}	82.63^{e}	143.4^{f}	51.73^{d}	27.53^{b}	19.80^{b}	3.97^{aA}	11.16^{abC}	6.75^{aB}	11.08^{abC}	22.47^{bD}	6.42^{aB}	$26.30^{b\alpha}$	$38.88^{c\alpha}$
25.	α-Guaiene	1450	1440	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$2.40^{c\beta}$	$1.56^{b\alpha}$
26.	Humelene	1469	1454	9.67^{b}	43.89^{d}	74.93^{e}	27.29^{c}	14.28^{b}	10.10^{b}	1.88^{aA}	5.71^{abC}	3.98^{abBC}	5.01^{abC}	12.33^{bC}	3.30^{aB}	$13.24^{b\alpha}$	$21.62^{c\beta}$
27.	Germacrene D	1498	1485	0^{a}	1.76^{b}	2.10^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.65^{a}	0.40^{a}	$5.0^{c\alpha}$	$13.02^{d\beta}$
28.	Germacrene B	1512	1511	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$3.33^{b\alpha}$	$5.11^{c\beta}$
29.	δ-Guaiene	1521	1505	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$5.46^{c\beta}$	$2.93^{b\alpha}$

	Compound	KI_{calc}	KI_{rep}	Leaf	Flower	5DAP	15DAP	30DAP	60DAP	90DAP	2DAH	5DAH	10DAH	15DAH	20DAH	SRw	SRp
30.	δ-Cadanine	1536	1523	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$3.36^{b\alpha}$	$4.06^{c\alpha}$
	Oxygenated monoterpenes																
31.	*cis*-β-Terpineol	1073		0.66^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
32.	(*Z*)-β-Terpineol	1102		0.85^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
33.	Linalool	1103	1107	0^{a}	8.09^{d}	3.57^{c}	3.59^{c}	0.53^{b}	0.18^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0^{a\alpha}$
34.	Borneol	1175	1169	1.38^{c}	3.18^{d}	0.61^{b}	1.23^{c}	0^{a}	0^{a}	0^{aA}	0.16^{aB}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	$0^{a\alpha}$	$0^{a\alpha}$
35.	Elemol	1552	1550	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0^{a\alpha}$	$0.39^{b\beta}$
	Oxygenated Sesquiterpenes																
36.	Caryophyllene oxide	1629	1606	0^{a}	1.39^{c}	1.65^{d}	1.45^{cd}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	0.23^{abB}	0^{aA}	0^{aA}	$0.38^{b\alpha}$	$0.59^{b\alpha}$
37.	τ-Muurolol	1659	1641	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0.44^{b\alpha}$	$1.59^{c\beta}$
38.	α-Cadinol	1673	1653	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	$0.57^{b\alpha}$	$1.21^{c\beta}$
	Lactones																
39.	γ-Butyrolactone	921	915	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0.18^{bB}	0.19^{bB}	0.77^{cC}	1.30^{dD}	$0^{a\alpha}$	$0.17^{c\beta}$
40.	α-Methylbutyrolactone	959	973	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0.14^{bB}	0.57^{cC}	$0^{a\alpha}$	$0^{a\alpha}$
41.	γ- Hexalactone	1064	1056	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0.36^{bB}	2.42^{eE}	1.13^{dD}	0.61^{cC}	$0^{a\alpha}$	$0^{a\alpha}$
42.	δ-Hexalactone	1101	1163	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	1.65^{dD}	1.00^{cC}	0.45^{bB}	$0^{a\alpha}$	$0^{a\alpha}$
43.	γ-Octalactone	1268	1261	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0.15^{aA}	1.08^{cC}	1.53^{dD}	0.56^{bB}	$0^{a\alpha}$	$0^{a\alpha}$

	Compound	KI_{calc}	KI_{rep}	Leaf	Flower	5DAP	15DAP	30DAP	60DAP	90DAP	2DAH	5DAH	10DAH	15DAH	20DAH	SRw	SRp
44.	δ-Octalactone	1296	1268	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0.12^{bB}	0.35^{cC}	1.24^{eE}	0.44^{dD}	$0^{a\alpha}$	$0^{a\alpha}$
45.	γ-Decalactone	1485	1467	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	0.04^{bB}	0.05^{bB}	0.17^{cC}	$0^{a\alpha}$	$0^{a\alpha}$
46.	δ-Decalactone	1520	1494	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	0.14^{bB}	0^{aA}	$0^{a\alpha}$	$0^{a\alpha}$
	Ketones																
47.	Mesifuran	1067	1065	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	2.29^{bB}	10.77^{cC}	28.27^{dD}	$0^{a\alpha}$	$0^{a\alpha}$
48.	Furaneol	1082	1060	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0.35^{aA}	4.56^{bB}	4.75^{bB}	6.15^{cC}	$0^{a\alpha}$	$0.07^{a\beta}$
	Non-terpene hydrocarbons																
49.	Toluene	760	773	1.16^{c}	0.57^{a}	1.41^{d}	0.97^{bc}	1.50^{d}	0.76^{ab}	0.65^{aA}	0.55^{aA}	1.10^{bcC}	0.91^{bB}	1.09^{bcBC}	0.55^{aA}	$0.72^{ab\alpha}$	$1.16^{c\beta}$
50.	Tridecane	1300	1300	1.27^{f}	0^{a}	0.1^{b}	0^{a}	0^{a}	0.32^{c}	0.46^{dD}	0.36^{cC}	0.14^{bB}	0^{aA}	0^{aA}	0^{aA}	$0.53^{e\beta}$	$0.10^{b\alpha}$
51.	Tetradecane	1396	1400	2.12^{e}	1.11^{d}	0.32^{b}	2.20^{e}	0.31^{b}	0.92^{cd}	0.91^{cC}	0.83^{cC}	0.41^{bB}	0.16^{abA}	0.23^{bA}	0.40^{bB}	$0.34^{b\beta}$	T
52.	Hexadecane	1600	1600	1.16^{e}	0^{a}	0^{a}	0^{a}	0^{a}	0.90^{d}	0.76^{cdC}	0.38^{bB}	0.59^{cBC}	0^{aA}	0^{aA}	0^{aA}	$0.44^{bc\beta}$	$0^{a\alpha}$
53.	Heptadecane	1700	1700	1.17^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.27^{bC}	0^{aA}	0.59^{cD}	0^{aA}	0^{aA}	0.17^{bB}	0^{a}	0^{a}
54.	Octadecane	1800	1800	0.96^{d}	0.82^{d}	0^{a}	1.95^{e}	0.76^{d}	0.85^{d}	0.54^{cB}	0.65^{cdB}	0.85^{dC}	0.20^{abA}	0.20^{abA}	0.27^{bA}	$0.41^{bc\beta}$	$0.17^{ab\alpha}$
	Miscellaneous																
55.	4-Ethoxy ethylbenzoate	1543		0.65^{c}	0.61^{bc}	0.28^{ab}	2.12^{e}	0.33^{b}	0.36^{bc}	0.35^{bcA}	0.62^{cB}	0.98^{dC}	0.14^{abA}	0.17^{abA}	0.13^{abA}	$0^{a\alpha}$	$0^{a\alpha}$
56.	Unidentified compound	1600		0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{aA}	0^{aA}	0^{aA}	0^{aA}	5.76^{cC}	4.70^{bB}	$0^{a\alpha}$	$0^{a\alpha}$

Monoterpene hydrocarbons

Monoterpene hydrocarbons quantitatively dominated the volatile blend of all Alphonso tissues; except that in 90DAP (79%), 2DAH (53%) and 20DAH (81%) fruits, in all other tissues, these compounds comprised more than 90% of the aroma (Fig 2b). In raw Sabja, this class occupied 59%, whereas in ripe one it occupied 24% of the total volatile blend (Table 1). Of the 14 monoterpene hydrocarbons detected in this analysis, α-pinene, β-myrcene, limonene, (*Z*)-ocimene, (*E*)-ocimene and *p*-cymene were present in all the sampled tissues, including Sabja; whereas, β-pinene and *allo*-ocimene were present in all Alphonso tissues but absent in Sabja. No monoterpene hydrocarbon was found specific to Sabja. In Alphonso, (*Z*)- and (*E*)-ocimene quantitatively dominated the monoterpene hydrocarbon presence followed by β-myrcene. In Sabja, β-myrcene was the most abundant monoterpene hydrocarbon. Except δ-3-carene, which was detected in all the ripening stages of Alphonso, rest of the monoterpene hydrocarbons showed high abundance in the developing fruits as compared to that in the ripening fruits; this was also true for Sabja fruits. In Alphonso fruit, most of these volatiles were present in their highest amounts in the early developmental period (5 to 15DAP); they decreased gradually till the maturation period and again increased in the mid-ripening phase (Table 1); this profile was mainly observed for α-pinene, β-pinene, β-myrcene, limonene, (*Z*)-ocimene, (*E*)-ocimene, *allo*-ocimene and *p*-cymene. All these compounds were found in at least 5 fold higher concentrations in young fruits (5 and 15DAP) as compared to any other Alphonso fruit tissues. When compared among the ripening stages (90DAP to 20DAH), these compounds peaked in the ripe stage (15DAH) (Table 1). Among all the sampled tissues, γ-terpinene, α-thujene, α-pinene, β-pinene, camphene, *p*-cymene and limonene peaked in flowers, whereas sabinene, 4-carene and δ-3-carene were

completely absent. 4-carene was detected only in leaf, 5DAP, 60DAP, 2DAH and raw Sabja fruit tissues wherein, leaf showed about two folds more amount than the other two tissues. Seven monoterpene hydrocarbons were detected in Sabja; except (*Z*)-ocimene all others were found more in the raw fruits than in the ripe ones. In general, 5 and 15DAP fruits contained more than 5 fold high concentration of monoterpene hydrocarbons than that in any other tissue; it was also on the upper side in mature and ripe Alphonso fruits to that in mature and ripe Sabja, respectively (Fig 3).

Oxygenated monoterpenes

Monoterpene alcohols (oxygenated monoterpenes) *cis*-β-terpineol and (*Z*)-β-terpineol were detected only in the leaf tissue. Linalool and borneol were most abundant in Alphonso flowers as compared to other tissues. In fruit, these three increased from 5 to 15DAP and then again decreased gradually till maturation. Similar to C6 GLV alcohols, the cumulative abundance of monoterpene alcohols was the highest in flowers; they were absent almost in all the ripening stages of Alphonso and also in the raw as well as ripe Sabja fruits (Fig 3).

Sesquiterpene hydrocarbons

In the current set of tissues, sesquiterpene hydrocarbons constituted the next quantitatively dominant class to that of monoterpene hydrocarbons (Fig 2c). Of the seven sesquiterpene hydrocarbons detected, only three were present in Alphonso tissues; these were β-caryophyllene, α-humulene and germacrene-D. The first two were detected in all the tissues with the highest amount in 15DAP fruit, whereas germacrene-D was detected only in flower, 5DAP, 15DAH and 20DAH stages. These three compounds were high during the early development (5 to 30DAP) of Alphonso fruit and gradually decreased in the later stages. When compared among the ripening stages (90DAP to 20DAH), these compounds peaked in the ripe stage (15DAH)

(Table 1). This trend also remained the same in Sabja; however, other two sesquiterpene hydrocarbons, δ-cadanine and unidentified SQTP were specific to this cultivar. Rest two sesquiterpene hydrocarbons α- and δ-guaiene were also specific to Sabja; they were observed to decrease during ripening (Table 1). Collectively, the trends of mono- and sesquiterpene hydrocarbons appeared similar (Fig 3).

Oxygenated sesquiterpenes

In the group of oxygenated sesquiterpenes, caryophyllene oxide was detected in flowers, young fruits (5 and 15DAP), and later in 10DAH fruit. It was more in raw fruits than the ripe ones. It was also detected in raw and ripe Sabja fruits; however, its quantity did not vary significantly among these two stages. Rest of the oxygenated sesquiterpenes elemol, τ-muurolol and α-cadinol belonged to the class of sesquiterpene alcohols. These three occurred exclusively in Sabja fruits and were observed to increase during the process of ripening.

Lactones

Seven of the total eight lactones detected in this analysis, were found only in the ripening Alphonso fruits (Table 1). In the perfect ripe fruit (15DAH), all eight lactones were present; γ-octalactone was present in the highest amount as compared to the other seven. Butyrlactone, α-methylbutyrlactone and γ-decalactone concentrations were the highest in over-ripe (20DAH) fruit, whereas δ-hexalactone and γ-hexalactone peaked in 10DAH fruit. All other lactones were present in their highest concentration at 15DAH stage making it a tissue, containing the highest cumulative amount of these flavorants (Fig 3). Butyrolactone was the only one found in Sabja.

Non-terpene hydrocarbons

Non-terpene hydrocarbons were present in low amount (up to about 2µg g^{-1}) (Table 1). Toluene was detected in all the sampled tissues. Its concentration was high

Compound class	Leaf	Flower	5DAP	15DAP	30DAP	60DAP	90DAP	2DAH	5DAH	10DAH	15DAH	20DAH	Sabja Raw	Sabja Ripe
Aldehydes	3.2^{bc} (0.38)	5.6^{c} (0.3)	1.2^{ab} (0.01)	8.0^{d} (0.09)	3.0^{bc} (0.18)	1.6^{ab} (0.13)	7.4^{dA} (7.96)	4.7^{cB} (7.94)	2.3^{abB} (0.63)	2.8^{bC} (0.38)	1.3^{abAB} (0.14)	1.0^{aB} (0.32)	13.7$^{c\beta}$ (6.68)	4.5$^{bc\alpha}$ (3.46)
Alcohols	1.2^{b} (0.15)	7.8^{e} (0.42)	2.0^{c} (0.04)	3.3^{d} (0.04)	0.6^{a} (0.03)	0.3^{a} (0.03)	0.3^{aB} (0.36)	0.4^{aB} (0.69)	0.3^{aB} (0.09)	0.3^{aAB} (0.04)	0.2^{aA} (0.02)	0.3^{aA}B (0.09)	0.9$^{ab\alpha}$ (0.44)	1.3$^{b\beta}$ (1.01)
Monoterpene hydrocarbons	795.6^{a} (94.86)	1690.2^{a} (91.48)	15433.0^{f} (98.52)	9075.2^{b} (98.87)	1669.1^{a} (97.16)	1170.7^{a} (97.01)	75.3^{aA} (80.11)	33.6^{aA} (56.82)	353.1^{aC} (94.84)	707.6^{aD} (95.49)	899.8^{aE} (93.17)	254.3^{aB} (81.92)	127.0$^{a\beta}$ (62.1)	31.7$^{a\alpha}$ (24.33)
Sesquiterpene hydrocarbons	27.4^{b} (3.26)	128.3^{e} (6.94)	220.4^{f} (1.41)	79.0^{d} (0.86)	41.8^{b} (2.43)	29.9^{b} (2.48)	5.9^{aA} (6.22)	16.9^{abC} (28.55)	10.7^{aB} (2.88)	16.1^{abC} (2.17)	35.^{4bD} (3.67)	10.1^{aB} (3.26)	59.1$^{c\alpha}$ (28.9)	87.2$^{d\beta}$ (67.01)
Oxygenated monoterpenes	2.9^{b} (0.34)	11.3^{d} (0.61)	4.2^{c} (0.03)	4.8^{c} (0.05)	0.5^{a} (0.03)	0.2^{a} (0.01)	0^{aA} (0)	0.2^{aB} (0.27)	0^{aA} (0)	0^{aA} (0)	0^{aA} (0)	0^{aA} (0)	0$^{a\alpha}$ (0)	0$^{a\alpha}$ (0)
Oxygenated sesquiterpenes	0^{a} (0)	1.4^{b} (0.08)	1.7^{b} (0.01)	1.5^{b} (0.02)	0^{a} (0)	0^{a} (0)	0^{aA} (0)	0^{aA} (0)	0^{aA} (0)	0.2^{aB} (0.03)	0^{aA} (0)	0^{aA} (0)	1.4$^{b\alpha}$ (0.68)	3.8$^{c\beta}$ (2.91)
Lactones	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{aA} (0)	0^{aA} (0)	0.8^{bB} (0.22)	5.7^{dD} (0.77)	6.0^{dD} (0.62)	4.1^{cC} (1.32)	0$^{a\alpha}$ (0)	0.2$^{a\beta}$ (0.13)
Ketones	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{aA} (0)	0^{aA} (0)	0.4^{aA} (0.09)	6.8^{bB} (0.92)	15.5^{eC} (1.61)	34.4^{dD} (11.09)	0$^{a\alpha}$ (0)	0.1$^{a\beta}$ (0.06)
Non-terpene hydrocarbons	7.8^{f} (0.93)	2.5^{c} (0.13)	1.8^{b} (0.01)	5.1^{e} (0.06)	2.6^{c} (0.15)	3.8^{d} (0.31)	3.6^{dC} (3.81)	2.8^{cB} (4.69)	3.7^{dC} (0.99)	1.3^{aA} (0.17)	1.5^{abA} (0.16)	1.4^{aA} (0.45)	2.4$^{c\beta}$ (1.19)	1.4$^{ab\alpha}$ (1.09)

Fig 3. Quantitative variation within different classes of compounds through leaf, flower, development and ripening of Alphonso and Sabja fruit. For each row, low to high variation represented by green to red color change, through yellow; the absolute cumulative quantities (µg g^{-1}) responsible for this color change are given in each square (the upper value). For each row, the values followed by the same alphabet do not differ significantly with each other, where the significance of comparison ($p \leq 0.05$) between all the 14 tissues is represented by small alphabets, that between the post-harvest stages of Alphonso is represented by the capital letters and between raw and ripe Sabja is denoted by Greek letters. Value in the parentheses denotes the relative percentage of each represented chemical class within the column or within the aroma of that particular tissue.

in 5 and 30DAP fruits and decreased gradually during maturation; during ripening, toluene concentration again increased till 15DAH stage and fell down in the over-ripe fruit. In Sabja, toluene increased while ripening. Odd chain *n*-alkanes, tridecane and heptadecane showed similar profiles. In leaf, these three compounds were found in the maximum amount and increase in their concentration was also noted near the fruit maturation. In the ripe Alphonso fruit these alkanes were present either at very low concentrations or were absent. Even chain *n*-alkanes, tetradecane and octadecane showed common pattern. In Alphonso fruit, their highest amounts were detected in 15DAP fruits which decreased gradually till ripening. Tetradecane concentration in leaf was equivalent to that in 15DAP fruit. Hexadecane also showed the highest concentration in leaf; however, its pattern in fruit tissues matched more with that of the odd chain *n*-alkanes. In Sabja, heptadecane was absent and other alkanes decreased with ripening, similar to Alphonso. *In toto*, ripening related decline was observed for these compounds in both, Alphonso as well as Sabja (Fig 3).

Ketones and miscellaneous compounds

Furaneol and mesifuran were detected as the ripening associated ketones in this analysis (Fig 3). Furaneol was also found in ripe Sabja fruit; however its concentration was about ten folds less than that measured in ripe Alphonso fruit. Mesifuran was not found in Sabja. 4-ethoxy ethylbenzoate was found only in Alphonso tissues, where its highest concentration was marked in 15DAP fruits followed by that in 5DAH fruits with a ripening associated fall.

Discussion

Fruit flavor is a dynamic commodity; its chemistry often depends upon the harvesting maturity and exact ripening stage. As the development and ripening

periods differ in different fruits and even in different cultivars, the parameters used in the determination of these stages are usually specific to a particular fruit or even, to a cultivar. For the unmistakable determination of these stages, analysis of different attributes of the fruit has been suggested and various techniques have been proposed (Lakshminarayana et al, 1970; Tandon and Kalra, 1983; Ueda et al, 2000; Almora et al, 2004; Saranwong et al, 2004). Here, we have chosen such characterization of the dynamics of aroma chemistry in Alphonso mango over the period of 110 days of development and ripening (90+ 20). This study has revealed numerous aspects of mango aroma and has also enabled us to propose various indicators for maturity and ripening. Occurrence and contribution of different odorants in the blend of developing and ripening mango fruits is discussed (Fig 3).

Aldehydes

Aldehydes form an important part of volatile blend of different mango cultivars (Idstein and Schreir, 1985; Pino et al, 2005; Pino and Mesa, 2006). In the present analysis we found three compounds of this group. The C6 GLV, (*E*)-2-hexenal contributed most, to the volatile blend of green and young fruit, nonanal contributed more to the leaf and floral volatiles and hexadecanal increased in the volatile mixture of mature fruits. Aldehydes contribute to the aroma either directly, or on derivatization in the vibrant cellular environment. Thus, their profiles depend upon the nature of surrounding that is never consistent in the developing and ripening fruit and the extent of their interaction with such surrounding. Secondly, apart from their contribution to aroma, their role in fruit's metabolism needs to be studied.

Alcohols

Alcohol members of the C6 GLV family are the instant example of the aldehyde derivatization. Results of this analysis suggest that such process is

prominent in flowers as two of the four GLV alcohols were found only in flowers, whereas remaining two also were found in their highest amounts in this tissue. GLV alcohols appear to contribute more to the floral odor than to the fruit aroma; in other words, in mango, they might have more relevant role in pollinator attraction than that in the dispersal agent attraction.

Terpene hydrocarbons and their derivatives

Monoterpene hydrocarbons are known to be quantitatively dominant flavorants (~90%) in ripe Alphonso mango (Idstein and Schreirer, 1985). Our analysis has further revealed that these compounds remain to be the dominant odorants throughout the development and ripening of Alphonso mango. We also observed the ripening associated decrease in the occurrence of majority monoterpenes; however, in spite of such relative decrease, most of them remained the major volatile components at the ripening stage (Table 1). Reduced concentration of monoterpene hydrocarbons in the ripening fruits could be directly attributed to the characteristic degeneration of plastids during the fruit ripening process, as monoterpenes are exclusively synthesized in these organelles (Aharoni et al, 2004). However, in strawberry, the specificities were relaxed; the cytosolic enzyme opted to produce monoterpenes and the plastidic localization of enzymes changed as a function of ripening (Medlicott et al, 1986; Parikh et al, 1990; Aharoni et al, 2004); it resulted in the retention of monoterpene, linalool in the ripe fruit. Alternatively, Dudareva et al (2005) showed that in snapdragon petals, plastidic pathway also supports cytosolic product formation. Thus flowers and fruits seem to have their own ways and means to produce these volatiles.

More such mechanisms might exist in mango considering the diversity of monoterpenes. Furthermore, ripening related appearance of δ-3-carene in Alphonso and such rise of (*Z*)-ocimene in Sabja, complicate the view of plastid degradation.

Surely, the theory of dual specificity of enzymes and also the phenomenon of mutated targeting of handful of enzymes fall short to explain the mango flavor dynamics. Mango is a tropical, climacteric fruit that produces myriad of aroma compounds therefore, several such mechanisms are likely to be involved in its metabolomics. Thus, monoterpene biosynthesis remains to be complex and a phenomenon of interest in the ripe mango. Overall, monoterpene hydrocarbon aroma could be described as the characteristic of leaf, 5DAP and 15DAP fruits, and as the chief component of mature as well as ripe fruits; secondly, in Alphonso, its synthesis was found to be a subject of spatial and temporal regulation.

In Alphonso, similar to that of monoterpene hydrocarbons, profiles of β-caryophyllene and α-humulene showed dominance in the developing fruit as compared to the ripe fruit, whereas germacrene-D showed its own temporal and spatial occurrence profile. Present analysis suggests that qualitatively, sesquiterpenes are the minor flavorants and synthesis of some of these compounds is synchronized with that of major monoterpene hydrocarbons in Alphonso. This might be possible with the help of dual specificity enzymes (Aharoni et al, 2004) and/or pathways (Dudareva et al, 2005). However, with the fact that, sesquiterpenes are synthesized in cytosol and the monoterpenes in plastids, the numerical dominance of monoterpenes over sesquiterpenes in the ripe Alphonso fruit is an interesting phenomenon to study. However, in Sabja, prominence of sesquiterpene hydrocarbons and their alcohols supports the view that cultivars differ at qualitative as well as quantitative levels of flavorants and provide interesting systems to study the newer mechanisms.

Oxygenated monoterpenes did not show any collective profile. Their occurrence was conspicuously spatial which suggests their differential role in various parts of mango plant. Most of these compounds contribute to the leaf aroma. Borneol

and linalool are probably meant for pollinator attraction; however, they also contribute to the volatile blend of the young fruits. Thus, to study the oxygenation mechanism in mango, Alphonso flowers can be used as an experimental tissue, as it is less tricky to handle than the fruits.

Lactones

Lactones are known to be the most deserved aroma compounds of several fruits (Wilson et al, 1990). These compounds impart the sweetness to the fruit aroma. This sweetness is known to be the characteristic of the flavor of many ripe fruits (Wilson et al, 1990). Several lactones have been detected from the ripe Alphonso fruit by Hunter et al (1974), Engel and Tressl (1983) and Idstein and Schreirer (1985). They are known for their low odor detection thresholds by virtue of which, they make substantial impact in the odor (Wilson et al, 1990). Our analysis has revealed that the occurrence of lactones in mango is associated with ripening; it is in congruence with the organoleptic perception of ripening specific sweetness. Biosynthesis of these components must be studied to reveal the secrets of fruity flavor.

Non-terpene hydrocarbons

Non-terpene hydrocarbons, especially *n*-alkanes are known for their high odor detection thresholds (Bicudo et al, 2002). Thus, without high concentration, these compounds do not contribute significantly to the odor character of any blend; therefore, though detected, their occurrence is not usually discussed with respect to aroma blend. Odd chain *n*-alkanes are known to be the intermediates of fatty acid decarboxylation pathway that is involved in the production of structural components like cuticular waxes (Kunst and Samuels, 2003). With reference to this pathway, though these alkanes are detected as volatiles, they are short-lived and are barely released as aroma components. Similarly, even chain n-alkanes are better known as

seed storage products (Lamarque et al, 1998) than as aroma ingredients. In the present analysis, the highest concentrations of *n*-alkanes from both these classes and low ones in the mature and ripe fruits support their role as structural components and suggest their significance as 'little' to the aroma.

Other volatile constituents

Furaneol is the major aroma compound in several fruits (Wilson et al, 1990; Bood and Zabetakis, 2002). It imparts sweet, herbaceous, strawberry flavor at its lower concentration, pineapple-like at the medium concentration and caramel- and burnt sugar-like at high concentration (Wilson et al, 1990). We found that Alphonso aroma is marked by its high concentration. Mesifuran is methyl ether of furaneol. Its odor detection threshold has been found about 15 fold less than that of furaneol; thus when furaneol is converted to mesifuran, its contribution to odor character is reduced (Wilson et al, 1990). In Alphonso, furaneol was detected as a ripening associated volatile and it was also found to be continuously converted to its methyl ether as its concentration always remained lower to that of mesifuran. However, in ripe Sabja, only furaneol was detected in low concentration than that in ripe Alphonso, whereas mesifuran was absent. This probably indicates the concentration dependent conversion of furaneol to mesifuran.

Aroma character of different tissues

This analysis also enabled to ascertain the change in aroma character during the development and ripening of Alphonso mango (Fig 3). Monoterpenes collectively impart strong turpentine, green, citrus odor with mild sweet character, whereas sesquiterpenes impart strong woody, earthy and oily character; these descriptions along with the green fruity notes from C6 GLV alcohols efficiently represent the young (5 and 15DAP) mango. Except that of *n*-alkanes, rest of this character weakens

in the mature fruit that possesses mildest aroma of all the developing and ripening time. Aroma of 2DAH fruit is very similar except a small rise in woody, earthy character added by the sesquiterpenes. This aroma drastically changes in the ripe fruit (15DAH), where green, citrus, minty, woody, earthy and oily aroma of the terpene hydrocarbons is raised and is prominently added a sweet, fruity, peach, coconut character by lactones. This blend also has a strong pineapple character and caramel notes that are imparted by furaneol and mesifuran. Overripe fruit has weakened ripe fruit aroma with dominating burnt sugar character from both the furanones.

Aroma of Alphonso leaf can be described as green, turpentine, citrus, mint and synthetic on the basis of its major volatile constituents, C6 GLVs, monoterpene hydrocarbons and *n*-alkanes. Flowers have enhanced mono- and sesquiterpene hydrocarbon character with added fruitiness of C6 GLV and terpene alcohols; synthetic, fuel like characters are almost missing in the flowers. It is interesting to note that in Alphonso, terpene and alcohol dominated floral odor is used to attract the pollinators whereas the strong, sweet, fruity odor of lactones with the background of mild terpene scent is used to attract the seed dispersal agents.

Raw Sabja fruit has dominant turpentine, green, citrus, mint character from monoterpenes, woody, earthy, pungent and oily smell from sesquiterpenes and mild green character from the C6 GLVs. Aroma characters from sesquiterpene hydrocarbons and their alcohols are enhanced in the ripe Sabja fruit and are added by sweet notes from butyrolactone, the only lactone detected in this fruit. Monoterpene aroma is weakened in the ripe Sabja fruit.

Sabja in comparison

As previously demonstrated by Bartley (1988) in Australian mangos, organoleptic perception that Sabja is insignificant and unpleasant flavored mango,

was used as the basis for its comparison with Alphonso in the present experiment. Indeed, we found that Sabja flavor was qualitatively as well quantitatively weak in comparison with that of Alphonso. Except butyrolactone, rest of the lactones were not detected in this cultivar; thus along with the weakness in the terpene flavor, it also lacks the sweetness of lactones. These results suggest that Sabja is an appropriate comparison in such experiment.

Important time points in mango development and ripening

This analysis of volatiles also revealed certain facts about mango development and ripening. Though this is a continuous and gradual process, due to the sudden rise or decline in the concentrations of certain volatiles, we realized that certain time zones in this process are particularly distinctive. 5DAP is obviously one of such special stages, as at this time cellular activities are busily transforming the newly fertilized ovary to a fleshy, seed protecting fruit. Level of activity at this stage was indicated by the highest volatile concentration; particularly, high concentrations of several mono- and sesquiterpene hydrocarbons, caryophyllene oxide supported this view; low concentrations or absence of aldehydes, alkanes and oxygenated monoterpenes indicated that the volatile synthesis must be selective and programmed. Similarly, 15DAP stage was marked by second-highest concentration of total volatiles, the burst of C6 GLVs, oxygenated monoterpenes, 4-ethoxy ethylbenzoate, and the structural components, the even chain *n*-alkanes. This stage alone or along with 5DAP can be termed as a ‘jump start’ stage in fruit development as these chemicals gradually decreased during the further development. As many of the volatiles described here are better known as defense chemicals of the plant, burst of these volatiles might also serve as a protection to the young fruit from insects and birds.

Conventionally, Alphonso mangos at Deogad are harvested at 90DAP. Results of our analysis supported this maturity for harvesting. We found that levels of C6 GLVs (except, (*E*)-2-hexenal), monoterpenes, their alcohols and sesquiterpenes lowered drastically at this stage, whereas odd chain *n*-alkanes peaked up; especially, shoot up of hexadecanal emerged as an indicator of maturity. We considered this harvesting day as a 'virtual zeroth' day and the 2nd day after harvesting as a 'real zeroth' day. This *priori* hypothesis was held true by some of the results. Total volatile concentration was the lowest at this stage; secondly, several monoterpenes that gradually decreased till the harvesting maturity, further lowered or zeroed down at 2DAH stage; however, past this stage, their amounts again started increasing. On the other hand, β-terpinene, 4-carene and δ-3-carene appeared *de novo*. Similarly, borneol, α-humulene and β-caryophyllene showed sudden increase at this stage. Synthesis of ripening related compounds also started after this time point. This can be looked upon as a completion of the perception of harvesting and also as a metabolic rearrangement required at a preparatory stage for ripening.

With regard to the commercial value, perfect indication of ripeness is most important. Lactones were the major components that indicated ripening with their peaked presence; specifically, δ-decalactone was detected only in 15DAH fruit. Furaneol and mesifuran were also found to be associated with ripening; however, they kept increasing even in the overripe mango. Along the process of ripening, the gradual increase in several monoterpenes, α-humulene and β-caryophyllene peaked at 15DAH and later decreased in the overripe (20DAH) mango. Ripening was also marked by the fall in the quantities of *n*-alkanes. Most of these observations were also true in case of maturity and ripeness of Sabja fruit. This fruit has different chemistry

and also different ripening time than Alphonso; it suggests that the abovementioned indicators might also be useful for the broader pool of mango cultivars.

All these indications are important when multiple cultivation locality study is undertaken. In Alphonso, period of development also varies according to cultivation localities and is usually determined using morphological markers. These markers are often prone to environment caused variation. Under such circumstances, as being multifactorial, the indicators obtained in the present analysis will be definitely advantageous over the morphological markers; at times they can also be used in combination with the former ones. Estimate of the gross volatile concentration can be an extremely useful and economic suggestion for the detection of precise harvesting maturity. Secondly, this analysis has revealed the dynamics of volatile blend through the development and ripening; in addition, it has pointed the stages of significant metabolism in terms of the assemblage of volatile blend. Based on this the comprehensive analysis for locality dependence may be carried out with the reduced number of time points; this will reduce the labor and the cost of experimentation in the bigger experiment. These findings can also help the further biochemical and molecular studies on the temporal and spatial substrate allocation for the flavor biogenesis. Mango growers may use these findings for harvesting and ripening of mangos, for protecting them at their sensitive developmental stages (stages near maturity that have low volatile concentration) from pests and pathogens as well as for the prediction of fruit quality in case of irregular climatic conditions, especially during the abovementioned time periods.

CHAPTER 5

Isolation of Various Flavor and Non Flavor Genes from Alphonso Mango and Profiling their Expression through the Development and Ripening of Fruit

Work described in this chapter has been communicated to *BMC Molecular Biology.*

Part of this work has been published in *Journal of Plant Biology* (Pandit et al, 2007).

Introduction

Fruit development and ripening are biochemically as well as physiologically programmed processes. During the development, fruit acts as a sink and grows by accumulating the in-flown material, while ripening is characterized by textural and rheological changes. Cell expansion and softening by cell-wall solubilization, dismantling of the photosynthetic apparatus, degradation of starch and chlorophyll and respiratory and/or ethylene climacteric are some of the major events of the ripening process (Rose et al, 1997; White, 2002).

These maturation and ripening events have been probed using a handful of genes, picked either from differential expression studies or from expressed sequence tags (ESTs) in several fruits like tomato (Alba et al, 2005), strawberry (Aharoni and O'Connell, 2002), melons (Nagasawa et al, 2005) pineapple (Moyle et al, 2005), and banana (Gupta et al, 2006; Mbe´guie´-A-Mbe´guie´ et al, 2007). From this, we understand that, most of the physiological processes involved in development and ripening of the fruit are specific to this propagule carrying organ. However, the fruit transcriptome shows quite a few gene reservations; instead, fruits express the usual plant transcriptome with its own reorganizations in the expression cascades (Aharoni and O'Connell, 2002; Bartley and Ishida, 2002). Except those on ethylene metabolism and response, these observations are true for both, climacteric and non-climacteric fruits. Roles of several genes involved in these processes have been justified whereas for many it still remains unexplained. In the tropical fruit like mango, such studies are in infancy; however, extensive commercial success of this fruit has pushed the demand for information from such research.

We found cultivar Alphonso as the most appropriate one for such an initiation, as it is the most popular and most exported mango cultivar of India because of its

delightful flavor, attractive color, ample, sweet, low fiber containing pulp and long shelf life (Tharanathan et al, 2006; Vasanthaiah et al, 2006). In spite of possessing so many virtues, this cultivar is troublesome for farmers because of its erratic and shy bearing, cultivation locality dependent variation in the fruit quality, susceptibility to fungal pathogens and insect pests, and physiological disorders like spongy tissue (Om Prakash, 2004; Ravindra and Shivashankar, 2004; Tharanathan et al, 2006; Vasanthaiah et al, 2006). Biochemical and molecular studies are, therefore, required to understand the basis of these demerits.

As a first step towards such characterization, we have studied the spatial (in leaf, flower and fruit) and temporal (during the development and ripening of fruit) expression of 19 different genes; *vis-à-vis*, these genes were studied in raw and ripe fruits of cultivar Sabja that have exceptionally mild aroma. Genes analyzed in this experiment, mainly include those related to terpene and aldehyde biosynthesis, along with certain other multifunctional genes that help to maintain the homeostasis during the rapid cellular activities in the fruit. The principle of relative transcript quantitation (Dallman and Porter, 1994) has been applied here to elucidate the expression profile of these genes. Most of the genes studied in this work have been reported for the first time from mango. Often, in case of multifunctional and multi-isoform genes, sequence based phylogenetic analyses help to determine their type, substrate specificity and product information (Li et al, 2001; Aharoni and O'Connell, 2002; Bartley and Ishida, 2002; Bowles et al, 2005). For a taxon like mango, such sequence based information is also helpful for characterizing its high cultivar diversity. Therefore, in the present work we have also conducted phylogenetic analyses for these genes.

Materials and methods

Plant material

Alphonso fruit takes about 90 days to mature after the fruit-set and further 15 to 20 days to ripe at 28^{o}C. Therefore, fruits of 5, 15, 30, 60 and 90 days after pollination (DAP) and of 2, 5, 10, 15 and 20 days after harvesting (DAH) were collected from the orchards of the Regional Fruit Research Station (RFRS) of Dr. Balasaheb Savant Kokan Krishi Vidyapeeth [(DBSKKV) (Dr. Balasaheb Savant Kokan Agricultural University)], at Deogad (Maharashtra, India). According to conventional indices for Deogad grown Alphonso fruit, 5 and 15DAP≡ early development; 30 and 60DAP≡ mid development; 90DAP/00DAH≡ harvesting maturity; 2DAH≡ harvesting- ripening intermediate; 5DAH≡ early ripening; 10DAH≡ mid ripening; 15DAH≡ ripe and 20DAH≡ overripe. Along with these fruit tissues, leaf and flower tissues were also collected. In this analysis, very low and unpleasant-flavored fruits of cultivar Sabja were used as controls. As Sabja fruit ripens within two days after harvesting, only two stages, mature unripe and ripe fruit were collected from the orchards at Vengurle (Maharashtra, India). All the tissues were preserved at -80^{o}C till use.

Isolation of RNA

Frozen tissue was crushed to a fine powder using mortar and pestle in liquid nitrogen. This powder was then homogenized to slurry with preheated (65°C) extraction buffer (10 mL extraction buffer g^{-1} of tissue); 20µL of 2-mercaptoethanol per mL of buffer (~0.2%) was added just before use. The homogenate was incubated at 65°C for 20 min, with intermittent and thorough vortexing. After the slurry cooled to room temperature (RT), an equal volume of chloroform: iso-amyl alcohol (24:2) was added followed by vigorous shaking with intermittent venting of the tube to form

an emulsion. Samples were centrifuged at 12000*g* for 10 min at RT. The aqueous phase was collected, to which, prechilled 10 M LiCl was added to a final concentration of 3 M. The RNA was allowed to precipitate at -20^{o}C for 30 min. The pellet was recovered by centrifugation at 12000*g* at 4°C for 10 min. The RNA pellet was dissolved in 10 mL diethyl pyrocarbonate (DEPC)- treated water, then extracted once with water-saturated phenol (pH 5.2 to 5.5), followed by extraction with chloroform: iso-amyl alcohol (24:2). To the aqueous phase, 3 M Na acetate (pH 5.4) was added, to a final concentration of 0.3 M, followed by a range of 0.6 to an equal volume of prechilled isopropanol. RNA was allowed to precipitate at -70^{o}C for 20 min. RNA was recovered by centrifugation at 12000*g* at 4°C for 10 min. The pellet was suspended in 1mL of 70% EtOH and again centrifuged at 12000*g* at RT for 10 min. The vacuum-dried pellet was dissolved in an appropriate volume of DEPC-treated water (100µL g^{-1} starting material). RNA was quantified spectrophotometrically at 260 nm, and the purity was determined by ratios of 260/230 nm and 260/280 nm. RNA quality was assessed by electrophoresing on 1% non-denaturing EtBr-stained agarose gel, using a 1kB ladder (Promega, USA) as the size marker.

All reagents were treated with DEPC and were autoclaved (15 psi, 121^{o}C for 20 min). The exception was Tris-Cl, which was prepared in DEPC-treated water and autoclaved. The entire procedure was conducted under RNase-free conditions (Pandit et al, 2007b).

Synthesis of cDNA

For all the tissues, first strand cDNA was synthesized over 1µg of total RNA using, Clontech's (Japan) Reverse Transcription system.

Sequence confirmation and annotation

For sequence confirmation, cDNA template was prepared by mixing the cDNA from above mentioned 12 Alphonso tissues in equal amounts. cDNA fragments of interest were amplified using different primer pairs (Table 1) and Advantage® Taq DNA polymerase (Clontech, Japan). Amplified fragments were cloned in pGEM-T Easy vector system (Promega, USA). At least five clones per primer pair were sequenced using Megabase 1000 DNA sequencer (Amersham biosciences, UK) and were compared to confirm their homogeneity and presence of uninterrupted translation frame in them. For annotation, these DNA as well as their respective amino acid sequences obtained by *in silico* translation were matched with the sequences available in the National Centre for Biotechnology Information (NCBI) database using the basic local alignment search tool (BLAST) algorithm (Artschul et al, 1990).

Relative quantification PCR

For all the relative quantification PCR, cDNA ensured to be synthesized from uniform amount of RNA from all samples was used as template. Two and four microliters from RT reaction were amplified separately in 20µl reaction with 1X green GoTaq™ buffer (Promega, USA), 2.0 mM $MgCl_2$, 2.5 mM dNTPs, 0.5 mM of each gene specific primer and GoTaq™ DNA polymerase (Promega, USA). The PCR cycle program consisted 30 or 35 cycles of denaturation at 94^{o}C, annealing followed by extension at 72^{o}C (each step of 45 s). PCR products were run through 2% agarose gel containing 0.5µg l^{-1} ethidium bromide along with 1µg *Hind*III digested Lambda DNA (λ/*Hind*III) (Genei, India). Concentrations of all the PCR products (ng), including that of internal standard were determined by plotting their band intensities on the standard curve made by those of λ/*Hind*III (the bands of known

concentrations). Imaging, intensity measurement and recording was done using ImageMaster VDS video documentation system (Amersham Biosciences, UK).

For all the primer pairs, except annealing temperature and number of cycles, rest of the PCR program was the same. Initially, amplification with each primer pair was attempted in 30 cycles' PCR; few primer pairs that failed to produce detectable concentration of amplicon were subjected to 35 cycles' PCR. Details of annealing and cycle number are given in table 1. Elongation factor 1α (EF1) gene was used as an internal control to monitor the uniformity of expression across the tissues for both, 30 as well as 35 cycle PCRs.

Statistical analysis

Statistical analyses were conducted using Systat statistical software (version 11, Richmond, CA, USA). Significance of the data was analyzed by ANOVA and Fisher's LSD. Multiple regression analysis was carried out to examine the synchrony between the relative quantitation PCR profiles of volatile producing genes and their putative products detected by gas chromatographic analysis (Chapter 4) from the selected tissues of Alphonso and Sabja mangos (leaf, flower, 5DAP, 15DAP, 90DAP, 2DAH, 15DAH, 20DAH, Raw Sabja and ripe Sabja).

Phylogenetic analysis

Sequence based phylogenetic analyses were carried out for *in silico* translated sequences of all mango genes using Clustal W algorithm option from the DNASTAR package. Mango sequences were typically compared with those from *Arabidopsis thaliana, Fragaria* spp., *Glycine max, Nicotiana tabacum, Oryza sativa* and *Vitis vinifera*. These reference species were chosen for the abundance of their sequences in the gene banks or their fruit related information content. In case of few genes, where the sequence information was not available from these particular plant species,

information from other species or plants from their respective families was used. In case of certain genes that are well characterized in bacteria, but are poorly known from plants, bacterial sequences were also used in the analysis.

Results

Sequence confirmation and annotation

Sequences of all the cDNA fragments that were amplified by the different primer pairs (Table 1) could be translated *in silico*, for uninterrupted amino acid stretches (Annexure 1). Sequences of these fragments were deposited to NCBI, and their accession numbers along with the results of the BLAST search are shown in Table 1. Amplicons annotated as mitochondrial small heat shock protein (sHSP) and metallothionein turned out to be the complete open reading frames of their respective genes. Ribosomal methyl transferase (MeTr), Geranyl pyrophosphate synthase (GPPS) and sesquiterpene synthase (SqTPS) fragments represented the 3' ends of their respective transcripts.

Sequence based phylogeny

Phylogenetic affinities of various mango genes

Phylogenetic analysis (Fig 1a-s) helped in typification of various genes. Mango MTPS sequence showed high similarity to limonene synthases from *Citrus* spp. and (-)-α-terpineol synthase from *Vitis vinifera* (Fig 1c). SqTPS gene was also placed in the cluster of *Citrus* spp. and *Vitis vinifera* genes that were responsible for the production of valencene and germacrene D, respectively (Fig 1f). GT cDNA isolated from mango resembled UDP-Glucose:cinnamate glucosyltransferase from *Fragaria* × *ananassa* and limonoid UDP glucosyltransferase from *Citrus unshiu*.

Table 1. Details of primers, annotation, and BLAST analysis for 20 different cDNA fragments that were profiled for expression through 14 mango tissues (leaf, flower, developing and ripening fruits of cultivar Alphonso and mature and ripe fruits of cultivar Sabja).

Accession no.	Primer sequence (5'- 3') (Forward and Reverse, respectively)	Annealing (^{o}C)	Cycle no.	cDNA fragment size (bp)	Annotation	Functional class	Significance E score (Nucleotide)	Nucleotide % similarity	Significance E score (amino acid)	Aminoacid % match (identities and positives, respectively)
EU513264	CTCATCGAGGAGGATGCTCTTGGG	70	30	125	Isopentenyl	Terpene	1e-31	88	2e-15	95
	TTGTAGAGAATCCGACCGAGTGGG				diphosphate isomerase	biosynthesis				100
					(IPI2)					
EU513265	TCTTGTTACGGGTGAAACCATG	58	35	554	Geranyl diphosphat	Terpene	3e-161	83	2e-57	83
	TTATTTGGTTCTTGTGATGACTC				synthase	biosynthesis				91
EU513266	GGTGTGTTGAAAAAGTTCAAGGACACGAC	70	35	224	Monoterpene cyclase	Terpene	1e-08	78	4e-08	50
	TGGAAGATTCATTGCGTGCTTCACTTGC					biosynthesis				81
EU513267	TCGAGATGATTCACACCATGTC	58	35	661	Geranylgeranyl	Terpene	1e-122	75	8e-79	88
	TATGGAATATAATTCAGCCAGAG				pyrophosphate	biosynthesis				93
					synthase					
EU513268	AGTATTCATTGCCACTTCATTGCCAG	68	30	331	Farnesyldiphosphate	Terpene	1e-94	88	1e-44	80
	AACACAGGCTGGATCTGCTTTCCC				synthase	biosynthesis				94
EU513269	GGCAAATCAAGGAAAATTATATCGTCTTG	67	35	580	Sesquiterpene cyclase	Terpene	2e-43	69	1e-25	40
	TCAGAGCTGTACGGAGTCTCTGAGCAATG					biosynthesis				59
EU513270	ATGAGATCCGAAAGAGAAACCCAGATCC	70	30	540	Isochorismatase	Benzenoid	6e-69	71	2e-71	71
	TTTCACAGTCAACCAAGTAAGCAAACCC				hydrolase family	metabolism				84
					protein					
EU513271	AATGGAGTCCGCAAGAGAAAGTTTTGG	70	30	373	UDP-glucosyl	multifunction	7e-79	78	3e-57	83
	ACCTCATCTACAAACTCTTGAATGTTCC				transferase					89

Accession no.	Primer sequence (5'- 3') (Forward and Reverse, respectively)	Annealing (ºC)	Cycle no.	cDNA fragment size (bp)	Annotation	Functional class	Significance E score (Nucleotide)	Nucleotide % similarity	Significance E score (amino acid)	Aminoacid % match (identities and positives, respectively)
EU513272	GACTATCCATATGCTGTGGATGG GGGTATTGGCCAAAGTTAACTGC	56	35	278	Lipoxygenase- 1	Aldehyde biosynthesis	1e-79	74	7e-47	85 95
EU513273	GTGCTCGTTGGGCATTTCATGCC CCCCCAAAGGCGTTGAAGCC	57	35	575	Fatty acid hydroperoxide lyase	Aldehyde biosynthesis	5e-89	73	7e-79	72 84
EU513274	GCTGCTTTCTATGAGGGTTATTATGC AACATCTCCCACAGCATAAACATCAGG	64	30	273	Cytosolic monodehydroascorbate reductase	Stress response	2e-91	87	1e-37	92 96
EU513275	ATGGCTTCCACTCCTTCAGCTCGCGAGG CTGCTGTTCACTGTCAGGCTTGGTGGC	71.5	30	783	14-3-3 protein	Stress response	0.0	81	1e-114	92 95
EU513276	ATGTCTTCTGGTTGTAACTGTG TCACTTGCCACATTTGCAGGGG	57	30	225	metallothionein 1a	Stress response	7e-19	71	3e-08	64 76
EU513277	TTCCGTGATACTCTCAGATATGTGTCC ATCTTGACAAATTAAATAAATCTCTCTGG	62	35	346	FtsJ-like methyltransferase family protein	Stress response	3e-26	86	9e-36	71 80
EU513278	GACCTCAGCCTCTCGCTTCTTCAACACC TCAATTTTTACCTGGAACACGTCACTCC	70.7	30	525	Mitochondrion-localized small heat shock protein 23.6	Stress response	1e-64	71	2e-46	64 76
EU513279	ATGTCCCAAAACTGTGACTGTGCTCCC AATTTCTGGCAATAATCAGTGTAATAACC	67	35	722	Chitinase CHI1	Stress response	1e-136	79	1e-84	79 85
EU513280	GTTGTGAAGGCGAAGCGGCAGGTGG CTAAGCAGAAGCAGCATCAAGAACC	68	30	168	Cysteine proteinase inhibitor	Stress response	4e-26	77	5e-12	70 85

Accession no.	Primer sequence (5'- 3') (Forward and Reverse, respectively)	Annealing (ºC)	Cycle no.	cDNA fragment size (bp)	Annotation	Functional class	Significance E score (Nucleotide)	Nucleotide % similarity	Significance E score (amino acid)	Aminoacid % match (identities and positives, respectively)
EU513281	AGATGTTGCAACCAAAGAAGCCGCCAGAG TAAGAGGTGATCACGATCAATATTCTGC	68.3	30	445	AP2/EREBP transcription factor ERF-2	Ethylene response	1e-13	82	8e-22	50 63
EU513282	ATGTAGCAGCCATTGAGGCTCTGGTTCG GTTCACTATGTCAACTTTCGCCTCTTGG	70	30	620	E3 ubiquitin ligase PUB. Armadillo/ U-box domain-containing protein	Protein turnover	4e-97	73	4e-75	76 89
EU513283	AATACGACTCACTATAGGGCAAGCAG ATACGACTCACTATAGGGCTCCTTCTC	70	30 and 35	132	Elongation factor 1-alpha	Protein synthesis	1e-50	94	1e-18	100 100

Gene of sHSP opted the cluster of 22/23 kd sHSPs (Fig 1o). Chitinase from mango matched with the type II acidic chitinases (Fig 1p). Present analysis revealed the similarity of mango ERF to ethylene response factor reported by Aharoni and O'Connell (2002).

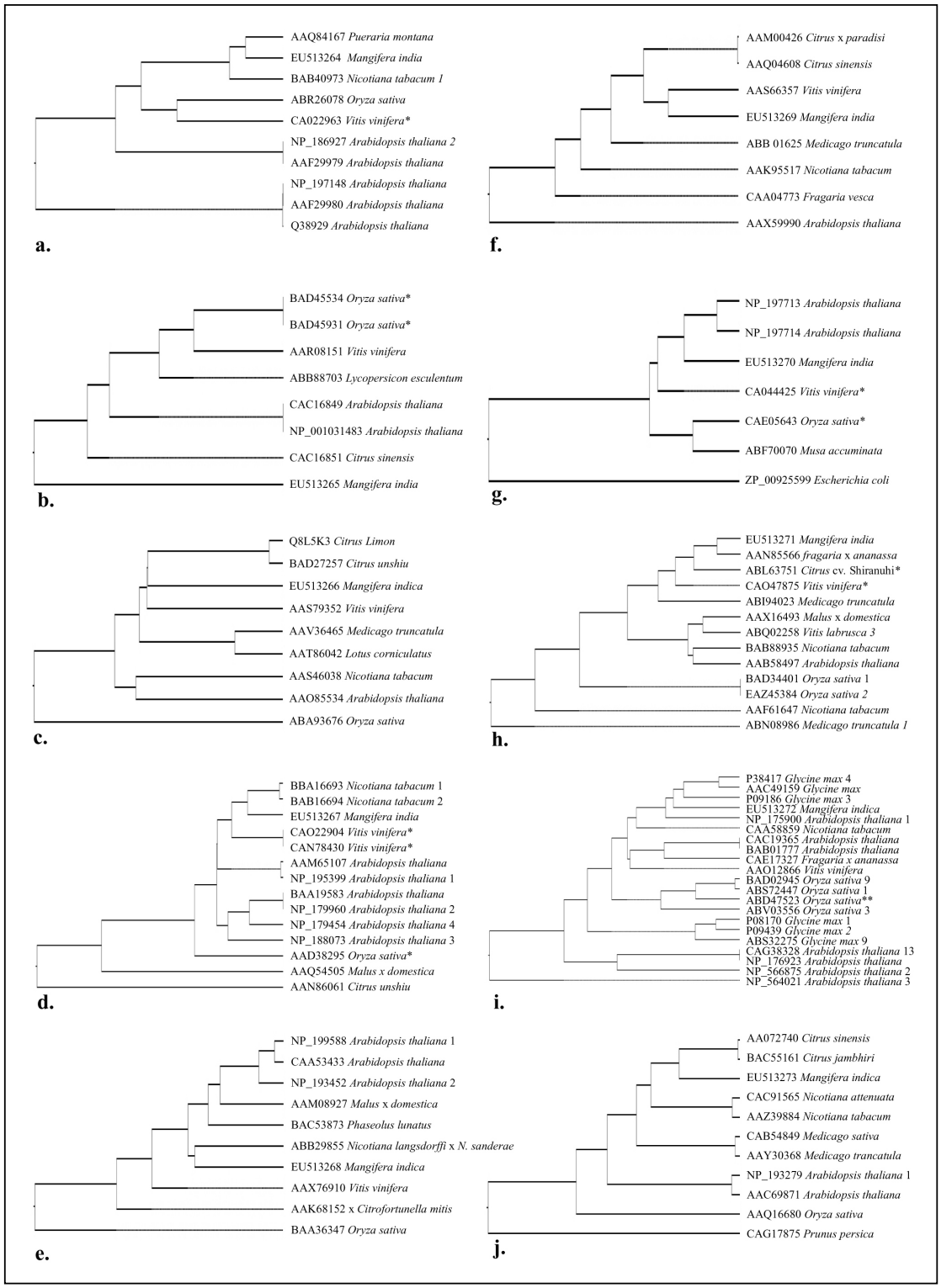
In the currently used set of reference sequences, majority of the mango gene sequences (10) showed high relative similarity to their respective homologs in the grapevine; six genes were found similar to their homologs from *Nicotiana* spp. and five to the respective homologs from *Arabidopsis thaliana* (Fig 1a- s). It was found that mango GPPS (Fig 1b) and CysPI (Fig 1p) sequences were highly divergent from all the used respective model plant sequences.

Flavor biosynthesis

Terpene flavor

Isopentenyl diphosphate isomerase (IPPI) gene expressed quite uniformly and abundantly (30 cycles PCR) through the sampled tissue range (Fig 2a). Among the Alphonso tissues, the highest transcript abundance was noted in flowers. IPPI transcript levels were similar in the mid developmental, ripe and overripe fruits; however, fruits in mid-ripening stages showed elevation in expression. Overall, the fruits of mid-ripening stage had higher transcript levels than the other stages. Similar observation was recorded in Sabja, where critical expression rise was noted on ripening.

In 2DAH fruits, GPPS expression was not detected. It showed increased transcript accumulation in mid-development and mid-ripening phases making a contour line similar to that of IPPI expression (Fig 2b). No significant change was observed in GPPS expression, while ripening of Sabja fruit.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AAF29980 Arabidopsis thaliana
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a.
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BAD45931 Oryza sativa*
AAR08151 Vitis vinifera
ABB88703 Lycopersicon esculentum
CAC16849 Arabidopsis thaliana
NP_001031483 Arabidopsis thaliana
CAC16851 Citrus sinensis
EU513265 Mangifera india
b.
Q8L5K3 Citrus Limon
BAD27257 Citrus unshiu
EU513266 Mangifera indica
AAS79352 Vitis vinifera
AAV36465 Medicago truncatula
AAT86042 Lotus corniculatus
AAS46038 Nicotiana tabacum
AAO85534 Arabidopsis thaliana
ABA93676 Oryza sativa
c.
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BAB16694 Nicotiana tabacum 2
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CAO22904 Vitis vinifera*
CAN78430 Vitis vinifera*
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NP_179960 Arabidopsis thaliana 2
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NP_188073 Arabidopsis thaliana 3
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d.
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NP_193452 Arabidopsis thaliana 2
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BAC53873 Phaseolus lunatus
ABB29855 Nicotiana langsdorffi x N. sanderae
EU513268 Mangifera indica
AAX76910 Vitis vinifera
AAK68152 x Citrofortunella mitis
BAA36347 Oryza sativa
e.
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AAQ04608 Citrus sinensis
AAS66357 Vitis vinifera
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ABB 01625 Medicago truncatula
AAK95517 Nicotiana tabacum
CAA04773 Fragaria vesca
AAX59990 Arabidopsis thaliana
f.
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NP_197714 Arabidopsis thaliana
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CAE05643 Oryza sativa*
ABF70070 Musa accuminata
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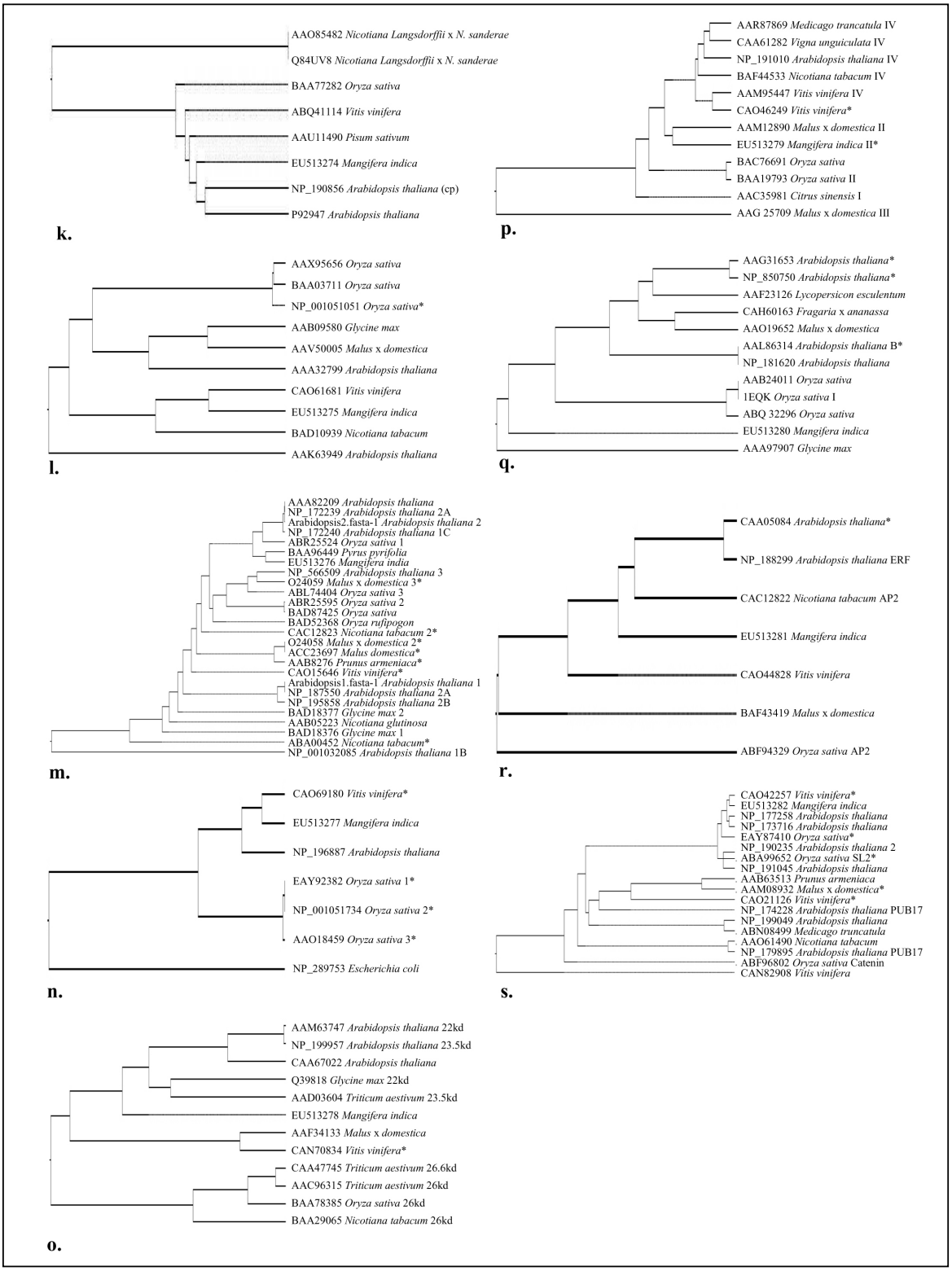
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l.
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CAH60163 Fragaria x ananassa
AAO19652 Malus x domestica
AAL86314 Arabidopsis thaliana B*
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1EQK Oryza sativa I
ABQ 32296 Oryza sativa
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q.
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ABR25595 Oryza sativa 2
BAD87425 Oryza sativa
BAD52368 Oryza rufipogon
CAC12823 Nicotiana tabacum 2*
O24058 Malus x domestica 2*
ACC23697 Malus domestica*
AAB8276 Prunus armeniaca*
CAO15646 Vitis vinifera*
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NP_195858 Arabidopsis thaliana 2B
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NP_001032085 Arabidopsis thaliana 1B
m.
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CAC12822 Nicotiana tabacum AP2
EU513281 Mangifera indica
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BAF43419 Malus x domestica
ABF94329 Oryza sativa AP2
r.
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EU513277 Mangifera indica
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AAC96315 Triticum aestivum 26kd
BAA78385 Oryza sativa 26kd
BAA29065 Nicotiana tabacum 26kd
o.

Fig 1 Sequence based phylogenetic analysis of 19 genes [**a.**≡ IPPI; **b.**≡ GPPS; **c.**≡ MTPS; **d.**≡ GGPPS; **e.**≡ FPPS; **f.**≡ SqTPS; **g.**≡ IsoCH; **h.**≡ GT; **i.**≡ LOX; **j.**≡ HPL; **k.**≡ MDHAR; **l.**≡ 14-3-3; **m.**≡ MT; **n.**≡ MeTr; **o.**≡ sHSP; **p.**≡ Chitinase; **q.**≡ CysPI; **r.**≡ ERF; **s.**≡ UbqPL; **t.**≡ EF1a (internal standard; 30 cycle PCR)] revealing the affinities of mango sequences. Sequences followed by * are the putative annotations, as reported in the NCBI gene bank. (In **k.** cp≡ chloroplastic form; in **p.** roman numbers following the botanical names denote the type of chitinase)

Expression of monoterpene synthase (MTPS) in Alphonso leaf, flower and raw fruit tissues was high; sharp decline in expression was marked at fruit maturity from where the expression remained consistently low till the overripe stage (Fig 2c). Raw as well as ripe Sabja fruits showed very low abundance of MTPS transcripts.

In Alphonso as well as Sabja, geranyl, geranyl pyrophosphate synthase (GGPPS) levels were maintained throughout the fruit life except, in 2 and 5DAH fruits, where they observed to be lowered (Fig 2d).

Farnesyl pyrophosphate synthase (FPPS) gene showed equal expression in leaves and flowers (Fig 2e). Among all the tissues, the highest expression of this gene was noted in 5DAP fruits. In Alphonso, except in 15DAH, FPPS expression could not be detected in any other ripening stages. In Sabja, ripening associated rise was observed in the expression of this gene.

The highest transcript level of SqTPS gene was marked in flowers, whereas it was not detectable in leaves (Fig 2f). Among the fruit tissues of Alphonso, 60 and 90DAP fruits showed the highest expression, whereas 2DAH fruit showed the lowest. In Sabja fruits, expression of this gene increased with ripening.

Isochorismate hydrolase (IsoCH) expression peaked in flowers (Fig 2g). During the maturarion period its expression showed steady rise till the late maturation

phase (60DAP); however, it displayed sharp downfall in the ripening period (in Alphonso as well as Sabja).

Glucosyltransferase (GT) expressed in high amounts in mango tissues thus, could be amplified with 30 cycles of PCR (Fig 2h). In fruit, the expression featured by sharp increase in early development as well as at ripening. In Sabja, marginal ripening related expression rise was recorded; however, the levels in both, raw and ripe fruits were lower than those in ripe Alphonso.

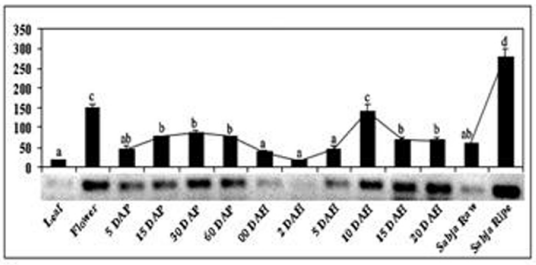
Green leafy aroma

To asses how the green smell of fruits is influenced by the genes involved in lipoxynase (LOX) pathway, expression of LOX and hydroperoxide lyase (HPL) was assessed (Fig 2i). Lox expression was about four fold higher in ripe fruits than that in leaves, flowers and developing and mature fruits. In Sabja, LOX expression was not detected.

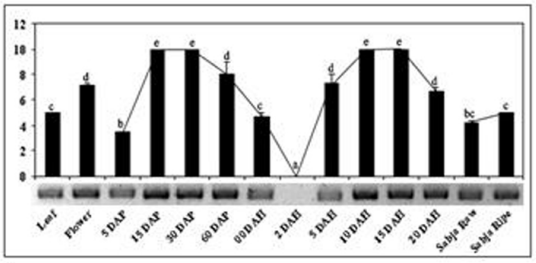
Following LOX, expression of HPL gene was also measured in mango (Fig 2j). It was moderately uniform through the development and ripening of fruit except in 5DAP and 2DAH stages. Comparable activity was observed in flowers as well as Sabja fruits. Alphonso leaves showed very low expression of HPL.

Genes related to the dynamic environment of fruit

Mono-dehydrogenase ascorbate reductase (MDHAR) mRNA levels were found sufficiently high to be detected within 30 cycles of PCR (Fig 2k). In most of the Alphonso as well as Sabja tissues, transcript levels were significantly uniform. Exceptional shoot up was observed at the fruit maturity.


Leaf
Flower
5 DAF
15 DAF
30 DAF
60 DAF
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

a.


Leaf
Flower
5 DAF
15 DAF
30 DAF
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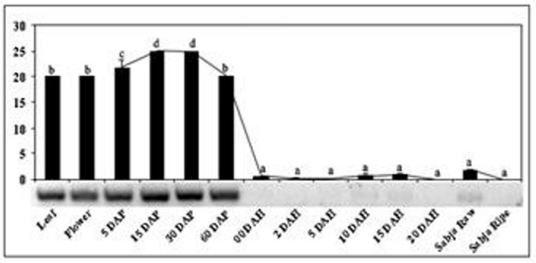
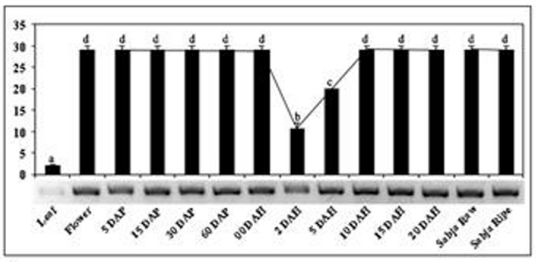
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

b.


Leaf
Flower
5 DAF
15 DAF
30 DAF
60 DAF
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

c.


Leaf
Flower
5 DAF
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30 DAF
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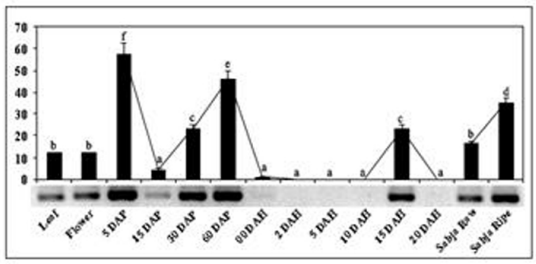
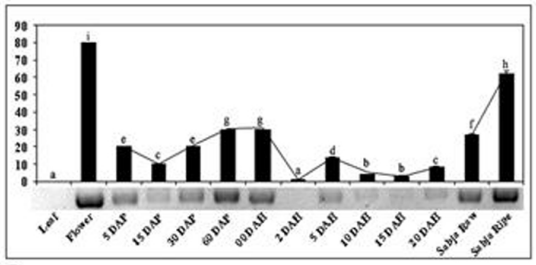
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

d.


Leaf
Flower
5 DAF
15 DAF
30 DAF
60 DAF
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

e.


Leaf
Flower
5 DAF
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30 DAF
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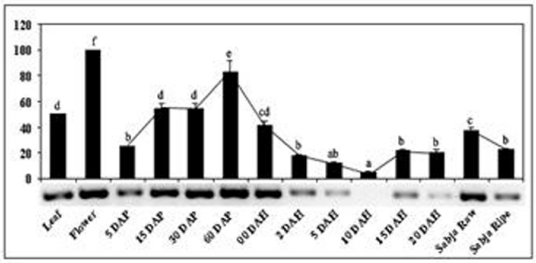
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

f.


Leaf
Flower
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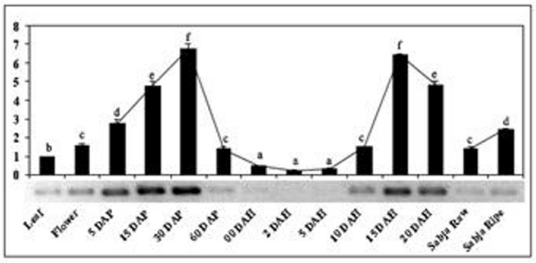
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

g.


Leaf
Flower
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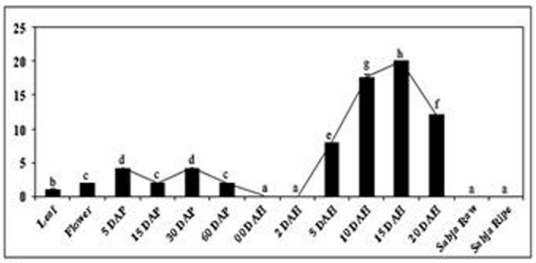
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

h.


Leaf
Flower
5 DAF
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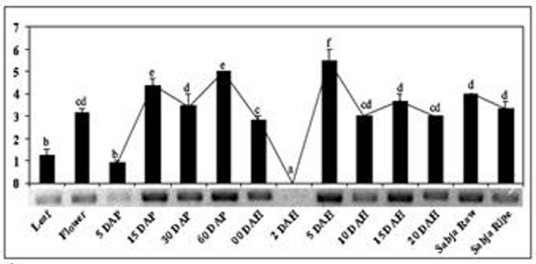
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

i.


Leaf
Flower
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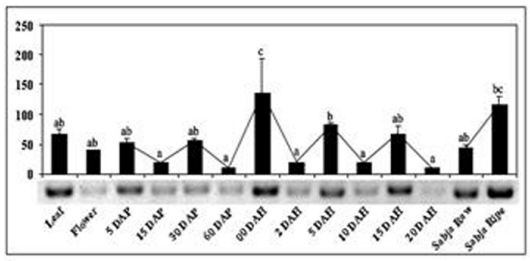
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

j.


Leaf
Flower
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00 DAH
2 DAH
5 DAH
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Sabja Raw
Sabja Ripe

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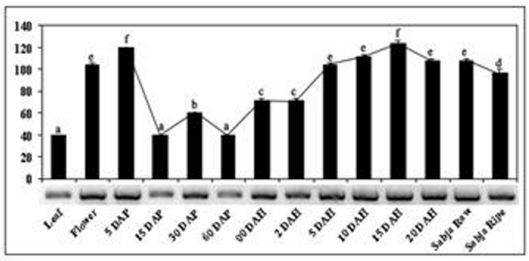
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Flower
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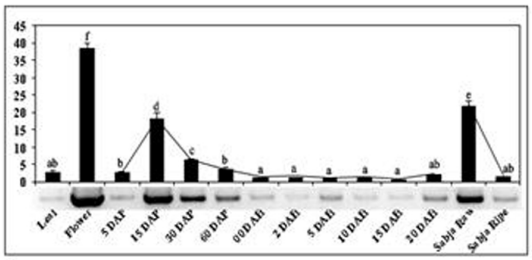
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

p.


Leaf
Flower
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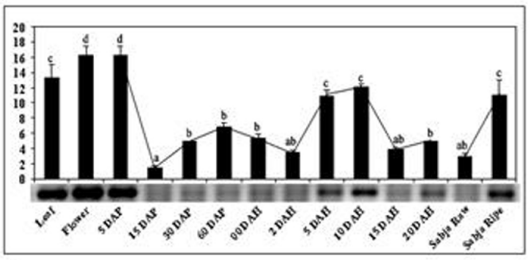
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

l.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Flower
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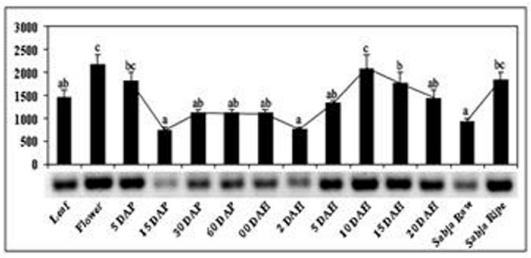
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

q.


Leaf
Flower
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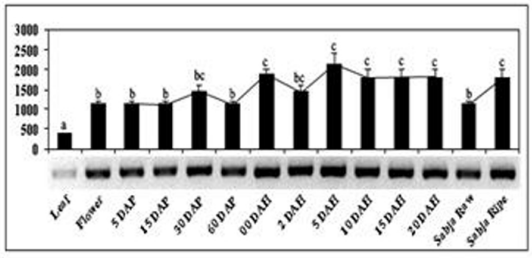
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

m.


Leaf
Flower
5 DAF
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00 DAH
2 DAH
5 DAH
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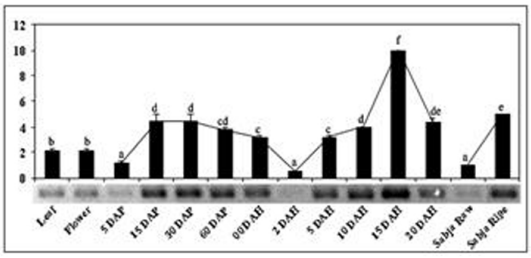
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

r.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Flower
5 DAF
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00 DAH
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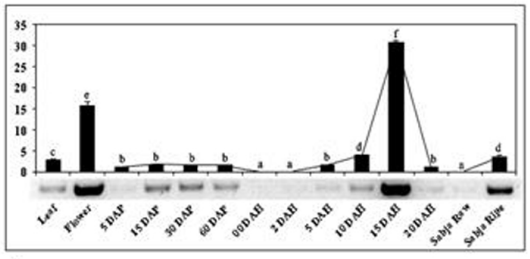
Sabja Raw
Sabja Ripe

n.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Flower
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00 DAH
2 DAH
5 DAH
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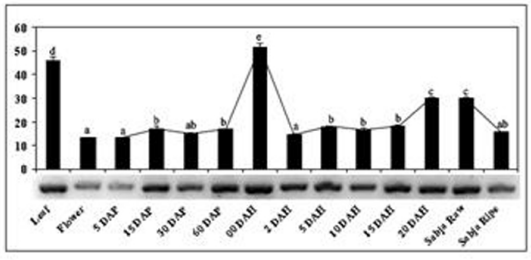
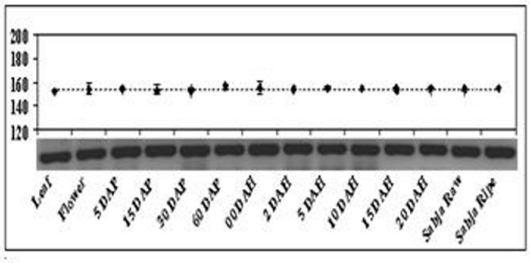
20 DAH
Sabja Raw
Sabja Ripe

s.


Leaf
Flower
5 DAF
15 DAF
30 DAF
60 DAF
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

o.


Leaf
Flower
5 DAF
15 DAF
30 DAF
60 DAF
00 DAH
2 DAH
5 DAH
10 DAH
15 DAH
20 DAH
Sabja Raw
Sabja Ripe

t.

Fig 2 Expression profiles of 20 genes [**a.**≡ IPPI; **b.**≡ GPPS; **c.**≡ MTPS; **d.**≡ GGPPS; **e.**≡ FPPS; **f.**≡ SqTPS; **g.**≡ IsoCH; **h.**≡ GT; **i.**≡ LOX; **j.**≡ HPL; **k.**≡ MDHAR; **l.**≡ 14-3-3; **m.**≡ MT; **n.**≡ MeTr; **o.**≡ sHSP; **p.**≡ Chitinase; **q.**≡ CysPI; **r.**≡ ERF; **s.**≡ UbqPL; **t.**≡ EF1a (internal standard)] through 14 tissues (leaf, flower, developing and ripening fruits of cultivar Alphonso and mature and ripe fruits of cultivar Sabja), wherein the peak expression levels of consecutive developing and ripening stages are connected by the trend line. In the profile of the internal standard, the dotted trend line covers all 14 tissues for effectively depicting the uniformity in expression. Amplicon concentrations expressed in ng/ 20µl reaction, on Y axis. Bars with the same alphabets are not significantly different from each other at $p \leq 0.05$; in none of the tissues, expression level of the internal standard was significantly different from that in the others.

14-3-3 cDNA isolated from Alphonso fruit showed high expression in the early developmental stages (Fig 2l); similar expression difference was also noted in the raw and ripe Sabja fruits. Alphonso flowers showed the highest expression of this gene.

High (30 cycle PCR) and constitutive expression of metallothionein (MT) gene was observed throughout the selected tissue range (Fig 2m). In flowers and 10DAH fruits the expression was the highest. The expression was more than 1.5 fold higher in ripe fruits (15DAH) than that in the mature unripe fruits (90DAP); similar rise was also noted during Sabja fruit ripening.

In Alphonso, expression of ribosomal methyl transferase (MeTr) was found quite consistent through the selected tissue range except the ripening associated sharp rise in ripe fruit (Fig 2n). Ripening associated expression burst of this gene was also observed in Sabja.

A gene for mitochondrial small heat shock protein (sHSP) or low molecular weight heat shock protein (LMW HSP) showed uniform expression in most of the

tissues (Fig 2o). Only in leaf, mature as well as overripe Alphonso fruit and in raw Sabja fruit this expression was observed to be raised.

Type II acidic chitinase (Fig 2q) expressed in low (35 cycle PCR) but ripening-upregulated manner in Alphonso fruit. Overall, the expression was higher during the ripening phase; however, flowers and 5DAP fruits also showed high expression. The perfect ripe fruit showed the highest expression.

Cysteine protease inhibitor (CysPI) gene expressed with moderate levels (30 cycle PCR) in all the sampled tissues of mango (Fig 2p). Similar to that of chitinase, in flowers and young fruits (5DAP) CysPI expression was higher than any other tissue. Significant elevations in the transcript levels were also observed in mid-developmental and mid-ripening stages of Alphonso fruit. Ripening associated expression rise was noted in Sabja fruit.

Genes related to ripening

A plant specific transcription activation factor associated with ethylene and abscisic acid response (ERF) was isolated from Alphonso fruit. This ethylene response factor (ERF) gene showed high (30 cycle PCR) and constitutive expression over the fruit life (Fig 2r). In Alphonso as well as Sabja it showed expression rise in ripening phase. Its expression in flowers was near equal to that in the developing fruits. In leaf, the expression was about two fold less than that in flowers.

Ubiquitin–protein ligase (E3) (UbqPL) gene showed very low expression throughout the selected tissue range; however, its high expression was noted in flowers and in the ripe fruits of Alphonso as well as Sabja (Fig 2s).

Internal standard

By 30 as well as 35 cycle PCR, elongation factor 1α gene expression was found to be uniform in all the 14 tissues (Fig 2t).

Regression between molecular and chemical activities

Table 2 presents the details of regression analysis. Regression between the gene expression profile of monoterpene synthase and certain monoterpenes yielded highly positive values. Analysis between sesquiterpene synthase and the three sesquiterpenes (α-humulene, β-caryophyllene and germacrene D) yielded low, positive values; among these three, germacrene D produced the highest value. However, sesquiterpenes yielded high positive values when analyzed with the profiles of IPPI and FPPS. LOX and HPL genes did not yield any significant regression values when analyzed with the variety of aldehyde products.

Table 2. Different genes from terpene biosynthesis pathway regressed with various terpene volatiles that were detected by gas chromatography, from the selected tissues (leaf, flower, 5DAP, 15DAP, 90DAP, 2DAH, 15DAH, 20DAH, raw Sabja and ripe Sabja).

	Regression (r^2)	**Probability (*p*)**	**Standard error (±SE)**
MTPS- MTP			
p-cymene	0.69	0.002	0.33
β-thujene	0.69	0.003	3.03
Allo-ocimene	0.57	0.01	3.33
Borneol	0.55	0.01	0.73
(*E*)-ocimene	0.52	0.02	125.14
Linalool	0.5	0.02	2.06
Limonene	0.49	0.02	3.03
(*Z*)-ocimene	0.47	0.02	3877.1
IPPI- SqTP			
Germacrene D	0.74	0.001	2.22
FPPS- SqTP			
α-humulene	0.59	0.01	15.5
β-caryophyllene	0.58	0.01	29.79
SqTPS- SqTP			
Germacrene D	0.33	0.1	3.53
MTPS-SqTP			
β-caryophyllene	0.43	0.03	1.04
α-humulene	0.43	0.03	0.548

Discussion

Fruits are mainly known by their aroma, taste and color. Flavor, the combination of aroma, taste and texture (Chapter 1) is the important criterion to decide the market value of any fruit when different varieties or cultivars are available for comparison. In mango, flavor is known to be dominated qualitatively as well as quantitatively by monoterpenes and sesquiterpenes (Chapter 3; Idstein and Schreirer, 1985; Pino et al, 2005; Pino et al, 2006). In several Indian mangos, odorant concentrations have been estimated to be high to infer that the significant part of the transcriptional machinery is involved in the aroma synthesis process (Chapter 3; Lizada, 1993). However, preharvest-environment dependent variations in various qualities of mangos have also been reported (Hofman et al, 1997), which indicate that the genes, other than those related to flavor, might play a regulatory role. Under such circumstances, the transcriptome analysis of mango to understand the transcriptional dynamics through its development and ripening would be an ideal approach. Present work covers several flavor related genes along with few genes that might affect the physiology of developing and ripening fruits.

Flavor biosynthesis

Terpenes

In Alphonso, more than 90% of total aroma compounds in fresh fruit pulp are mono- and sesquiterpene hydrocarbons (Chapter3; 4; Engel and Tressl, 1983; Idstein and Schreier, 1985). In addition, the color conferring compounds like anthocynins and isoflavonoids are also the products of same terpenoid biosynthesis pathways (Bohlmann et al, 1998). Therefore, study of this pathway is the most appropriate initiation for the transcriptomic studies in Alphonso.

Isopentenyl pyrophosphate isomerase (IPPI) marks the key point of terpene biosynthesis pathway. Cytosolic as well as plastidic isoprenoid biosynthesis has IPP as a common and exchangeable precursor. The type of terpene product (C5, C10, C15 etc.) is usually decided by the localization of these IPP units. Monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) and polyterpenes (Cn) are the customary products of the isoprenoid metabolism. These types cover the volatiles meant for ecological interactions, photosynthetic component phytol, pigments like carotene, nutritional elements like vitamin A, D, K, and other terpene derivatives. To ensure the production of such a diverse array of compounds to their respective required scales, plants must keep their IPP reservoir filled. In the organs like fruit where these chemicals are found in the highest amounts, the IPP metabolism would be a very interesting subject of investigation. Expression profile of IPPI gene in developing and ripening mango supported this hypothesis of reservoir. Highest transcript abundance was noted in flowers where majority of IPP pool is supposed to be reserved for the production of fragrance compounds. High regression values with sesquiterpene hydrocarbons assign this IPPI as a cytosolic form.

After the formation of IPP, geranyl pyrophosphate (GPP) formation is catalyzed by GPPS in the terpene biosynthesis pathway. GPP is the substrate for monoterpene synthases that produce major class of defense and/ or flavor volatiles as well as a precursor for the synthesis of other longer terpene chains. Indeed, the profile of GPPS gene expression (Fig 2b) suggests that it is an important factor to the fruit, as except that in the developmental and ripening transitions, for rest of the fruit life it is maintained at the elevated levels.

Cyclization or derivatization of GPP units is catalyzed by MTPS in plastids. We observed the ripening associated fall in the expression of MTPS that could well be

correlated with the quantitative gas chromatographic profiles of major monoterpene odorants reported earlier in chapter 4. This fall in expression of MTPS while ripening of fruits can be primarily attributed to the degradation of plastids during this phase. Indeed, as monoterpenes are synthesized mainly in chloroplasts, they are entitled to diminish from the blend of ripe fruit. However, several fruits have evolved their own molecular means to retain these compounds as their major flavorants. In cultivated strawberry, Aharoni et al (2004) explained this phenomenon, wherein during the process of ripening, cytosolic sesquiterpene synthases adopt GPP as an additional substrate to produce monoterpenes in the cytosol. Aharoni et al (2004) also demonstrated the role of mutations in the signal peptides that modifies the subcellular localization of the sesquiterpene synthase having a dual specificity; this creates diversity of locations for terpene biosynthesis and thus, can be a major factor in retaining monoterpenes in ripe fruits. Secondly, it has been demonstrated that the chromoplasts synthesize and retain monoterpenes in the chloroplast lacking tissues, especially by non-mevalonate pathway (Mettal et al, 1988; Fellermeier et al, 2001; Adam et al, 2002; Gao et al, 2002). This is very much relevant in the ripening fruits, where chromoplasts take over upon the degradation of chloroplasts (Medlicott et al, 1986; Parikh et al, 1990). Concept of cellular compartmentalization has been further obscured, as the contribution of plastidic pathway to the formation of sesquiterpene, nerolidol was discovered and was strongly supported by the cross talk that was detected between the cytosolic and plastidic pathways (Dudareva et al, 2005). With the given, high diversity of monoterpenes in Alphonso and other cultivars of mango (Chapter 3), several such mechanisms are likely to exist and mango will be an interesting system to study the biosynthesis of these compounds. As, several monoterpene hydrocarbons have been detected from developing and ripening

Alphonso fruit (Chapter 4), the product specificity for the presently cloned gene also needs to be studied in detail.

Apart from the use as a substrate for the formation of monoterpenes, GPP is also used in the synthesis of geranyl geranyl pyrophosphate (GGPP). GGPPS catalyses the condensation of two GPP units to form GGPP. This is a vital compound as a precursor of ubiquinone, the component of electron transport chain (Trumpower, 1981). GGPP is also important in forming essential pigments like chlorophylls and carotenoids, geranyl geranylated proteins (Rho, Rac, Rab etc.) that are translation regulators and hormones like gibberellins (Brown and Goldstein, 1993). We found in Alphonso as well as Sabja mangos that GGPPS levels are maintained throughout the fruit life; however, external stimuli such as harvesting were found to lower this expression.

In the cytosolic compartment, two molecules of IPP and one of dimethylallyl diphosphate (DMAPP) are condensed by FPPS to produce farnesyl pyrophosphate (FPP). FPP is also utilized in the synthesis of sterols and ubiquinone. Expression profile of FPPS gene in Alphonso tissues indicates that FPP is made available for the further processes on the temporal basis; further assimilation is probably regulated from this point in the pathway. This view is also supported by the high and positive regression values between the expression pattern of this gene and the GC profile of sesquiterpene hydrocarbons (Table 2). The downstream activities must be low near the harvesting period where this gene showed sharp decline in the expression.

FPP is further used as a substrate for sesquiterpene biosynthesis by SqTPS. In Alphonso fruit, ripening associated fall was observed in the expression of this gene. Lowest activity recorded in 2DAH fruit suggested that SqTPS gene responds to external stimuli like harvesting. Based on the GC profiles and the expression profile

of SqTPS, it is predicted that few more SqTPS forms must be present in Alphonso. The gene profiled here might have prominent role in Alphonso flowers and Sabja fruits. We had predicted that (chapter 4) with the help of enzymes that support cytosolic monoterpene synthesis (Aharoni et al, 2004) and the plastidic pathways that help sesquiterpenes produce (Dudareva et al, 2005), might be active in mango as the biogenesis of chief mono- and sesqui-terpenes were found to be synchronized during the development and ripening. Here, this view was further supported by the regression between the MTPS profile and the quantitative occurrence of major sesquiterpenes, β-caryophyllene and α-humulene (Table 2).

This analysis of terpene biosynthesis genes suggests that in addition to the volatile terpenes, in ripe fruit IPP and GPP are invested in different products. IPP and GPP allocation for biosynthesis of major non volatile isoprenoid constituents such as pigments and vitamin A must be studied in order to obtain a clear picture. Mono- and sesquiterpene products of the assessed genes contribute more to the raw mango flavor than the ripe one; this is in congruence with the results obtained from the gas chromatographic data of volatile emissions (Chapter 4). With the phylogenetic analysis, both, MTPS and SqTPS genes of mango were indicated to be involved in the formation of cyclic products. However, considering the terpene abundance in the mango flavor, more terpene synthase genes other than the assessed ones, are expected to be active during mango ripening.

Sequence based phylogeny revealed that mango GPPS gene was significantly different from the other plants' GPPS genes (Fig 1b). IPPI (Fig 1a), FPPS (Fig 1e) and GGPPS (Fig 1d) genes from mango were similar to their respective homologs in *Nicotiana* spp. Terminal genes in the terpene synthesis pathway MTPS (Fig 1c) and

SqTPS (Fig 1f) allied with the cluster of *Citrus* spp. and *Vitis vinifera* genes, respectively.

Terpene derivatives like ubiquinone, carotenoids and chlorophylls have been discussed above; moreover, terpenes contribute even further to the fruit metabolism by undergoing glucosylations and hydroxylations to produce taste and flavor compounds. Glucosylation of terpenes is catalyzed by glucosyl transferase (GT) enzymes (Schwab, 2003). Along with terpenes, these enzymes are known to accept wide range of substrates. Glucosylation affects organoleptic properties of the compounds, enhances their solubility, stabilizes the volatiles, assigns substances for further easterification and also helps different compounds to maintain homeostasis (Bowles et al, 2005; Poppenberger et al, 2005). Most of the functions of GTs are relevant and important for the fruit. GT cDNA isolated from mango resembled UDP-Glucose:cinnamate glucosyltransferase from *Fragaria* × *ananassa* and limonoid UDP glucosyltransferase from *Citrus unshiu* suggesting its role in flavor metabolism. Unique, biphasic expression profile of this gene in Alphonso fruit indicated the possibility of multiple substrate use by this enzyme as discussed by Schwab (2003). Secondly, it suggested a strong role in volatile, especially monoterpene glucosylation in the raw and ripe fruit.

An enzyme not directly related to terpene pathway, however, has a peripheral role to it in synthesizing ubiquinone is isochorismate hydrolase (IsoCH). This enzyme is also known to be involved in the metabolism of various cofactors, vitamins and other secondary (volatile) metabolites such as benzenoids (D'Auria and Gershenzon, 2005).Its expression showed steady rise from the early development till the late maturation phase (60DAP). Unlike GGPPS, which is also associated with the ubiquinone metabolism, IsoCH expression displayed sharp downfall in the ripening

period (in Alphonso as well as Sabja). Results suggested its probable role in the synthesis of components that are essential for fruit development rather than for the secondary metabolism. Moreover, IsoCH expression in flowers that was the highest among all the tissues needs to be studied with respect to floral benzenoid production.

Green aroma volatiles

Lipoxygenase (LOX) pathway is known to be involved in the production of C6 aldehydes and alcohols that impart green smell to the fruits. The C6 volatiles are also involved in the defense against insect herbivores (biotic stress) and jasmonate response. LOX oxidizes lipids and produces fatty acid hydroperoxides that are in turn converted to aldehyde volatiles by hydroperoxide lyase (HPL) (Feussner and Wasternack, 2002). Aldehydes are further derivatized to alcohols and esters that also are the important components of mango aroma. Ripening induced expression of mango LOX gene indicated the important role of LOX pathway products and their derivatives in the aroma of ripe fruit. In Sabja, as the PCR amplification profile suggests, this particular LOX allele was either absent or was not expressed.

Unlike LOX, expression of HPL gene was moderately constant through the development and ripening of fruits (Fig 2j). It can be concluded that in mango, HPL might be present in multiallelic or familial forms as described by Matsui (2006). Secondly, some green leaf volatiles must be emitted in development or ripening independent manner by mango.

Genes related to the dynamic environment of fruit

MDHAR

Along with flavor and taste, nutritional qualities of fruit decide its market success. Antioxidant levels have always been an important measure of fruit's nutritional quality (Huang et al, 2007). MDHAR is an important antioxidant enzyme

possessed by the fruits. It is involved in the recycling of antioxidative ascorbate radicles to cope up with the frequent oxidative spurts during the development and ripening of fruit (Jimenez et al, 2002). High MDHAR transcript levels indicate the high antioxidant levels in mango fruit and consequently ensure the high quality of nutrition. Expression shoot up observed at the fruit maturity was a very important finding for molecular and biochemical characterization of Alphonso fruit, as the appropriate harvesting maturity directly affects the quality of ripe fruit.

Multifunctional genes

By and large, the fruit research has focused the improvement of organoleptic values of the fruit and mechanism of ethylene- induced climacteric. Recent studies that generated comprehensive EST collections, added significant amount of information about the rest of fruit physiology. In these as well as in other exploratory exercises, the members of plant's stress responsive arsenal were frequently observed to be employed for the fruit metabolism (Aharoni and O'Connell, 2002; Moyle et al, 2005). Though in fruit, 'stress' is probably not the appropriate term the role of such genes seems to be pivotal. Most probable rationalization for this fact can be sought through the multifunctionality and familial nature of such genes (Schwab, 2003). Unlikeness in the physiology of fruit and other plant organs, as well as the share of plant life that is assigned for fruiting in most of the plants also explain this involvement of multifunctional, stress responsive genes; It is probably the economy of transcriptome size that the plants try to adopt and maintain through such genes.

14-3-3 domain proteins provide the best example. 14-3-3 family proteins are known to be involved in protein-protein interactions and signaling pathways where they perform multitude of functions as activators, repressors, chaperons and adaptors (Chung et al, 1999). With these myriad functions, these proteins are obviously the

handiest tools for stress management. Indeed, in plants as well as in animals they are the prime isolates of the stressed tissues (Roberts et al, 2002). These proteins have also been isolated from various fruits like tomato (Lemaire-Chamley et al, 2000), strawberry (Aharoni and O'Connell, 2002) and apple (Goulao and Oliveira, 2007). Though their role in fruit is unclear, their differential expression has been noted during the development and ripening of the fruit (Laughner et al, 1994; Laughner et al, 1995; Lemaire-Chamley et al, 2000). The form of 14-3-3 isolated from Alphonso fruit appears to be involved more in floral activities, fruit set and early development when the cellular activities are accelerated (Fig 2l). It suggests a specialized role of this gene in mango as it was detected within 30 cycles of PCR in all the sampled tissues.

Similar to 14-3-3, MTs form another group of composite-crisis managers. Elementary function of MTs is metal homeostasis; nonetheless, they act in almost all abiotic stress conditions (Mir et al, 2004) and are also found involved in the response to biotic stress (Potenza et al, 2001). Such a pronounced expression enforces their categorization as stress response genes. However, these genes are also associated with the ethylene response and fruit ripening (Clendennen and May, 1997; Liu et al, 2002; Mir et al, 2004). Transcript abundance of this gene was the highest among all the genes profiled in the current set of mango tissues (Fig 2m). Such a high expression of MT has always been associated with fruit life irrespective of the climacteric [banana (Clendennen and May, 1997; Liu et al, 2002), kiwifruit (Zhu et al, 2000)] or non climacteric [grape (Davies and Robinson, 2000) pineapple (Moyle et al, 2005), strawberry (Nam et al, 1999)] nature of the fruit where it was considered to be involved in the homeostasis of metallic cofactors, required by the variety of enzymes. In pineapple, its expression was correlated with the high oxidative environment

(Moyle et al, 2005). This can also be a putative role for MTs in several other fruits including mango as these ripening fruits often produce high amount of active oxygen species (Huang et al 2007). Secondly, the expression profiles in Alphonso as well as Sabja indicated important role of this gene in mango ripening.

Another stress related candidate isolated from Alphonso fruit was the ribosomal methyl transferase (MeTr) (Table 1). This enzyme is known to methylate the ribosomal 50s subunit upon heat shock as a signal to halt the protein synthesis; it uses S-adenosyl methionine (SAM) as a cofactor. It has been characterized mainly from bacteria; however, it is reported to be conserved from bacteria to humans (Bűgl et al, 2000). Recently, EST database constructions have reported similar sequences in plants like, Arabidopsis, Rice and grapevine; nonetheless, it is yet to be characterized in plants. Its low expression (35 cycle PCR) in most of the tissues suggested that it might not be a regulator of choice for protein synthesis under normal cellular conditions. However, upregulation in ripe Alphonso and Sabja fruits pointed its role in the thermal elevation at ripening, as an outcome of the climacteric. Thus, MeTr appeared to be a part of climacteric; based on these results, we propose its utility as a marker of ripeness.

A gene for mitochondrial small heat shock protein (sHSP) or low molecular weight heat shock protein (LMW HSP) was also isolated in the present experiment (Table 1). This mango sHSP belonged to the group of 22/ 23 kD sHSPs as suggested by the phylogenetic analysis (Fig 1o). Upon heat shock, this type of HSPs (17- 30 KD) are known to protect respiratory polypeptides from degradation by chaperonic action (Waters, 2005). Fruits develop hyperthermia during development and especially, ripening; therefore, proteins of such class are the handiest tools to cope with the situation. Interestingly, MeTr class of HSPs marked ripening phase with the

rise in expression whereas sHSP expression peak marked the maturity (90DAP). It indicates that in fruit, the hyperthermia is negotiated on the temporal basis and with the help of different chaperonic tools. Being a chaperone, its constitutive and high expression also shows high mitochondrial-proteomic activity in mango tissues, especially leaf and mature fruit.

Biotic stress managers

Along with abiotic stress managers, biotic stress related genes such as type II acidic chitinase (Fig 2q) expressed in low (35 cycle PCR) but ripening-upregulated manner in Alphonso fruit (Table 1). These chitinases are pathogenesis- related (PR) proteins that are induced upon abiotic (include chemical elicitation) as well as biotic (mainly fungal) stress. They catalyze the hydrolysis of polymeric chitin from the fungal cell wall and help plants defending against them (Payne et al, 1990; Flach et al, 1992; Khan and Shih, 2004). For fruit, this action may not be relevant and therefore, their presence can be explained either as a part of precautious defense arsenal or as an unknown stress manager; such an occurrence of PR proteins, including chitinase has been previously discussed in bell pepper, cherry and grape (Fils-Lycaon et al, 1996; Meyer et al, 1996; Robinson et al, 1997). Ripe mangos are abundant of sugary substances that can attract fungal pathogens; they probably deal with this posed threat by upregulation of chitinase transcription during ripening. Abundance of chitinase transcripts in flowers can also be explained in the similar way, in relation to the sugary nectar. Secondly, the annotation of this gene as ‘acidic’ chitinase was relevant to the acidic environment of the fruit, which suggested that among the several types, this type of chitinase was retained in the fruits as an adaptation. This enhances the possibility of broad-spectrum and pivotal role of this gene in the fruit.

In mango, we also profiled the gene for cysteine protease inhibitor (CysPI) or cystatin. These too, are the common isolates of different fruits but without any clarified role (Ryan et al, 1998; Shatters Jr. et al, 2004; Rassam and Laing, 2004); nonetheless, overall in plants, these molecules either regulate endogenous proteinase activity or protect from foreign proteinases of pathogens and insects (Bode and Huber, 1992). In fruit, the prior role was thought to be prominent (Ryan et al, 1998; Rassam and Laing, 2004). CysPI expressed with moderate levels (30 cycle PCR) in all the sampled tissues of mango (Fig 2p); here, its role as endogenous protector seems applicable, as the transcript abundance elevated in the busy periods such as early fruit formation, early ripening as well as in the short lived flower, notably, without any posed external biotic threat. Furthermore, this gene's phylogenetic uniqueness suggested that it might play novel and specialized role in the developent and ripening of mango. Unlike Alphonso, expression rise was observed during ripening in Sabja fruit where, the role of cystatins needs to be characterized.

Ethylene response

In addition to the flavor and stress related genes, a cDNA fragment annotated as plant specific transcription activation factor, associated with ethylene and abscisic acid response (ERF) was also isolated (Table 1; Fig 2r). In both, Alphonso as well as Sabja fruit tissues, high expression of ERF suggested that it is one of the key transcription factors and is involved in multitude of activities including the ethylene mediated climacteric. Aharoni and O'Connell (2002) isolated this transcription factor from strawberry and suggested its involvement in late achene development, the ethylene responsive phase.

Protein turnover

Proteins involved in almost all cellular activities are a subject of regulation by ubiquitin pathway (Hochstrasser, 1996). Ubiquitination system includes three main steps, ubiquitin activation by ubiquitin-activating enzyme (E1), binding of this complex to ubiquitin-conjugating enzyme (E2), and coupling of this complex to the target protein by ubiquitin–protein ligase (E3) (UbqPL). For most of the proteins, such assembly results in proteolytic degradation to terminate their role in the cell process. Thus in a broader sense, expression rise of ubiquitin pathway genes usually indicates the termination of physiological processes. Interestingly, mango ripeness was also marked by the expression rise of UbqPL (Fig 2s) suggesting its connection with the climacteric. In flowers, the high ubiquitination activity could be attributed to their ephemeral existence. Further, in mango, more inclusive role of ubiquitination and protein turnover could be depict, as these processes were also indicated to be involved in the physiological disorder of Alphonso mango, the spongy tissue (Ravindra and Shivashankar, 2004; Vasanthaiah et al, 2006) .

Overall, this analysis revealed the temporal and spatial regulation of flavor and stress related genes during the development and ripening of Alphonso mango. Zeroing down of the expression of many genes at 2DAH stage advocated our *priori* hypothesis about the ‘zero day’ stage that 90DAP fruit upon harvesting may carry the continuation of *in planta* processes and therefore, can be regarded as ‘virtual’ zero day, whereas within two days, almost complete halting of *in planta* processes may take place to consider 2DAH as a ‘real’ zero day stage. This finding was congruent with the GC analysis (Chapter 4) and thus, is important for the planning of further biochemical and molecular studies in Alphonso fruit. Expression peaks of sHSP and MDHAR genes and the characteristic expression drop of chloroplastic GPPS and

MTPS genes at 90DAP, ascertained this stage as a perfect physiological maturity for harvesting. Similarly, 15DAH could be marked as a perfect ripe stage by the expression peaks of FPPS, LOX, MeTr, Chitinase and UbqPL genes.

Sesquiterpene dominance in Sabja that was revealed by the GC analysis (Chapter 4) was confirmed in this molecular analysis. Secondly, low relative expression of monoterpene synthase gene ensured that the sesquiterpene dominance was relative (as shown by the GC analysis) and was due to the lack of major flavorants, monoterpenes (Chapter 3). Absence of HPL expression supported the observation of qualitative weakness in the flavor of Sabja (Chapter 3, 4); nevertheless, such a conclusion must be supported by the analysis of additional flavor genes.

CHAPTER 6

Summary, Conclusions and Future Prospective

Mango, 'the King of Fruits' is a delicious and widely cultivated fruit for a fresh market use. It is the second most important tropical fruit after banana and is one of the oldest cultivated tropical fruits, originated in Indo-Burmese region. Present dissertation discusses various aspects of mango with emphasis on the cultivar Alphonso. These aspects include the genetic diversity among the mango cultivars (Indian as well as non Indian), volatile flavorant diversity among them, aroma profile of developing and ripening Alphonso mango and its gene expression dynamics.

Genetic diversity analysis

India has a large diversity of mango cultivars with an estimated number exceeding one thousand. In India, where such a high diversity of mango cultivars originated and exists, ambiguities in cultivar identification and nomenclature are also common especially in case of the cultivars that exhibit prominent similarities in their morphological features. It is essential to authenticate the identities of such cultivars as well as analyze the diversity among the existing cultivars. DNA based markers can be the best tools to resolve such ambiguities. In the present work polymerase chain reaction (PCR) based inter simple sequence repeat (ISSR) marker system was used to probe the relationships among 70 mango cultivars that included North Indian, South Indian and also non Indian cultivars along with an outgroup *Nothopegia colebrookiana* Blume.

Out of 100 ISSR primers, 40 showed amplification in 11 genotypes that were used for the initial screening. Of these 40, 33 primers generated reproducible polymorphic DNA amplification patterns in all the 71 genotypes. These 33 primers yielded a total of 420 scorable bands on amplification and their sizes ranged between 200 bp to 2000 bp. Twelve different cultivar specific bands were obtained from the amplification profiles with eight ISSR primers. Separation of non-Indian cultivars

from the Indian cultivars was revealed in the cluster analysis, however, no such separation was revealed between the north Indian and the south Indian cultivars. Indian cultivars formed small subgroups and no prominent lineages could be sketched. This could be because of the cultivar spreading in the recent past and higher rate of new cultivar generation in India. Cultivars those were suspected to be synonymous, opted different subgroups rejecting the claims of ambiguous synonymy. Lastly, ISSR emerged as an informative marker system for genetic diversity analysis of mango.

Qualitative and quantitative analysis of flavorant diversity in mango

Morphological and molecular diversity analyses help significantly in cultivar identification; however, use of biochemical features, the actual desired traits, will definitely supplement this task and confer a functional dimension. The volatile component of flavor presents a good experimental system for such endeavor as it includes array of chemicals from various classes such as alcohol, aldehydes, ester, ketone and terpene. In the present study, 27 mango cultivars that include Indian as well as non-Indian ones were analyzed based on the gas chromatographic profiles of their ripe fruit extracts. Based on qualitative as well as quantitative presence of compounds among these cultivars, the diversity and the relationship were assessed.

More than hundred compounds could be detected from the selected pool of mango cultivars. Volatiles from classes such as alcohol, aldehydes, ester, ketone and terpene were found to be mixed in the flavor of all the analyzed cultivars. Mono- and sesqui-terpenes dominated the gas chromatographic profiles of all cultivars. Eighty five different volatile constituents were detected from the blends of 27 cultivars. Aroma of cultivar Alphonso contained the highest number of volatiles (36), whereas Musharad and Pairi fruits contained the lowest number (17). On the quantitative basis,

δ-3-carene, (*Z*)-ocimene, (*E*)-ocimene, β-myrcene, limonene and β-caryophyllene were the major aroma compounds in mango, whereas furaneol, *allo*-ocimene, (Z)-2, 6-dimethyl-3, 5, 7-octatriene-2-ol and all lactones had qualitative importance. Aroma composition of 20 cultivars showed dominance of monoterpene hydrocarbons, whereas remaining seven cultivars showed dominance of sesquiterpene hydrocarbons in their blend. Multivariate analysis revealed that Indian and non Indian cultivars form separate groups whereas the line of distinction between the North- and South-Indian cultivars is obscure. In this analysis quantitative dominance as well as the uniqueness of flavorants contributed to the cultivar grouping.

Aroma profiling through the development and ripening of Alphonso fruit

Alphonso is the most popular and most exported cultivar of India. It is also the flavor of choice for the mango lovers all over the world. In addition, Alphonso is blessed with attractive color, ample, sweet, low fiber containing pulp and long shelf life. In spite of possessing so many virtues, this cultivar is troublesome for farmers because of its erratic and shy bearing, and cultivation locality dependent variation in the fruit quality. It is known that the environment that reigns during the fruit development enforces such alteration in the quality of ripe fruit. To understand the complexities of this problem, the chemistry of developing fruit should be studied in relation to the influencing environment. Current work deals with the biological dimension of this problem, wherein we have attempted to baseline the nature of biochemical changes that occur during the development and ripening of Alphonso mango.

Among the sampled Alphonso fruit tissues, the highest concentration of volatiles was detected in 5DAP (15665µg g^{-1}) and the lowest in 2DAH (62µg g^{-1}); ripe fruits (15DAH) (966µg g^{-1}) had more than ten folds higher content of volatile

odorants than the raw fruits (90DAP) (94µg g^{-1}). Monoterpene hydrocarbons quantitatively dominated the volatile blend of all Alphonso tissues; except that in 90DAP (79%), 2DAH (53%) and 20DAH (81%) fruits, in all other tissues, these compounds comprised more than 90% of the aroma.

This analysis revealed that the monoterpenes remain quantitatively dominant components throughout the fruit life. Based on the volatile profiles, fruit setting and early development were predicted to be vibrant whereas the stages near maturity were thought to be calm. Ripeness was found to be attained at the 15^{th} day after harvesting where the levels of most of the flavorants were elevated. It was characterized by the strong presence of lactones, furaneol and mesifuran in addition to the terpenes. Floral blend was found to be dominated by the C6 GLV and monoterpene alcohols suggesting the spatially differential role of aroma volatiles. Overall, the results suggested that the flavor of the Alphonso is a combination of freshening sap scent and the ripening induced fruity odor.

Gene expression dynamics in developing and ripening Alphonso mango

Along with the qualitative and quantitative profiles of various Alphonso flavorants, expression profiles of various flavor and non flavor genes were analyzed in the same set of developing and ripening fruit tissues using relative quantitation PCR.

This analysis supported the findings of the Gas chromatographic analysis. Expression peaks of sHSP and MDHAR genes and the characteristic expression drop of chloroplastic GPPS and MTPS genes at 90DAP, ascertained this stage as a perfect physiological maturity for harvesting. Similarly, 15DAH was marked as a perfect ripe stage by the expression peaks of FPPS, LOX, MeTr, Chitinase and UbqPL genes. Overall, the expression profiles suggested that the aroma related genes might be under

the same control that the stress related genes were. The fruit might be a mimic of stressful system, rather than really being stressed. The stress related genes were probably used to manage the vibrant activities in this busy tissue by its own way. Lastly, this analysis also indicated that the aroma pathways might be regulated far upstream from the actual product formation steps.

Future prospects

Present work on mango aroma has given primary information about genetic as well as aroma diversity among different cultivars, qualitative and quantitative variation of volatiles during mango development and ripening and the expression of various genes during these processes. However, it has built a platform to carry out more in depth studies and has provided following directions for future studies.

- Genetic diversity studies with larger sample set and with other markers to have a complete understanding of the genetic structure of mango germ pool.
- Aroma composition studies for bigger set of cultivars and correlation of this information with that from the genetic diversity studies.
- Detailed analysis of Alphonso flavor with different volatile extraction and detection methods.
- Sorting of odor active compounds to facilitate different formulations of mango flavor.
- Identification of newly detected compounds and ascertaining their contribution to aroma.
- Characterization of glycosidically bound aroma compounds.
- Profiling of new genes and different isoforms of the present set to have further depth in the knowledge about active metabolic pathways.

- Characterization of currently analyzed genes to understand the kinetics and regulation of volatile biosynthesis.
- A broader study, involving multiple Alphonso-growing localities, to understand the environmental regulation of flavor biogenesis.

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ANNEXURE I

Annexure I. **Sequences of 20 different cDNA fragments, their genebank accession numbers (NCBI) and their respective amino acid sequences.**

>EU513264 ***Mangifera indica*** **putative isopentenyl diphosphate isomerase (IPPI) mRNA, partial cds**.

CTCATCGAGGAGGATGCTCTTGGGGTGAGAAACGCTGCACAAAGAAAGCT
TTTGGATGAGCTGGGCATTGTTGCTGAAGATGTGCCGGTTGATCAGTTCAC
CCCACTCGGTCGGATTCTCTACAA

Protein:

LIEEDALGVRNAAQRKLLDELGIVAEDVPVDQFTPLGRILY

>EU513265 ***Mangifera indica*** **putative geranyl diphosphate synthase (GPPS) mRNA, partial cds.**

TCTTGTTACGGGTGAAACCATGCAAAAGACTACTTCATCTGATCACGGTGT
CGCATGGAATATTATATGCAAAAAACATACTACAAGACTGCTTCATTGAT
ATCAAACAGCTGCAAGGCAATTGCTCTTCTTGCTGGGCAATCAGCAGAAG
TTGCAATGTTGGCTTTCGAGTTGGAAAAAATCTGGGACTGGCCTACCAATT
AATAGATGACGTTCTTGATTTCACGGGCACATCAGCTTCACTTGGAAAGG
GATCTTTATCGGACATACGGCATGGAATTGTAACGGCTCCTATACTGTTTG
CAATGGAAGAATTCCCCCAGTTGCGTGCAGTTATTAATCAGGGCTTTGAA
AATCCTTCAAACGTCGATGTTGCCCTTGAATACCTTGGCAAGAGTCGGGG
AATACAAAGGACGAGAGAGCTAGCGATGAACCATGCCAACCTTGCTGCA
GCTGCCATCGATGCTCTACCCGAAACTCACAATGAAGAAGTAAGAAAGTC
AAGACGGGCACTTTTAGATCTAACTCAAAGAGTCATCACAAGAACCAAAT
AA

Protein:

LLRVKPCKRLLHLITVSHGILYAKNILQDCFIDIKQLQGNCSSCWAISRSCNVG
FRVGKNLGLAYQLIDDVLDFTGTSASLGKGSLSDIRHGIVTAPILFAMEEFPQL
RAVINQGFENPSNVDVALEYLGKSRGIQRTRELAMNHANLAAAAIDALPETH
NEEVRKSRRALLDLTQRVITRTK.

>EU513266 *Mangifera indica* putative monoterpene synthase (MTPS) mRNA, partial cds.

GGTGTGTTGAAAAAGTTCAAGGACACGACGGGCAGTTTCAAAGAGTGTCT
TCGCGACGATATCAGGCTATGCTGGCCCTTTATGAAGCTTCATATCATGGG
TTTGATGGAGAAAATGTCATGGAGGAGGCTTGGCAGTTTACATCTAAATA
TCTGAAAGAGGTTGACACAAAGGATATAGACCAGAATATGGCATTGCAAG
TGAAGCACGCAATGAATCTTCCA

Protein:

CVEKVQGHDGQFQRVSSRRYQAMLALYEASYHGFDGENVMEEAWQFTSKY
LKEVDTKDIDQNMALQVKHAMNLP

>EU513267 *Mangifera indica* putative geranyl geranyl diphosphate synthase (GGPPS) mRNA, partial cds.

TCGAGATGATTCACACCATGTCCTTAATTCATGATGATTTACCTTGTATGG
ATAATGATGACCTTCGTCGTGGCAAACCCACAAACCACAAAGTTTTCGGA
GAAGATGTCGCAGTTTTAGCCGGAGATGCACTGCTTGCTTTTGCATTTGAA
CACATGGCTGTTTCTACTGTTGGCATTCCGCCTTCGAGGGTGGTCAAAGCA
GTTGGAGAATTAGCGAAATCGATTGGCATTGAGGGTCTTGTTGCCGGCCA
AGTTGTGGATATAAACTCTGAAGGTTTAAAAGAAGTGGGTTTAGATCATC
TTGAATTTATTCATCAGCATAAGACAGCTGCATTACTGGAAGGATCAGTG
GTTCTTGGAGCAATATTGGGTGGTGGAAGTGATGATGAAGTTGAAAAGCT
GAGAACTTTTGCTCGGTGTATTGGGTTGTTGTTTCAGGTGGTTGATGATAT
TCTTGATGTGACCAAATCATCTCGGGAATTGGAAAGACTGCTGGTAAGGA
TTTGGTGGCTGATAAAGTCACTTATCCTAAGTTGCTGGGGATTGAAAAATC
AAGGGAATTACCTGACAATTGCATTAAAGATGCTCAACAACAATTGTCTG
GATTTGATCAGGAGAAAGCCGCTCCTTCGATTGCTCTGGCTGAATTATATT
CCATA

Protein:

EMIHTMSLIHDDLPCMDNDDLRRGKPTNHKVFGEDVAVLAGDALLAFAFEH
MAVSTVGIPPSRVVKAVGELAKSIGIEGLVAGQVVDINSEGLKEVGLDHLEFI
HQHKTAALLEGSVVLGAILGGGSDDEVEKLRTFARCIGLLFQVVDDILDVTKS
SRELERLLVRIWWLIKSLILSCWGLKNQGNYLTIALKMLNNNCLDLIRRKPLL
RLLWLNYIP

>EU513268 *Mangifera indica* putative farnesyl diphosphate synthase (FPPS) mRNA, partial cds.

AGTATTCATTGCCACTTCATTGCCAGATAGTTCAGTACAAACTGCTTATTA
CTCTTTCTACCTTCCGGTTGCTTGTGCTTTACTTATGGCAGGCAAAAATCTT
GATGATCACATTGATGTCAAGAACATTCTTATTGAAATGGGAATCTATTTT
CAAGTACAGGATGATTATCTAGATTGTTTTGGCACTCCTGAAGTGATTGGT
AAGATTGGAACTGATATTGAAGATTTTAAGTGCTCTTGGTTGGTTGTGAAA
GCAATGGAACGTTGTAACGAAGAACAGAAGCAATTGTTAATTGAGAATTA
TGGGAAAGCAGATCCAGCCTGTGTT

Protein:

VFIATSLPDSSVQTAYYSFYLPVACALLMAGKNLDDHIDVKNILIEMGIYFQV
QDDYLDCFGTPEVIGKIGTDIEDFKCSWLVVKAMERCNEEQKQLLIENYGKA
DPACV

>EU513269 *Mangifera indica* putative sesquiterpene synthase (STPS) mRNA, partial cds.

GGCAAATCAAGGAAAATTATATCGTCTTGATTTTGCAAAAGAGGCGATGG
AGGATATAGTTAGAAATTATCACACTGAAGCCAAATGGTGTCATGAAAAT
TATTTTCCAACATCGGATGAGTACATGAGTGTAGCATTGGTTACCAGTGCG
TACCAATTGCTACCCACAACATCTTTGGTAGAAATGGGAGATGTGCAACC
AAAGAAGCCTTTGAATGGCTATTCAGCTACCCTAAGGTTTTTGAAGGCTGC
CACAAATAATTGCAGACTCATGGATGACATAGTGGACACAAGCATGAGCA
AAGAGAGGACATGTGCCTCAGCAATTGAATGTCACATGAAGGAACATGGT
GTTCAGGAAAAGAGACGATTAAAGTGTTTCTTGAGCAAATTGCAAATGCA
TGGAAAGATATTAATGAAGCTTTCCTTAAACCAACTGCTGCTCCAGTGCCT
CTGCTTGATCGTATTCTTAATTTTTCACGTGTATAGACCTTCTTACAAAGAC
GATGACTGCTACACCAATTCTTATTTGACCAAAGACCATGTTGCTTCATTG
CTCAGATACTCCGTACAGATCTGA

Protein:

ANQGKLYRLDFAKEAMEDIVRNYHTEAKWCHENYFPTSDEYMSVALVTSAY
QLLPTTSLVEMGDVQPKKPLNGYSATLRFLKAATNNCRLMDDIVDTSMSKER
TCASAIECHMKEHGVQEKRRLKCFLSKLQMHGKILMKLSLNQLLLQCLCLIV
FLIFHVYRPSYKDDDCYTNSYLTKDHVASLLRYSVQI.

>EU513270 *Mangifera indica* putative Isochorismatase hydrolase mRNA, partial cds.

ATGAGATCCGAAAGAGAAACCCAGATCCTAAGACTTGTGTTTTATTAGTG
ATCGACATGCAAAACTATTTCTCCGCCATGGCCAAACCCATTCTCGACAAC
CTTCTCACCACCATCCGCCTCTGCCGACGCGCCTCCATCCCCGTCTTCTTCA
CCCGCCACTGTCACAAGTCCCCCGCCGACTACGCCATGCTTGGCGAGTGG
TGGAATAACGACCTTGTTTACGACGGCACCGTGGAGGCCGAGCTCATGCC
CCAGATTAAAGAGGTGGCGAGCGCTGATGAAGTGATCGAGAAGAATACTT
ACAGCGCGTTTGTTAGCACGCGCTTGCAGGAGCGGTTGGTGGAGATGGAT
GTGAAGGAGGTGATAGTGAGTGGAGTTATGACTACTTTGTGTTGTGAAAC
GACGGCGCGTGAGGCGTTTGTGAGAGGGTTTAGGGTGTTTTTTTCGACGG
ATGCGACAGCCACGTCAGATATTGAACTACATGAGGCTACCTTGAAGAAC
TTGGCATATGGGTTTGCTTACTTGGTTGACTGTGAAA

Protein:

EIRKRNPDPKTCVLLVIDMQNYFSAMAKPILDNLLTTIRLCRRASIPVFFTRHC
HKSPADYAMLGEWWNNDLVYDGTVEAELMPQIKEVASADEVIEKNTYSAFV
STRLQERLVEMDVKEVIVSGVMTTLCCETTAREAFVRGFRVFFSTDATATSDI
ELHEATLKNLAYGFAYLVDCE

>EU513271 *Mangifera indica* putative glucosyl transferase mRNA, partial cds.

AATGGAGTCCGCAAGAGAAAGTTTTGGTTCACCCGAGTGTCGCTTGCTTTG
TAACACACTGCGGATGGAACTCGACAATGGAGTCGTTAACTTCCGGCATG
CCGGTTGTGGCTTTCCCGCAGTGGGGCGATCAAGTCACTGATGCTGTGTAC
TTAGTGGAAGTATTCAAGACCGGGATCCGAATGTGCCGTGGAGAGGCCGA
AAACAGGATAATCCCTCGTGAGGAGATTGAGAAATGCCTGCTGGAGGCGA
TATCGGGGCCTAAGGCGGCGGAGATGAAGCAAAACGCATTGAAGTGGAA
GAAGGTAGCGGAGGAAGCGGTGGCAGAAGGTGGCTTCTCCGACAGGAAC
ATTCAAGAGTTTGTAGATGAGGT

Protein:

WSPQEKVLVHPSVACFVTHCGWNSTMESLTSGMPVVAFPQWGDQVTDAVY
LVEVFKTGIRMCRGEAENRIIPREEIEKCLLEAISGPKAAEMKQNALKWKKVA
EEAVAEGGFSDRNIQEFVDE

>EU513272 *Mangifera indica* putative lipoxygenase (LOX) mRNA, partial cds.

GACTATCCATATGCTGTGGATGGGCTTGAAATCTGGTTTGCAATAAAAAA
CTGGGTCAAAGACTATTGCTACTTCTACTACAAAAGCGATGAAATGATGC
AAAAGGATAGTGAACTGCAATCCTGGTGGAAGGAACTACGCGAGGAGGG
TCATGGTGACAAGAAAGATGAGCCCTGGTGGCCTAAAATGCAAAATCGTG
AAGAGCTGATAGAGGCATGCACCATAATCATATGGATAGCTTCCGCTCTC
CATGCTGCAGTTAACTTTGGCCAATACCC

Protein:

DYPYAVDGLEIWFAIKNWVKDYCYFYYKSDEMMQKDSELQSWWKELREEG
HGDKKDEPWWPKMQNREELIEACTIIIWIASALHAAVNFGQY

>EU513273 *Mangifera indica* putative hydroperoxide lyase (HPL) mRNA, partial cds.

GTGCTCGTTGGGCATTTCATGCCTAGTGTCAAATTTACTGGAAATTTAAGA
ACTTGCGCTTATCTTGATACTTCTGAGCCACAACACGCTAAGATCAAGAAC
CTCGTCCTTGACATTCTGAAACGCAGTTCAACAGTGTGGCTTACAGCGCTC
AAGTCGAACCTCGACACATTGTTTGACACCATTGAAACGAATATCTCCGA
GAAGGGTTCTGCAAGCTTTTTATTCCCTTTACAAAAATGCTTGTTCAACTT
CCTCACAACGGCCATCGTTGGAGCTGATCCCACAACCGACCCTAACATCG
CCGACTCCGGCTATGCCATGCTGGACCGCTGGCTCGCCCTACAGATCCTCC
CCACCGTCAAAATTGGAATCTTACAGCCTCTTGAAGAGATTTTTCTTCACT
CTTTTGCTTACCCCTTTGCCCTCGTAAGTGGAGGCTACAATAAGCTTTATA
ACTTCGTTGAAAAACAAGGCAACGAGGTGGTGCAACGAGGTGTCACCGA
GTTTGGACTCACTAAAGAAGAAGCTACCCATAATTTGTTGTTCACGCTAGG
CTTCAACGCCTTTGGGGG

Protein:

VLVGHFMPSVKFTGNLRTCAYLDTSEPQHAKIKNLVLDILKRSSTVWLTALK
SNLDTLFDTIETNISEKGSASFLFPLQKCLFNFLTTAIVGADPTTDPNIADSGYA
MLDRWLALQILPTVKIGILQPLEEIFLHSFAYPFALVSGGYNKLYNFVEKQGN
EVVQRGVTEFGLTKEEATHNLLFTLGFNAFG

>EU513274 *Mangifera indica* putative monodehydrogenase ascorbate reductase (MDHAR) mRNA, partial cds.

GCTGCTTTCTATGAGGGTTATTATGCTAATAAGGGAGTAAAAATTATCAA
GGGAACTGTTGCAGTTGGATTTACAGCTGATGCTAATGGAGAGGTGAAAG
AAGTTAAACTAAAGGATGGCAGGGTTCTGGAAGCTGACATTGTTGTTGTT
GGTGTTGGAGGTAGACCTCTCATATCATTAGTTAAGGGGCAACTTGAAGA
GGAGAAAGGTGGAATTAAGACTGATGCATTCTTCAAGACAAGTGTTCCTG
ATGTTTATGCTGTGGGAGATGTT

Protein:

AAFYEGYYANKGVKIIKGTVAVGFTADANGEVKEVKLKDGRVLEADIVVVG
VGGRPLISLVKGQLEEEKGGIKTDAFFKTSVPDVYAVGDV

>EU513275 *Mangifera indica* putative 14-3-3 domain protein (14-3-3) mRNA, partial cds.

ATGGCTTCCACTCCTTCAGCTCGCGAGGAGAACGTCTACATGGCCAAGCTT
GCTGAGCAAGCTGAGCGTTACGAGGAGATGGTTGAGTTCATGGAAAAGGT
TTCAGCTTCCTCTGAGAATTCTGAAGAGCTCAACGTAGAAGAACGTAACC
TCCTCTCCGTTGCCTACAAGAATGTTATCGGGGCGCGTAGAGCCTCATGGC
GTATAATATCCTCCATTGAACAGAAAGAGGAGAGCCGTGGAAACGAAGG
CCACGTCTCTACGATCCGAGATTACCGTTCAAAGATCGAGACCGAGCTGT
CCTCGATCTGTGACGGGATCTTGAAGCTGCCCGACTCTCGGCTCATTCCCT
CGGCTTCATCTGGTGACTCCAAAGTTTTTTATTTGAAGATGAAAGGAGATT
ACCATAGGTACTTGGCCGAGTTCAAGACCGGAGCCGAGCGAAAAGAAGC
TGCTGAGAGTACTCTCACTGCCTACAAATCGGCTCAGGATATTGCAAACG
CAGAACTGGCTCCCACTCATCCAATTCGTCTAGGACTGGCTCTCAACTTCT
CTGTGTTCTACTATGAGATTCTGAATTCTCCTGATCGCGCTTGCAATCTTGC
CAAGCAGGCTTTTGACGAGGCAATTGCCGAGTTAGATACCCTTGGTGAAG
AGTCATATAAGGACAGCACTCTGATCATGCAGCTACTCCGTGATAATCTC
ACTCTCTGGACATCCGACATACCGGATGATGGAGCTGATGAAATTAAAGA
AGCCACCAAGCCTGACAGTGAACAGCAG

Protein:

MASTPSAREENVYMAKLAEQAERYEEMVEFMEKVSASSENSEELNVEERNL
LSVAYKNVIGARRASWRIISSIEQKEESRGNEGHVSTIRDYRSKIETELSSICDGI
LKLPDSRLIPSASSGDSKVFYLKMKGDYHRYLAEFKTGAERKEAAESTLTAY

KSAQDIANAELAPTHPIRLGLALNFSVFYYEILNSPDRACNLAKQAFDEAIAEL
DTLGEESYKDSTLIMQLLRDNLTLWTSDIPDDGADEIKEATKPDSEQQ

EU513276 *Mangifera indica* putative metallothionein mRNA, complete cds.

ATGTCTTCTGGTTGTAACTGTGGCTCCAACTGCTCCTGCGGCAGCGACTGC
AAATGTGGCAAGTACTCTGATCCGGCTTTCACGGAGGTGGCAACCACCGA
GACACTCATCGTCGGGGTTGCTCCGGTGAAGATGCACCTTGAGGGATCTG
AGATGAACTATGGGACAGAGAACTGCGGCTGTGGAGACAACTGCTCCTGC
AACCCCTGCAAATGTGGCAAGTGA

Protein:

MSSGCNCGSNCSCGSDCKCGKYSDPAFTEVATTETLIVGVAPVKMHLEGSEM
NYGTENCGCGDNCSCNPCKCGK.

>EU513277 *Mangifera indica* putative ribosomal methyl transferase mRNA, partial cds.

TTCCGTGATACTCTCAGATATGTGTCCTTCAGTTTCTGGAATTACAACTAA
AGATGCAGCTTTATCTGCTGAGTTAGGGATGCGAGCTCTTGATTTGGCTGT
TGGTTGTGCTGCCTCACCTCATCCAGTTGGTGATCAAGGGGAGAGACATCT
GAATGATTCAAATTCTGATCCAGATGAAAATGGTGTTTTGAAACCAGGTG
GTCACCTTGTCATTAAGCTTCTAGAGAGTGAGGATGTGAAAGAATTTAGC
CAAATTTGCAAACCACTCTTCAGAAAGGCATCATGGTTGCGGCCTAAAGC
TACAAGATCATCATCCAGAGAGATTTATTTAATTTGTCAAGAT

Protein:

SVILSDMCPSVSGITTKDAALSAELGMRALDLAVGCAASPHPVGDQGERHLN
DSNSDPDENGVLKPGGHLVIKLLESEDVKEFSQICKPLFRKASWLRPKATRSS
SREIYLICQD

>EU513278 *Mangifera indica* putative mitochondrial small heat shock protein (sHSP) mRNA, partial cds.

GACCTCAGCCTCTCGCTTCTTCAACACCAACGCCGTTCGTCACCGGGACGA
TGAGTCCGACGCCCGCGACCTCGACGTTGACCGTCGATCTGTTCCTCACCG
CCGCGATTTCTTCTCAGATGTGTTTGATCCGTTCTCTCCAACAAGGAGCTT
GAGCCAGGTTCTGAACCTGATGGACCAAATGACTGAGAATCCGTTCTTTG
CTGGGACACGTGGCGGCCTACGCCGAGGCTGGGATGCAATAGAAGACGA

AAACGCTCTGAAACTCCGAATCGACATGCCAGGGCTGGGAAAGGAAGAT
GTGAACGTGTCAGTGGAACAGAGCACACTGGTGATCAAAGGTGAAGGAG
CGAAAGAAGCTGATGATGAAGAAAGCATTCGAAGGTACACTAGCAGAAT
CGATCTGCCTGAGAAGATGTACAAGACCGATGGGATCAAGGCGGAGATG
AAGAACGGTGTGTTGAAGGTGGTGGTGCCCAAGGTAAAGGAAGAGGAGA
GGAGTGACGTGTTCCAGGTAAAAATTGA

Protein:

TSASRFFNTNAVRHRDDESDARDLDVDRRSVPHRRDFFSDVFDPFSPTRSLSQ
VLNLMDQMTENPFFAGTRGGLRRGWDAIEDENALKLRIDMPGLGKEDVNVS
VEQSTLVIKGEGAKEADDEESIRRYTSRIDLPEKMYKTDGIKAEMKNGVLKV
VVPKVKEEERSDVFQVKI

>EU513279 *Mangifera indica* putative type II acidic chitinase mRNA, partial cds.

ATGTCCCAAAACTGTGACTGTGCTCCCAACTTGTGTTGCAGTCAGTTTGGT
TACTGTGGCACCGGCGAAGCCTACTGTGGATTGGGGTGTAAGGGGGGTCC
TTGCACCTCGACGCCATCGACACCGTCACCTACACCAACCGGTGGTGGTTC
AGTTGCCAGTATTGTTACGGCTGATTTCTTTGATGGGATAAAGAATCAAGC
TGCTGCAAGCTGTGCTGGAAAGAGCTTCTACACAAGAGATGGATTTCTTA
ATGCAGCCAATTCGTTTCCTCAGTTTGGATCAGGCTCTGCCGACGAATCCA
AGCGTGAGATTGCTGCATTTTTTGCCCACGTTACTCATGAAACTGGACATT
TATGCTACACCGAAGAGATTGACAAGTCAAATGCCTACTGTGACCAATCA
AACACACAGTACCCATGTGTCCCCGGAAAGAAGTATTACGGGCGCGGACC
GATGCAGCTCACCTGGAACTACAACTACGGTGCCTGTGGAACAGCCGTCG
GGTTCGATGGACTCAACGCTCCCGAAACAGTGTCTAATGACCCTGCTGTCT
CCTTTAAGGCTGCCTTGTGGTTCTGGATGACCAATGTTCACTCAGTCATGA
ACCAAGGCTTTGGGGCTACCATTCAGAAAATTAATGGCGCTCTTGAATGC
GGTGGAAAGCAACCTGATAAAGTTAATGCTCGTATTGGTTATTACACTGA
TTATTGCCAGAAATT

Protein:

MSQNCDCAPNLCCSQFGYCGTGEAYCGLGCKGGPCTSTPSTPSPTPTGGGSV
ASIVTADFFDGIKNQAAASCAGKSFYTRDGFLNAANSFPQFGSGSADESKREI
AAFFAHVTHETGHLCYTEEIDKSNAYCDQSNTQYPCVPGKKYYGRGPMQLT

WNYNYGACGTAVGFDGLNAPETVSNDPAVSFKAALWFWMTNVHSVMNQG
FGATIQKINGALECGGKQPDKVNARIGYYTDYCQK

>EU513280 *Mangifera indica* putative cystatin mRNA, partial cds.

GTTGTGAAGGCGAAGCGGCAGGTGGTTTCTGGAACTGTGTATTATCTAAC
TCTGGAGGCGAAAGAGGGGGATCAGAAGAAGCTTTATGAAGCCAAAGTG
TGGGAGAAGCCTTGGTTGCACGTCAAGGAGTTGCAGGAGTTTAAGGTTCT
TGATGCTGCTTCTGCTTAG

Protein:

VVKAKRQVVSGTVYYLTLEAKEGDQKKLYEAKVWEKPWLHVKELQEFKVL
DAASA.

>EU513281 *Mangifera indica* putative ethylene response factor mRNA, partial cds.

AGATGTTGCAACCAAAGAAGCCGCCAGAGCCTACGACGAAGCCGCCAAG
CGCATACGTGGAGATAAAGCCAAGCTCAACTTTGCTGAACCACCAGCACC
TCCGCCTACTCCACCACCTCCGCCTACTCCACCACCTCAAGACGAACCTCC
GTCTAAAAAGCGCTGCTGCATATCCCCACCCGAGTTGACTCAGCCGAGTTT
ACCATCACCCTACGCGGATTTCGGGTTTGAAAACGAGTTTTATCATCAACC
CAATGAAGTTGGGCGGAATAACAACGAGCTGGAGCTGAAAGAGCAAATC
TCCAGCTTGGAGGCGTTCCTGGGGCTGGATCCAACGACTCAGCAGCTGAA
CGGAAACGGCGACTGTTACTCGGCTGATTTATGGATGCTGGATGACGTGG
TGGCGCCTGTTTATCAGCAGAATATTGATCGTGATCACCTCTTA

Protein:

DVATKEAARAYDEAAKRIRGDKAKLNFAEPPAPPPTPPPPPTPPPQDEPPSKK
RCCISPPELTQPSLPSPYADFGFENEFYHQPNEVGRNNNELELKEQISSLEAFLG
LDPTTQQLNGNGDCYSADLWMLDDVVAPVYQQNIDRDHLL

>EU513282 *Mangifera indica* putative ubiquitin protein ligase mRNA, partial cds.

ATGTAGCAGCCATTGAGGCTCTGGTTCGCAAGCTCTCAAGCCGGTCAGTT
GAGAAGCAAAGAGCTGCTGTGGCTGAAATAAGATCACTATCCAAAAGAA
GTACAGATAACAGGATACTAATTGCAGAAGCAGGGGCGATTCCAATTCTT
GTCAACCTTTTAACAACAGATGACACTGTGACACAAGAACATGCTGTGAC

TTCAATTCTTAATCTGTCGATATACGAAGACAACAAAGGACTCATTATGCT
TGCTGGTGCCATTCCTTCCATAGTTCAAATCCTGAGAGCTGGAAGCATGGA
AGCAAGAGAGAATGCAGCAGCAACCCTTTTTAGCCTATCACATCTGGATG
AGAACAAGATAATAATTGGTGCATCAGGGGCGATACCAGCTCTGGTAGAT
TTGCTCCAAAATGGGAGTTCAAGAGGAAAGAAAGATGCTGCAACTGCATT
GTTCAATCTTTGCGTTTATCCAGGCAACAAGGGAAGGGCTGTAAGGGCCG
GAATTATATCTGCTTTGTTGACAATGCTTACAGATTCGAGAAATTGTATGG
TTGATGGGGCTCTGACTATACTCTCAGTGCTTGCAAGTAACCAAGAGGCG
AAAGTTGACATAGTGAAC

Protein:

VAAIEALVRKLSSRSVEKQRAAVAEIRSLSKRSTDNRILIAEAGAIPILVNLLTT
DDTVTQEHAVTSILNLSIYEDNKGLIMLAGAIPSIVQILRAGSMEARENAAATL
FSLSHLDENKIIIGASGAIPALVDLLQNGSSRGKKDAATALFNLCVYPGNKGR
AVRAGIISALLTMLTDSRNCMVDGALTILSVLASNQEAKVDIVN

>EU513283 *Mangifera indica* putative elongation factor 1-α mRNA, partial cds.

CATGAACCACCCTGGCCAGATTGGTAACGGATATGCCCCAGTGCTCGACT
GCCACACTTCCCACATTGCTGTCAAGTTTGCTGAGATCTTGACCAAGATTG
ACAGACGATCTGGCAAGGAGCTTGAGAAGGA

Protein:

MNHPGQIGNGYAPVLDCHTSHIAVKFAEILTKIDRRSGKELEK

BIODATA

PERSONAL INFORMATION

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		National Chemical Laboratory
Gender	**:** Male	Pune- 411008
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Date of birth	**:** 08/ 05/1978	
Marital status	**:** Single	**Address (Res.)**
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		Parvati, Pune- 411009
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ACADEMICS

Examination	University	Year	Percentage	Class
B.Sc. (Botany)	University of Pune, Pune (India)	May 1999	75.88	First class with distinction
M.Sc. Botany (Plant Biotechnology)	University of Pune, Pune (India)	May 2001	70.30	First class with distinction and 4th University Rank

Ph. D. PROJECT

Nov. 2002 to Nov. 2007

Funded by: Council of Scientific and Industrial Research, India

Title: 'Genetic Analysis of Alphonso Mango Flavor Biogenesis'

Guide: Dr. Vidya S. Gupta

Submitted to: University of Pune (Maharashtra, India)

Work Details:

1. Gas chromatography- Mass spectrometry based characterization of developing and ripening Alphonso mangoes for the pulp flavor.
2. Targeting the genes, involved in synthesis of major flavor compounds in Alphonso using RT-PCR, Differential Display RT-PCR and cDNA library of ripening mango fruit.
3. Expression analysis of isolated flavor genes.
4. Tracking the expression dynamics of different flavor and non-flavor genes through the development and ripening of Alphonso mango.
5. Gas chromatography- Mass spectrometry based characterization of flavor profiles of thirty mango cultivars.
6. Inter simple sequence repeat (ISSR) marker based diversity analysis of mango cultivars to decipher the relationships among them and to infer the variations in their flavor signatures with respect to their genetic relationships.

M. Sc. DISSERTATION

Nov. 2000 to April 2001

'Subcloning of Glutathione peroxidase cDNA from pBLUESCRIPT to pGEX, an expression vector'.

M. Sc. SUMMER PROJECT

May to July 2000

'Use of Molecular Tools for Plant Genetic Diversity Analysis'

(Plant Molecular Biology Unit, Division of Biochemical Sciences, National Chemical Laboratory, Pune, India).

OTHER QUALIFICATIONS

1. Completed a course in **Plant Tissue Culture** from Modern College Career Guidance Academy, Modern college, Pune, India.
2. Completed, a course in **Computer Hardware & Networking** at Jetking, School of Electronic Technology, Pune, India.

TEACHING EXPERIENCE

Contributed to the B.Sc and M.Sc Biotechnology program as a visiting lecturer at Modern College, Pune, India during the academic year 2001- 2002.

ACHIEVEMENTS

1. Qualified **CSIR-UGC Joint National Eligibility Test in Life Sciences for Junior Research Fellowship and All India Lecturership** held on 30th Dec. 2001. (Award includes two years junior research fellowship and an assessment based promotion to senior research fellowship for next three years to conduct doctoral studies anywhere in India.)
2. Received the best poster award (Feb 2008) (Title: Revealing the secrets of mango flavor) in the area of biological sciences, at the national science day poster presentation session, organized at National Chemical Laboratory, Pune.

TECHNICAL EXPERIENCE

1. Worked as a project assistant II on the project, **'genotyping of biodiversity for conservation and prospecting of biological wealth in Southwestern and Northeastern parts of India using molecular approaches'**, funded by Department of Biotechnology, Government of India, at Plant Molecular Biology Unit, Division of Biochemical Sciences, National Chemical

Laboratory, Pune. The work includes collection of different plant species from Northeastern and Western Ghats of India followed by their cytotyping and DNA fingerprinting (July 2001 to Nov 2002).

2. Worked in **Modern college plant tissue culture laboratory,** Pune from June 1997 to July 1998 and got acquainted with all the basic techniques including media preparation, aseptic manipulation and plant acclimatization.
3. Worked as a Plant Tissue Culturist from Aug 1997 to Aug 1998 in **Dutch-Indo Tissue Lab,** Pune**.** This work constituted commercial scale multiplication of different plant species.
4. Worked as a resource person for a floral section in a project, '**Biodiversity monitoring of Eastern and Western Ghats of India'** funded by the ministry of environment and forests along with the Department of Biotechnology and Department of Space, Government of India. (Jan 1997 to Dec 2000).
5. Learnt isolation, expression cloning and assays for **terpene synthase genes** at Max-planck institute for chemical ecology, Jena (Germany) under the guidance of Prof. Jonathan Gershenzon during August 2006 to December 2006.

PUBLICATIONS

Based on Ph.D. work

1. **Pandit SS**, Mitra SS, Giri AP and Gupta VS (2007). A Quick Method for Isolating RNA from Raw and Ripe Fleshy Fruits as well as for Co-isolating DNA and RNA from Polysaccharide- and Polyphenol-Rich Leaf Tissues. *J. Plant Biol.* 50(1), 60-64.
2. **Pandit SS**, Mitra SS, Giri AP, Pujari KH, Patil BP, Jambhale ND and Gupta VS (2007) Genetic Diversity Analysis of Mango Cultivars Using Inter Simple Sequence Repeat (ISSR) Markers. *Curr Sci*, 93 (8): 1135-1141.
3. **Pandit SS**, Mitra SS, Giri AP and Gupta VS (2008) Dominant Markers-Biology and Statistics; Qualitative Processing for the Quantitative Data. Communicated to *BMC Genetics.*
4. **Pandit SS** *et al* (2008) Dynamics of gene expression during the ripening of Alphonso mango. Communicated to *BMC Molecular Biology*.

5. **Pandit SS** *et al* (2008) Flavor changes during the ripening of Alphonso mango. Communicated to *Food Chemistry*.
6. **Pandit SS** *et al* (2008) Relationships among popular mango cultivars based on their flavor profiles. Communicated to *Journal of Agricultural and Food Chemistry.*

Other publications

7. Ghate HV, Borowiec L, Rane NS, Ranade SP and **Pandit SS** (2003) Tortoise beetles and their host plants from Pune (Maharashtra State, India) and nearby places (Coleoptera: Chrysomelidae: Cassidinae); *Genus* 14 (4): 519-539.
8. BahulikarRA, Lagu MD, Kulkarni BG, **Pandit SS**, Suresh HS, Rao MKV, Ranjekar PK and Gupta VS (2004) Genetic diversity among spatially isolated populations of *Eurya nitida* Korth. (Theaceae) based on inter-simple sequence repeats; *Curr sci*, 86 (6): 824- 831.
9. Anish Kumar N, Naik DD and **Pandit SS** (2007) High-frequency *in vitro* flowering in six species of *Ceropegia*; *J. Plant Biol,* 50 (3): 374- 377.

SELECTED PRESENTATIONS:

1. **Diversity of leaf eating tortoise beetles around Pune.**
   Ghate HV, Ranade SP, Rane NS and **Pandit SS**
   'National symposium on basic and applied aspects of plant and microbial biotechnology'; 4th and 5th Feb 2000. Organized by Department of Botany, Modern College, Pune, India.

2. **Prospects of '*Elaeagnus latifolia*: an untamed forest resource.**
   Pandit SS, Lawre SL and Dhumal KN
   'National seminar on Frontiers of Research and Development in Medicinal Plants'; 16th to 18th Sept 2000. Organized by Central Institute of Medicinal and Aromatic Plants' (CIMAP), Lucknow, India. (Abstract: Journal of medicinal and aromatic plant sciences (JMAPS). 2000, Vol. 22, Supplement- 1, Page 91).

3. **Diversity in Indian mango assessed using ISSR and morphological markers.**

   Pandit SS, Mitra SS, Pujari KH, Giri AP, Patil BP, Chidley HG, Jambhale ND, and Gupta VS

   International conference on 'Plant genomics and biotechnology: chalanges and opportunities'; 26th to 28th Oct 2005. Organised by Indira Gandhi Agricultural University, Raipur, India.

REFERENCES:

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