# Molecular and Genetic Regulation of Sensory Quality of Climacteric Fruit

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**Abstract**

**The sensory quality of fruit has become a major criterion in making the purchasing decision by consumers. Breeding programs have mainly been directed, from the post-harvest stand point, towards improving shelf-life. Chance seedlings or mutants with improved agronomic traits and/or extended shelf-life have been used for introgressing the long shelf-life character and eventually improved sensory quality traits in commercial genotypes of apple, melon or tomato. Because the plant hormone ethylene plays a central role in both storability and ripening of climacteric fruit, the generation by biotechnology of ethylene-inhibited fruit has offered a powerful tool to better understand, at the molecular and genetic level, the inter-relations between storability and sensory quality. In the melon, inhibition of ethylene synthesis results is a strong inhibition of the synthesis of aroma volatiles while the accumulation of sugars is not affected or is even improved. The softening of the flesh is strongly affected but not abolished. Mid or long shelf-life melons generated by classical breeding present the same behavior. The generation of recombinant inbred lines by crossing a typical climacteric melon (Cantaloupe Charentais of the *cantalupensis* group) with a non climacteric melon (PI161375 of the *agrestis chinensis* group) allowed to demonstrate that the climacteric character is conferred by 2 duplicated loci only, which are of great importance for the regulation of storability and sensory quality. Due to the importance of aroma volatiles in sensory quality and to the strong negative correlation between aroma production and ethylene synthesis, we have developed a research program aimed at isolating genes involved in the synthesis of aroma volatiles. We will report on the recent advances in the field with special emphasis on the characterization of genes responsible for the synthesis of esters, a family of compounds crucial for the flavor of many fruit.**

## INTRODUCTION

The sensory quality of fruit involves a range of attributes such as sweetness, acidity, aroma, firmness and color. In the last decades, consumers have often complained about the poor eating quality of fruit put into the market and flavor has now become a major criterion in making the purchasing decision. The sensory quality of fruit depends on many factors, including variety, culture conditions, picking date, and post-harvest handling and storage methods. However, among these factors, the genotype is probably the most critical. Breeding programs have mainly been directed, towards improving yield, size, resistance to pathogens. Increasing shelf-life has also been an important goal and fruit with better storability such as mid or long shelf-life tomatoes, melons or apples, have been generated. However, this has generally been accompanied by a loss of flavor suggesting that an antagonism exists between extension of shelf-life and quality. In this paper, taking apple, melon and tomato as examples, we will give an overview of (i) the recent progress made in breeding for storability and quality (ii) the methods used for the identification of genetic factors of fruit quality (iii) the understanding of the antagonism between storability and sensory quality and (iv) the molecular regulation of sensory quality with emphasis on the identification of genes involved in the biosynthesis of aromas.

## BREEDING FOR STORABILITY AND QUALITY

Breeding for quality and storage life has long been a secondary goal as compared to yield and disease resistance. However mutants or chance seedlings that give fruit with long storage life or non-ripening character have been used in the last twenty years to generate commercial varieties with good keeping character and sometimes improved sensory quality. In the case of apple for instance, the chance seedling Golden Delicious has been widely developed because of its good agronomic characters (high yield, absence or low alternate bearing, long storage life, cultivation in a wide range of climate and culture conditions) but fruit have limited sensory qualities, specially when produced at high yield. Crosses have been made between Golden Delicious and old apple varieties with good sensory attributes to generate new apple varieties that combine good agronomic and good sensory characters (Table 1). Similarly, the poor keeping qualities of Delicious (mealiness after few months of storage) has been extended by crossing with long keeping apples (Rall's janet) giving rise to the Fuji group of apples (Table 1) (Vaysse et al., 2000).

In tomato, many long or mid-shelf-life modern varieties have been obtained by introgression of the *rin* and *nor* genes from a ripening-inhibited and non-ripening natural mutant respectively (Tigchelaar et al., 1978). It has been recently discovered that the *rin* gene corresponded to a MADS box type transcription factor that regulates the ripening process and confers insensitivity to ethylene (Giovannoni, 2004). However, the long shelf-life characters is associated with poor sensory qualities.

In Charentais type melons, long or mid-shelf life commercial genotypes are available. They have been generated using a non-ripening melon named "Vauclusien". In these hybrids, the development of abscission zone is impaired or delayed, which renders the determination of harvest time difficult. Fruit ripening is impaired, as compared with the original Charentais type, in terms of degreening of the rind and production of aroma volatiles although fruit generally have a higher sugar content.

## IDENTIFICATION OF GENETIC FACTORS OF FRUIT QUALITY AND SHELF-LIFE

A relationship between the rates of ripening and ethylene production has been established for climacteric fruit. For instance, apple fruit with low ethylene production have a long storage life (Gussman et al., 1993). The amount of ethylene in ripening apple parallels the transcription level of the ripening-specific 1-aminocyclopropane-1-carboxylate (ACC) synthase gene, *Md ACS1* (Harada et al., 2000). An allele of the gene (*MdACS1-2*) which contains an inserted retrotransposon-like sequence at the 5'-flanking region, is transcribed at a lower level than the wild type allele *MdACS1-1*. Cultivars that are homozygous for this allele have a long storage life (Sunako et al., 1999) and a reduced preharvest drop (Sato et al., 2004). The presence of the *MdACS1-2* allele can therefore be used as a molecular marker for the early detection of apple cultivars with low ethylene production and low fruit drop rate.

In melon, a correlation exists between ethylene production and post-harvest decay (Zheng and Wolff, 2000). Using probes made from cDNAs encoding ACC oxidase it has been demonstrated that low ethylene production was associated with the presence of putative RFLP-ACO allele Ao, whereas high ethylene production was associated with allele Bo in the homozygous conditions (Zheng and Wolff, 2000). These RFLP markers are available for marker-assisted-selection.

QTL analysis of fruit quality in fresh market tomato indicated that a few chromosome regions are capable of controlling the variations in sensory traits (Causse et al., 2002). Major QTLs were detected for fruit weight, diameter, colour, firmness, meltiness and for six aroma volatiles. QTL approaches of fruit quality have been undertaken for apple and peaches (see papers in this volume).

Beside understanding the genetic control of fruit quality traits, all these data provide breeders with markers for selection.

## GENETIC CONTROL OF THE CLIMACTERIC CHARACTER IN MELON

Fleshy fruits are divided into two groups, climacteric and non climacteric, based upon the presence or absence of an autocatalytic burst of ethylene during ripening. A large diversity of fruit exist within *Cucumis melo* fruit comprising both climacteric and non climacteric types. They offer a material of choice for the study of the genetic control of the climacteric character.

In climacteric melon, such as cantaloupes, exogenous ethylene can prematurely induce abscission, ethylene production and ripening. Non climacteric melon fruit such as corean type melons of the *Cucumis melo* subsp. *agrestis* do not abscise and do not show any sensitivity to ethylene in terms of softening, ethylene production, abscission and expression of ethylene-ripening-related genes although the seedlings display the usual ethylene-induced triple response (Périn et al., 2002). Genetic analysis on a population of recombinant Cantaloupe Charentais x *C. melo agrestis* (PI 161375) inbred lines in segregation for fruit abscission and presence of climacteric ethylene production indicated that both characters are controlled by two independent loci only, *abscission layer* (*Al*)-3 and (*Al-4*). The intensity of ethylene production is controlled by at least 4 QTLs localized on other genomic regions The non climacteric phenotype is related to the recessive allelic forms from PI161375 (Périn et al., 2002).

## PHYSIOLOGICAL BASES FOR THE ANTAGONISM BETWEEN STORABILITY AND SENSORY QUALITY

Extension of shelf-life by breeding has involved, often without knowing, a reduction in ethylene biosynthesis or action. The generation by biotechnology of ethylene-inhibited fruit has offered a powerful tool to better understand, at the molecular genetic level, the inter-relations between storability and sensory quality.

In the melon, inhibition of ethylene synthesis results is a strong inhibition of the synthesis of aroma volatiles while the accumulation of sugars is not affected or is even improved (Ayub et al., 1996; Guis et al., 1997; Bauchot et al., 1998). The softening of the flesh is strongly affected but not abolished (Ayub et al., 1996; Flores et al., 2001). Mid or long shelf-life melons generated by classical breeding present the same behavior. These data indicate that in climacteric fruit, ripening processes related to sensory quality are made of ethylene-regulated and ethylene-independent components (Theologis et al., 1993; Lelièvre et al., 1997). Slowing-down ethylene production or action through breeding or biotechnology has both beneficial and negative effects on sensory quality.

## GENES OF AROMA BIOSYNTHESIS IN THE MELON

Due to the importance of aroma volatiles in sensory quality and to the strong negative correlation between aroma production and ethylene synthesis, we have developed a research program aimed at isolating genes involved in the synthesis of aroma volatiles.

The aroma volatiles evolved by Charentais melons are mainly made of a complex mixture of esters, of saturated and unsaturated aldehydes and alcohols and of sulphur compounds (Homatidou et al., 1992; Wyllie et al., 1995; Beaulieu and Grimm, 2001). The aliphatic and branched esters represent the largest portion of volatiles and are essential contributors to the aroma. The biosynthetic chain of esters comprises two reduction steps from fatty acids into aldehydes and from aldehydes into alcohols. The biosynthesis of esters itself corresponds to the acylation of alcohols through alcohol acyl transferases, AAT (Fellman et al., 2000).

Our studies have been first devoted at understanding which step in the ester biosynthetic pathway is under the control of ethylene. Using fruit disks incubated in the presence of various precursors, the steps at which ester formation was inhibited in ethylene-suppressed fruit was the reduction of fatty acids and aldehydes. As for the acyl transfer to alcohols to form esters, it was inhibited only partly, indicating that this step had both ethylene-dependent and -independent components (Flores et al., 2002).

We have then initiated a programme for the isolation of genes encoding AATs. So far some AAT genes had been isolated from flowers that encode an acetyl-CoA : benzyl alcohol acetyltransferase in *Clarkia breweri* (Dudareva et al., 1998) and an acetyl CoA : geraniol/citronellol acetyltransferase in rose (Shalit et al., 2003). Also a gene showing AAT activity denominated SAAT has been isolated from strawberry (Aharoni et al., 2000). In the melon, a gene putatively encoding an AAT protein had been isolated from Charentais fruit but its functional identification was lacking (Aggelis et al., 1997). We have characterised two cDNAs (Cm-AAT1 and Cm-AAT2) with strong sequence homology (89% identity at the protein level) that belong to a large and highly divergent family of multifunctional plant acyl transferases and that show at most 34% identity with the only other fruit acyl-transferase characterised so far in strawberry (Aharoni et al., 2000). RT-PCR studies indicated that both genes were specifically expressed in fruit at increasing rates in the early and mid phases of ripening. Expression was severely reduced in ethylene-suppressed melons and in wild-type fruit treated with 1-MCP (El Yahyaoui et al., 2002). Cloning of the two genes in yeast revealed that the CM-AAT1 protein exhibited alcohol acyl-transferase activity while no such activity could be detected for CM-AAT2, despite the strong homology between the two sequences. CM-AAT1 was capable of producing esters from a wide range of combinations of alcohols and acyl-CoAs, but not from ethanol (El Yahyaoui et al., 2002).

Recently, two new cDNAs (Cm-AAT3 and Cm-AAT4) have been isolated from melon fruit that show 73% and 28% similarity with Cm-AAT1, respectively. The percentage similarity over the whole amino acid sequences is 29%. Cm-AAT3 show the highest similarity to the tobacco Nt-hsr201 protein involved in the hypersensitivity response (Czernic et al., 1996), and Cm-AAT4 with an acyltransferase from *Rosa hybrida* Rh-AAT1 and SAAT from strawberry (Aharoni et al., 2000; Shalit et al., 2003). Both of them, together with Cm-AAT1 and Cm-AAT2, share three conserved regions common to the BAHD acyltransferase gene superfamily (Aharoni et al., 2000; St-Pierre and De Luca, 2000). Preliminary studies after heterologous expression in yeast indicate that the encoded proteins have a narrower range of substrate specificity than CM-AAT1, in particular Cm-AAT4.

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## Tables

Table 1. Origin of some modern varieties of apples (adapted from Vaisse et al., 2000).

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| Hybrids of Golden Delicious | **Elstar**: Golden Delicious x Ingrid Marie<br>**Gala**: Kidds Orange red x Golden Delicious<br>**Jonagold**: Golden Delicious x Jonathan<br>**Pink Lady**: Golden Delicious x Lady Williams<br>**Tentation**: Golden Delicious x Grifer<br>**Corail**: Clivia x Golden Delicious<br>**Delbart Jubilé**: Golden Delicious x Lundbytrop<br>**Delbarstivale**: Stark Jongrimes x Golden Delicious<br>**Belchard-Chantecler**: Golden Delicious x Reinette Clochard |
| Hybrids of Delicious | **Fuji group**: Rall's Janet x Delicious |