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Molecular Control of Fruit Ripening and Sensory Quality of Charentais Melon

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Abstract

Traditional Charentais melons have a typical climacteric behavior with ethylene playing a major role in the regulation of the ripening process. Genetic studies using climacteric and non-climacteric types of *Cucumis melo* demonstrated that the climacteric character is dominant and conferred by 2 duplicated loci only which are of great importance for the regulation of storability and sensory quality. Commercial varieties of Charentais melon with long shelf-life have been generated, some of them by crossing with a non-ripening Charentais genotype (Vauclusien). The introduction of the long shelf-life character resulted in undesirable loss of aroma volatiles production. The inhibition of ethylene synthesis by knocking-down ACC oxidase gene expression has been achieved in Charentais melon. It results in a strong inhibition of the synthesis of aroma volatiles while the accumulation of sugars is not affected or is even improved and the softening of the flesh is strongly affected but not abolished. It was also demonstrated that ethylene-inhibited fruit exhibited better resistance to chilling injury. 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 volatile esters, compounds that are essential for the flavor of Cantaloupe melons. We report here on the recent advances in the field with special emphasis on the characterization of two families of genes encoding aldehyde reductases and alcohol acyl transferases.

INTRODUCTION

The sensory quality of fruit encompasses a range of properties such as sweetness, acidity, aroma, firmness and color that are associated with specific metabolic pathways and are generally coordinated during the ripening process. The development of good quality attributes depends on many factors including variety, culture conditions, harvest date, and storage conditions. In climacteric fruit the plant hormone ethylene is controlling most of the ripening events and therefore most of the metabolic pathways responsible for the development of sensory quality (Lelièvre et al., 1997; Giovannoni, 2004). In addition ethylene is controlling the rate of ripening and the duration of shelf-life. Increasing storability has been one of the major goals of the breeders in the last decades and this has generally been accompanied by a loss of flavor. In the case of Cantaloupe Charentais melons for instance, traditional fast ripening genotypes with sharp climacteric phase have been replaced on the market by mid or long shelf-life varieties which are considered of lower sensory quality by the consumers. More specifically, aromas which represent a major quality parameter of Charentais melon are substantially reduced in long keeping genotypes. In this paper we present information on (i) the genetic determinism of the climacteric character, (ii) the physiological basis of the antagonism between extended shelf-life and some quality attributes and (iii) the molecular mechanisms of aroma biosynthesis in melon.

ETHYLENE, STORABILITY AND SENSORY QUALITY

In melon, as in other climacteric fruit, an inverse relationship exists between the intensity of ethylene production and the duration of shelf-life (Gussman et al., 1993; Zheng and Wolff, 2000). Melon with high and sharp climacteric ethylene production such as Cantaloupe Charentais (cantalupensis group) have shorter shelf-life than American Cantaloupes or rockmelons (reticulatus group) that produce less ethylene. Non-climacteric melon such as cassaba and piel de sapo (inodorus group) have extended shelf-life. The climacteric character is therefore a strong determinant of the ripening rate and storability. In order to better understand the genetic basis of the climacteric character Périn et al. (2002) have generated a segregating population resulting from a cross between a typical climacteric Charentais type melon and a non-climacteric melon of the *agrestis* subspecies (PI 161375). By studying the segregation of the formation of the abscission layer (Al) of the peduncle and ethylene production, it was found that the climacteric character was controlled by two independent and dominant loci (*Al-3* and *Al-4*), and that the intensity of ethylene production was controlled by at least 4 QTLs localized on other genomic regions. None of the QTLs matched with known genes of the ethylene biosynthetic or transduction pathways. These data suggest that the difference between the climacteric and non-climacteric character is weak at the genetic level indicating that number of regulatory genes determining the climacteric character is probably low. However many genetic factors seem to control the intensity of ethylene production.

In order to evaluate the effects of ethylene on melon fruit ripening, Cantaloupe Charentais melons have been transformed with an antisense construct of an ACC oxidase (ACO) cDNA driven by the 35S promoter has been used for generating (Ayub et al., 1996). ACC oxidase is the enzyme controlling the last step of ethylene biosynthesis. A line of the antisense ACO melon showed a reduction of ethylene production by more than 99.5%, which resulted in an inhibition of rind yellowing, fungal attack and peduncle detachment as well as a slowing down of flesh softening and an inhibition of the climacteric respiration (Guis et al., 1997). Low concentrations of ethylene (2.5 to 5 $\mu\text{L L}^{-1}$) applied to antisense ACO melons were able to restore the original ripening phenotype (Flores et al., 2001). Some ripening pathways, such as coloration of the flesh and accumulation of sugars and organic acids, were not affected by ethylene suppression. The sugar content could even be higher since antisense ACO fruit could be kept on the vine for longer without abscission and risk of over-ripening. The softening of the flesh was not completely abolished by ethylene suppression. It is partly ethylene independent. In addition to the ripening phenotype, the antisense ACO melons have shown increased tolerance to low temperature disorders during and after storage at 2°C than the wild-type plants (Ben Amor et al., 1999). Such tolerance was even improved under modified atmosphere packaging (Flores et al., 2004). Chilling tolerance was correlated with a reduced accumulation of ethanol and acetaldehyde and a higher activity of enzymes capable of removing activated oxygen species, such as catalase and superoxide dismutase (Ben Amor et al., 1999).

ETHYLENE AND AROMA VOLATILES PRODUCTION

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. Long or mid-shelf life commercial Charentais melons genotypes generated by conventional breeding are available. Some of them have been obtained 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. Aubert and Bourger (2004) have studied the production of aroma volatiles in 15 Charentais melon cultivars having differential ripening rates and storability. Aubert and Bourger (2004) have found a considerable reduction (49-87%) of aroma volatiles production in long shelf-life cultivars as compared to the original type and

mid-shelf-life. In particular, most of the esters with low odor threshold (potent odorants) were reduced by 2 to 30 fold.

Interestingly, Bauchot et al. (1998) came to similar conclusions using hybrids of Charentais melons obtained by crossing with an antisense ACO line. The transgenic hybrids evolved 60 to 85% less total volatiles than the non-transformed hybrids (Bauchot et al., 1998). Volatiles with low odor values, such as ethyl, 2-methylpropyl and 2-methylbutyl acetates, were half to fifth lower than in controls whereas potent odorants, such as ethyl-2-methylpropanoate and ethyl-2-methylbutanoate, were about 3% that of controls. Altogether, these data suggest that ethylene is controlling preferentially the synthesis of the most potent odorants.

In order to determine 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).

GENES OF AROMA BIOSYNTHESIS

The Alcohol Acyl Transferase Gene Family

The formation of esters is catalysed by alcohol acyl-transferase enzymes (AAT) that transfer an acyl-CoA to an alcohol (Harada et al., 1985). These enzymes are capable of combining different alcohols and acyl-CoAs resulting in the synthesis of a wide range of esters accounting for the diversity of esters emitted by the fruit. We have demonstrated that, in Charentais melon (*Cucumis melo cantalupensis* group), AAT are encoded by a gene family of at least four members with amino acid identity ranging from 84% (Cm-AAT1/Cm-AAT2) and 58% (Cm-AAT1/Cm-AAT3) to only 22% (Cm-AAT1/Cm-AAT4). All encoded proteins, except Cm-AAT2, were enzymatically active upon expression in yeast and show differential substrate preferences (Yahyaoui et al., 2002; El-Sharkawy et al., 2005). Cm-AAT1 protein produces a wide range of short and long-chain acyl esters but has strong preference for the formation of *E*-2-hexenyl acetate and hexyl hexanoate (Fig. 1). Cm-AAT3 also accepts a wide range of substrates but with very strong preference for producing benzyl acetate. Cm-AAT4 is almost exclusively devoted to the formation of acetates, with strong preference for cinnamoyl acetate (Fig. 1). Site directed mutagenesis demonstrated that the failure of Cm-AAT2 to produce volatile esters is related to the presence of a 268-alanine residue instead of threonine as in all active AAT proteins. Mutating 268-A into 268-T of Cm-AAT2 restored enzyme activity, while mutating 268-T into 268-A abolished activity of Cm-AAT1. Activities of all three proteins measured with the preferred substrates sharply increase during fruit ripening. The expression of all *Cm-AAT* genes is up-regulated during ripening and inhibited in antisense ACC oxidase melons and in fruit treated with the ethylene antagonist 1-methylcyclopropene (1-MCP), indicating a positive regulation by ethylene. The data presented in this work suggest that the multiplicity of *AAT* genes accounts for the great diversity of esters formed in melon.

The Alcohol Dehydrogenase Genes

Alcohol dehydrogenases (ADH) participate in the biosynthetic pathway of aroma volatiles in fruit by interconverting aldehydes to alcohols and providing substrates for the formation of esters (Speirs et al., 1998). We have isolated two highly divergent ADH genes (< 8% identity at the amino acid level) of Cantaloupe Charentais melon (*Cucumis melo* var. *cantalupensis*). Cm-ADH1 belongs to the medium chain zinc-containing class of ADH and is highly similar to all *ADH* genes expressed in fruit isolated so far (Chase, 1999). Cm-ADH2 belongs to the short chain classes of ADH that has never been described in fruit. The two encoded proteins are enzymatically active upon expression in yeast and purification. Cm-ADH1 has strong preference for NADPH as a co-factor

whereas Cm-ADH2 preferentially uses NADH. Both work better as reductases with Km's 10 to 20 times lower for the conversion of aldehydes to alcohols than for the dehydrogenation of alcohols to aldehydes. They both show strong preference for aliphatic aldehydes. Cm-ADH1 is capable of reducing branched aldehydes such as 3-methylbutyraldehyde, whereas Cm-ADH2 cannot. Both *Cm-ADH* genes are expressed specifically in fruit and up-regulated during ripening. Gene expression is strongly inhibited in antisense ACC oxidase (AS) melons and in fruit treated with the ethylene antagonist 1-methylcyclopropene (1-MCP). Similarly total ADH activity of ripening fruit is also inhibited by 1-MCP and in AS fruit, indicating that ethylene plays a major role in regulating ADH activity (Fig. 2). In addition ADH activity in ripening fruit is mostly NADPH-dependent indicating that ADH1 is more active than ADH2. These data suggest that the two ADHs play specific roles in the regulation of aroma biosynthesis in melon.

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Figures

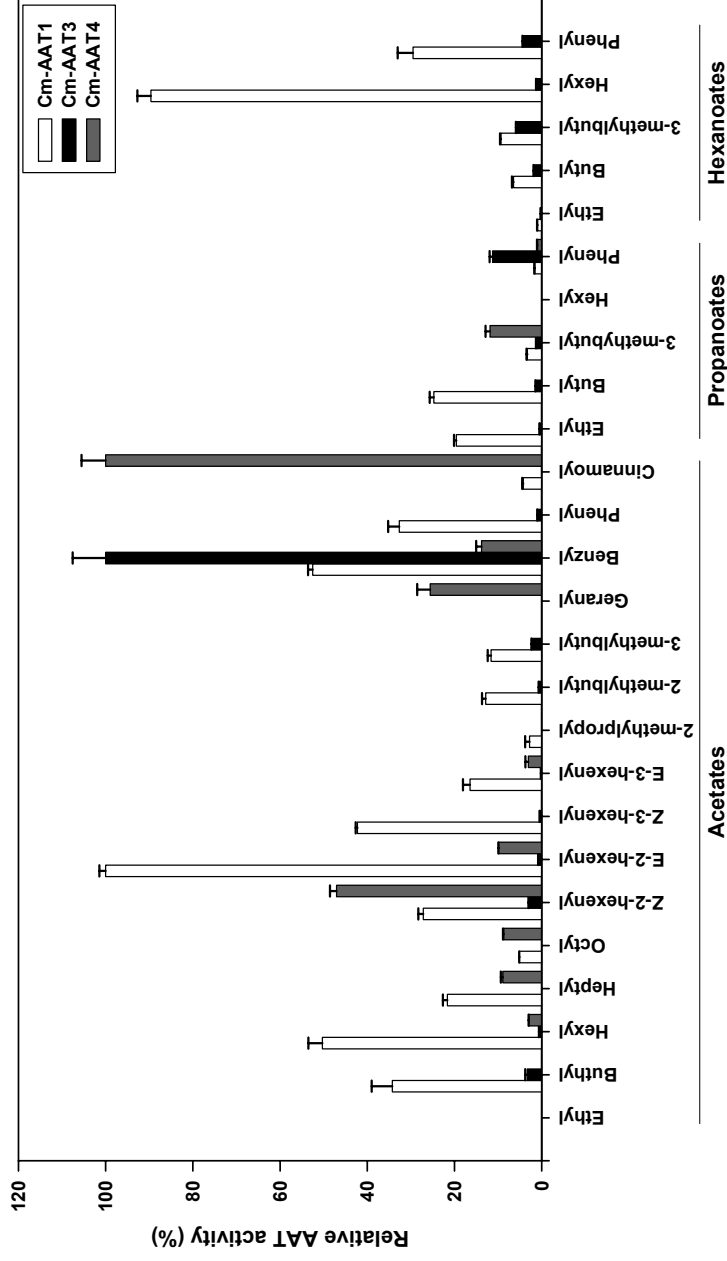


Fig. 1. Substrates specificity of the recombinant Cm-AAT enzymes toward different alcohols and acetyl-CoAs. Activity was measured after purification of the protein. Activity was expressed in % of the most activity substrate: 2490 pkat. mg prot⁻¹ (Cm-AAT1), 16285 pkat. mg prot⁻¹ (Cm-AAT3) and 3961 pkat. mg prot⁻¹ (Cm-AAT4).

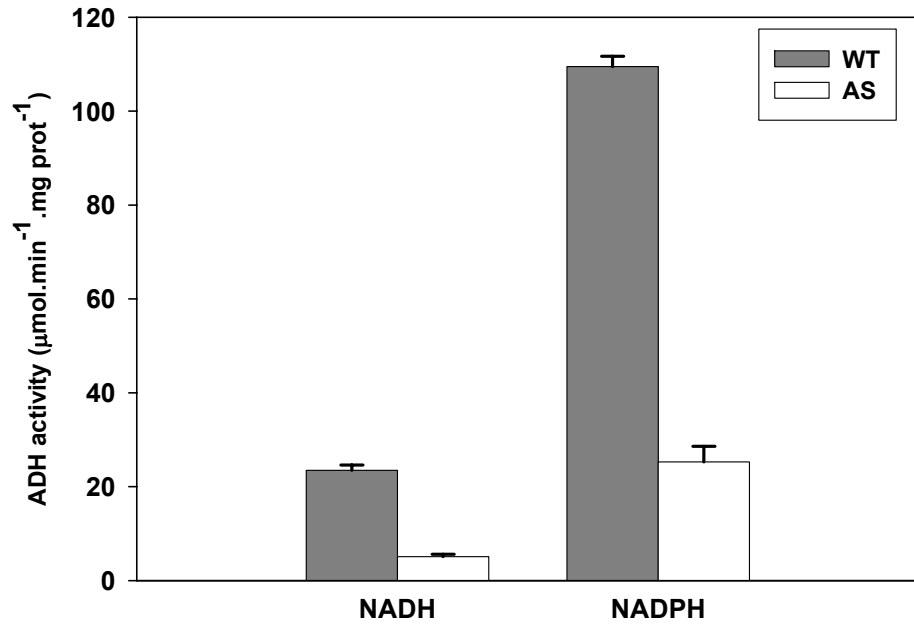


Fig. 2. ADH activity in protein extracts of ripe WT and AS melon fruit (42 days after pollinisation) evaluated as conversion of acetaldehyde into ethanol in the presence of NADH or NADPH.