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## Title

### ***Unraveling the key step in the aroma puzzle: Insights into alcohol acyltransferases in strawberries***

## Abstract

Alcohol acyltransferases (AATs) play a crucial role in catalyzing the transfer of acyl groups, contributing to the diverse aroma of fruits, including strawberries. In this research we identified nine AAT genes in strawberries through a comprehensive analysis involving phylogenetics, gene structure, conserved motifs, and structural protein model examinations. The study used the 'Camarosa' strawberry genome database, and experiments were conducted with fruits harvested at different developmental and ripening stages. The transcriptional analysis revealed differential expression patterns among the AAT genes during fruit ripening, with only four genes (SAAT, FaAAT2, FaAAT7, and FaAAT9) showing increased transcript accumulation correlated with total AAT enzyme activity. Additionally, the study employed in silico methods, including sequence alignment, phylogenetic analysis, and structural modeling, to gain insights into the AAT protein model structures with increase expression pattern during fruit ripening. The four modeled AAT proteins exhibited structural similarities, including conserved catalytic sites and solvent channels. Furthermore, the research investigated the interaction of AAT proteins with different substrates, highlighting the enzymes' promiscuity in substrate preferences. The study contributes with valuable information to unveil AAT gene family members in strawberries, providing scientific background for further exploration of their biological characteristics and their role in aroma biosynthesis during fruit ripening. © 2024 Elsevier Masson SAS

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Funding text 1: In conclusion, this study provides insights into the molecular intricacies of the alcohol acyltransferase (AAT) gene family during strawberry fruit ripening. Nine AAT genes in strawberries were identified, with seven new genes being described in the present work, showcasing diverse responses during ripening, and expanding the knowledge beyond previously reported sequences. The analysis of the promoter region suggests a hormonal fine-tuning transcriptional regulation. In fact, the transcriptional profiling during fruit ripening revealed distinct expression patterns, categorizing FaAAT genes into four groups with potential roles in aroma biosynthesis. The correlation between gene expression and total AAT activity underscored their significance in ester production. Additionally, the structural modeling of FaAAT proteins provided valuable insights into their catalytic mechanisms and substrate interactions. Despite the lack of experimental structural data, our computational models showed remarkable similarities to the known AAT structural model and revealed conserved features essential for catalytic activity,

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such as solvent channels and catalytic residues. Furthermore, protein-ligand interaction analysis, supported by MD simulations, elucidated the substrate preferences and binding dynamics of FaAAT enzymes. These findings highlighted the versatility of AATs in accommodating various alcohol substrates and provided mechanistic insights into ester biosynthesis in strawberries. Finally, our study contributes to a comprehensive understanding of the role of AAT enzymes in aroma biosynthesis during the strawberry fruit ripening process, laying the foundation for further research into enhancing fruit flavor and quality through targeted manipulation of AAT gene expression and enzyme activity. Finally, the most important AAT for strawberry aroma appears to be SAAT (Strawberry Alcohol Acyltransferase), along with FaAAT2. These enzymes exhibit increased expression levels during late ripening stages, correlating with the production of volatile esters responsible for the characteristic aroma of ripe strawberries, and are in line with what was previously described by other authors. Additionally, FaAAT7 and FaAAT9 have been identified as novel AAT genes with expression profiles similar to SAAT and FaAAT2, indicating their potential significance in aroma biosynthesis. Therefore, SAAT, FaAAT2, FaAAT7, and FaAAT9 are crucial players in shaping the aromatic profile of ripe strawberries. The Agencia Nacional de Investigaci\u00F3n y Desarrollo (ANID, Chile) [grants REDES #190093 to LM-Q, PR, and JPF; FONDECYT #1220782 to LM-Q.; FONDECYT #1211057 to PR; ANILLO #ATE220014 to LM-Q and PR; FONDECYT Postdoctoral #3220284 to AM-Y; FONDECYT #11220444 to DB, and FOVI230136 project to DB] supported the work. DS acknowledges ANID for a doctoral scholarship #21241441. The funders had no role in study design, data collection and analysis, publication decision, or manuscript preparation.; Funding text 2: The Agencia Nacional de Investigaci\u00F3n y Desarrollo (ANID, Chile) [grants REDES #190093 to LM-Q, PR, and JPF; FONDECYT #1220782 to LM-Q.; FONDECYT #1211057 to PR; ANILLO #ATE220014 to LM-Q and PR; FONDECYT Postdoctoral #3220284 to AM-Y; FONDECYT #11220444 to DB, and FOVI230136

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