

POLYMORPHISM OF THE ENZYMES THAT DETERMINE FRUIT SOFTENING IN STRAWBERRY

M. DRAYE, D. CUVELIER, A. ANTOINE and P. VAN CUTSEM

Unité de Recherche en Biologie cellulaire végétale, FUNDP, B-5000 Namur, Belgium

Strawberry (*Fragaria X ananassa*) is one of the most appreciated fruits throughout the world, but its fragility also makes it one of the most difficult fruits to handle. Indeed, during ripening, the fruit accumulates sugars and aromatic compounds but also undergoes a softening process under the effect of a small group of enzymes able to hydrolyze cell wall components. Different types of enzymes can be distinguished : polygalacturonases (PG) that hydrolyze demethylesterified pectins; pectins methylesterases (PME) that support the action of the PG by deesterifying pectins (Gaffe *et al.*, 1997); and finally cellulases that act on xyloglucans. We are presently dealing with cellulases that seem to be the predominant enzymes in cell wall softening during ripening of the strawberry receptacle (Harpster *et al.*, 1998), and with PMEs.

Cellulases and PME sequences were cloned in order to investigate their polymorphism. Briefly, RNA from a mature fruit (red stage) was extracted by LiCl precipitation. Using total RNA as starting material, single strand cDNA was synthesized by reverse transcription. Double strand cDNA was obtained by PCR using specific primers and cloned in PCR II vector. *E. coli* (TOP10 strain) were transformed and PCR screened with the specific primers to recover recombinant colonies. The inserts of interest were sequenced, aligned with the Clustal algorithm and compared.

Fourteen different partial sequences of cellulases (about 1345 bp each) and thirteen different partial sequences of PME (213 to 246 bp each) were obtained. The cellulase sequences differ by a few base pairs (Single Nucleotide Polymorphism) and they all belong to the same family of ripening-specific cellulases. A strategy was developed to check that the polymorphism was not due to Taq polymerase misincorporation during PCR amplification. Eight different cellulase promoters have been cloned and sequenced and they are presently studied. PME sequences were compared and they appear to be more divergent than cellulases and we were able to distinguish between five different PME sub-families.

5' and 3' -RACE PCR is being performed to obtain full-length cDNAs for both cellulases and PMEs. Transcription of the isoforms will be specifically studied by RT-PCR to determine those most significant in fruit softening (Stenman *et al.*, 1999).

Acknowledgements

This research is funded by the Belgian Ministry of Agriculture (convention N° S-5850-1).

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