The Effect of 1-MCP on the Expression of Several Ripening-related Genes in Strawberries

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Abstract. To elucidate the role of ethylene in nonclimacteric fruit development and ripening, quantitative (cDNA–amplified fragment length polymorphism) cDNA–AFLP was used to visualize differential gene expression in four stages of ripening of strawberries (Fragaria ×ananassa Duch. ‘Elsanta’) treated with 1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene action. The proportion of clones affected by 1-MCP treatment was much higher in green than in white, pink, and red receptacle tissue. Three major cell-wall-related genes were affected by 1-MCP and, thus, are putatively ethylene dependent: a ripening-repressed beta-galactosidase (Faβgal3), up-regulated by 1-MCP; a putative endo-1,3-1,4-beta-D-glucanase (EGase), up-regulated in green and down-regulated in red fruit by 1-MCP; and a pectate lyase B (pPb), expressed only in the red stage and significantly down-regulated by 1-MCP. Furthermore, we have identified genes encoding an alcohol dehydrogenase, a protein kinase-related protein, and a putative glutathione S-transferase, all ripening-induced and down-regulated by 1-MCP, suggesting that their regulation is at least partly ethylene dependent.

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Materials and Methods

‘Elsanta’ strawberries were harvested from a commercial farm near Ghent, Belgium. In total, 5 to 10 green, white, pink, and fully ripe red fruit (without removing the calyces) were treated with 1-MCP (15 nL·L–1) in 5 L sealed jars for 10 h at 22 °C. 1-MCP was released from SmartFresh powder (Rohm and Haas, Philadelphia, Pa.). Control fruit were kept in the same conditions except for 1-MCP treatment.

cDNA–AFLP experiment. Total RNA was extracted from the fruit flesh according to Salzman et al. (1999). For RNA extractions minimum five fruit per ripening stage were used. First-strand cDNA synthesis was carried out starting from 10 μg of total RNA, and quantitative cDNA–AFLP analysis was conducted as described by Breyne et al. (2003). Restriction enzymes BstYI and Msel (New England Biolabs, Beverly, Mass.) were used, and for preamplifications an Msel primer without selective nucleotides was applied in pair with a BstYI primer containing a C as a selective nucleotide. (The primer sequences were 5'-GACTCGTAGTAGCTCC-3' for BstYI and 5'-GATGAGCTGCTGATA-3' for Msel). In the cDNA–AFLP reactions 48 primer combinations were tested, both primers contained two selective nucleotides. Amplification products were separated on 5% polyacrylamide gels using the SequiGen system (Bio-Rad, Hercules, Calif.). Dried gels were exposed to BioMax films (Kodak) and scanned with a PhosphorImager 445 SI (Amersham Biosciences, Little Chalfont, U.K.).

Characterization of AFLP fragments and quantitative measurement of the expression profiles. Based on the autoradiogram, the TDFs (transcript derived fragments) were excised from gels and hydrated in 100 μL water for 1 h before PCR amplification of 2 μL by using the same primers as in the selective amplification. Sequence information was obtained either by direct sequencing of the re-amplified product with the BstYI primer or after cloning the fragments in pGEM-T Easy (Promega, Madison, Calif.). The reactions were analyzed with an ABI Prism 310 Genetic Analyzer. Nucleotide and translated amino acid sequences were analyzed for homology to known gene sequences using the BLASTN and BLASTX programs. Quantitative measurement of the expression profiles was done as described by Breyne et al. (2003). Scanned gel images were quantitatively analyzed with the AFLP QuantarPro image analysis software (Keygene N. V., Wageningen, The Netherlands) by which all visible AFLP fragments were scored and individual band intensities were measured in each lane. The raw data were corrected for differences by using a total lane intensity correction. The intensity values were summed per lane for each primer combination and each of the sums was divided by the maximal summed value to yield the correction factors. Finally, raw data were divided by these correction factors. For the corrected data, the coefficient of correlation was calculated
variation (CV) (CV = standard deviation/mean) was calculated for each TDF, and a threshold of 2 was chosen for significantly differentially expressed genes.

**Results and Discussion**

From 86 green receptacle specific clones, 43 were down-regulated by 1-MCP, 33 were up-regulated, and 10 clones showed no change in expression pattern after the treatment. Among the ripening induced clones a much lower percentage was regulated by 1-MCP. From 84 ripening specific TDFs, 20 were down-regulated, 9 were up-regulated, the expression pattern of the rest was not effected by 1-MCP.

The difference in response to 1-MCP of the green (88%) and ripening fruit (34%) might be due to their different sensitivity to ethylene, the consequence of the reduced endogenous ethylene production in the ripe fruit compared with the green receptacle. We focused on one ripening repressed and several ripening induced genes with modified expression pattern in response to 1-MCP treatment. Fruit softening, especially in strawberries, is an important postharvest quality trait, thus one of our main interest was to determine the effect of 1-MCP on genes related to cell-wall metabolism. We identified 3 cDNA–AFLP clones encoding enzymes responsible for cell wall loosening during cell expansion and fruit growth or ripening. One of them—C13M214M008—is ripening repressed, expressed only in the green receptacle, shows homology to beta-galactosidase (FaGal3) from strawberries (AJ278705), a cell wall hydrolase, mediating reversible wall loosening and thus allowing turgor-driven cell growth and expansion during fruit growth beside other cell wall modifying proteins. Trainotti et al. (2001) reported, that the transcript amount of this gene appears to be very high in flowers and in young fruits (both small and large green ones), then it gradually decreases during ripening and becomes almost undetectable in red fruits. In the experiment described by Trainotti et al. (2001), the exposure to ethylene of small green fruits and continuance of growth in the presence of ethylene did not seem to particularly affect the expression of FaGal3. In contrary, in our case, FaGal3 is significantly up regulated by 1-MCP (Fig. 1A).

The other clone (C23M21M011) accounts for a putative protein similar to endo-1,3-1,4-beta-D-glucanase from rice (AAU10802). It is present in the green receptacle, where it is up regulated by 1-MCP, and in ripe fruit, down regulated by 1-MCP. Though the cDNA–AFLP fragment with homology to endo-1,3,1,4-beta-D-glucanase shows no homology to any of the previously described EGase strawberry genes (Trainotti et al., 1999; Llop-Tous et al., 1999). To prove that we have identified a new member of the EGase gene family, the isolation of the full-length cDNA is in progress. The two, already described, EGase genes, FaEG1 and FaEG3, are predominantly expressed during the ripening process, in the case of FaEG3, the gene expression was also observed in large green fruit and, at low levels, in young vegetative green tissues (Spolaore et al., 2003; Trainotti et al., 1999). There are no data about the ethylene regulation of these genes in strawberry. The sequence analysis of the FaEG1 and FaEG3 genes encoding beta-galactosidase (FaGal3) and pectate lyase (FaPect) in green, white, pink, and red fruit based on the relative, variance-normalized cDNA–AFLP expression data. Gray bar = control fruit, black bar = 1-MCP treated fruit.

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**Fig. 1.** Quantitative expression profile of genes encoding beta-galactosidase (A) and pectate lyase (B) in green, white, pink, and red fruit based on the relative, variance-normalized cDNA–AFLP expression data. Gray bar = control fruit, black bar = 1-MCP treated fruit.

**Fig. 2.** Quantitative expression profile of genes encoding alcohol dehydrogenase (A), protein kinase-related (B), and putative glutathione S-transferase (C) in green, white, pink, and red fruit based on the relative, variance-normalized cDNA–AFLP expression data. Gray bar = control fruit, black bar = 1-MCP treated fruit.
promoters revealed the presence of ethylene responsive element, ATTTCCAAA, identified in the promoter region of an ethylene responsive glutathione-S-transferase gene from Dianthus Caryophyllus (Izthaki et al., 1994) suggesting that these genes might be ethylene regulated. The fact that in our experiment 1-MCP affects the transcript level of endo-1,3,1,4-beta-D-glucanase, AAW82451 (putative glutathione-S-transferase), and AY769612 (putative protein kinase).

In this paper we described several genes identified by cDNA–AFLP, showing altered expression in response to 1-MCP, an ethylene inhibitor, thus gaining insight into some potentially ethylene dependent processes in strawberry ripening. However, the effect of 1-MCP on prolongation of postharvest life of strawberries is contradictory (Bower et al., 2003), we found, that 1-MCP is suitable for scientific studies of nonclimacteric maturation at molecular level.

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