Plant Physiol. (1995) 108: 1323–1324

Plant Gene Register

Pummelo Fruit Transcript Homologous to Ripening-Induced Genes[1]

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Organic acid accumulation in the vacuole of juice cells of citrus fruits is a developmentally regulated process, the degree and timing of which varies greatly among species and varieties and is highly susceptible to environmental conditions (Clements, 1963; Ting and Vines, 1966; Vandercook, 1977). Total titratable acidity of the fruit juice of the acidless pummelo 2240 (*Citrus maxima* [Burm.] Merrill) is only 0.10% on a weight basis; HPLC analysis indicates that the citric acid concentration of pummelo 2240 fruit juice is 10 times lower than that in normal fruits. The acidless phenotype of pummelo 2240 is caused by a mutation involving a single nuclear gene, *acitric*, that does not affect other traits (Cameron and Soost, 1977; M.L. Roose, unpublished results). To generate citrus varieties of moderate acidity, pummelo 2240 was used in crosses with varieties of intermediate and high acid levels (Soost and Cameron, 1961). F_2 and backcross populations obtained from these crosses include low-acid individuals that are homozygous for *acitric* and high-acid individuals that carry at least one copy of the wild-type allele. As part of a continuing effort to identify *acitric*, two-dimensional IEF/SDS-PAGE protein patterns of tonoplast-enriched fractions obtained from the juice of low- and high-acid fruits were compared. A moderately abundant polypeptide of 10 to 15 kD and approximately neutral pI was identified in the juice of immature low-acid fruits that was not detected in juice from high-acid fruits at the same developmental stage. Mouse polyclonal antibodies raised against the electroeluted protein reacted with a single low-molecular-weight protein of juice tissues and seeds; the protein was not detected in roots, epicotyl, fruit rind, or unstressed leaves.

Full-length cDNA clones encoding the purified polypeptide were isolated that showed strong nucleotide sequence similarity to the *Lycopersicon esculentum* genes *asr1* and *asr2* (GenBank accession Nos. L08255 and L20756). The tomato genes encode 13-kD proteins, whose expression is induced in leaves by water stress and in fruit pericarp by ripening (Iusem et al., 1993). The citrus gene, designated *asr1*, encodes a 98-amino acid polypeptide of 10.87 kD. Like the tomato *asr1* product, the citrus protein is rich in Ala, Glu, His, and Lys (Table I).

Table I. *Characteristics of citrus cDNA encoding a differentially accumulating fruit protein*

Organism:
Citrus maxima [Burm.] Merrill; University of California at Riverside Agricultural Research Station accession No. 2240.

Techniques:
The polypeptides of tonoplast-enriched fruit juice fractions, obtained by differential centrifugation through a discontinuous Ficoll gradient (Chedhomme and Rona, 1985), were resolved by IEF/SDS-PAGE. The differentially accumulating protein was electroeluted and injected into Balb/c mice. Polyclonal antibodies were used to screen a λ-ZAP (Stratagene) expression library constructed from poly(A)$^+$ mRNA isolated from the juice cells of an immature acidless fruit (99 d postanthesis). Both DNA strands were sequenced by the Sanger dideoxy chain termination method using vector- and gene-specific primers.

Characteristics of the cDNA:
The cDNA is 575 nucleotides long, excluding the poly(A) tail. The open reading frame, encoding a 98-amino acid polypeptide, starts at nucleotide 31 with a Met codon located within a consensus translation-initiation sequence (Kozak, 1991). Third position G/C content is 50%. The 3′ untranslated region includes a canonical polyadenylation signal at nucleotide 519.

Gene Copy Number:
One copy per haploid genome, as determined by Southern analysis.

Characteristics of the Deduced Amino Acid Sequence:
The open reading frame encodes a 10.87-kD protein of net charge −6 and pI 6.5; the protein is rich in Ala (13%), Gly (11%), Glu (17%), His (12%), and Lys (13%).

Characteristics of *asr1* Product:
Amphiphilic protein, as determined by fractionation in Triton X-114 solution (Bordier, 1981). Moderately abundant in juice tissues of young, immature low-acid fruits and older, maturing high-acid fruits. Present at low levels in seeds; not detected in roots, epicotyl, albedo, or unstressed leaves. Unknown subcellular localization.

Examination of older, maturing high-acid fruits by immunoblotting established the presence of the protein in their juice tissues at levels similar to those observed in younger, low-acid fruits. It is not known with certainty whether citrus *asr1* is, like the tomato genes, induced by ripening. The process of fruit ripening in citrus is significantly different from that in tomato; citrus fruits are nonclimacteric and ripen much more slowly. Thus, equally significant differences between the regulation of *asr1* and

[1] Funding was provided by the California Citrus Research Board and a research assistantship to C.C. by the Department of Botany and Plant Sciences, University of California, Riverside.

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its tomato homologs can be expected. Genomic polymorphisms detected in the test F_2 population with radioactively labeled *asr1* cDNA probes segregated independently of fruit acidity, indicating that *asr1* is not linked to *acitric*. It is possible, however, to hypothesize a connection between ripening induction and the early expression of *asr1* in low-acid juice vesicles. In most citrus fruits, including pummelos and mandarins, ripening is accompanied by a steady decrease in acidity; therefore, it is conceivable that expression of ripening-induced genes in low-acid fruits occurs at a developmental stage during which acidity is still very high in normal fruits. The differential expression of *asr1* in low- and high-acid fruits may reflect the inability of the former to accumulate large amounts of citric acid.

Received December 27, 1994; accepted January 31, 1995.

The GenBank accession number for the sequence reported in this article is U18972.

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