

Article

Supplementary Materials: Beyond Purple Tomatoes: Combined Strategies Targeting Anthocyanins to Generate Crimson, Magenta, and Indigo Fruit

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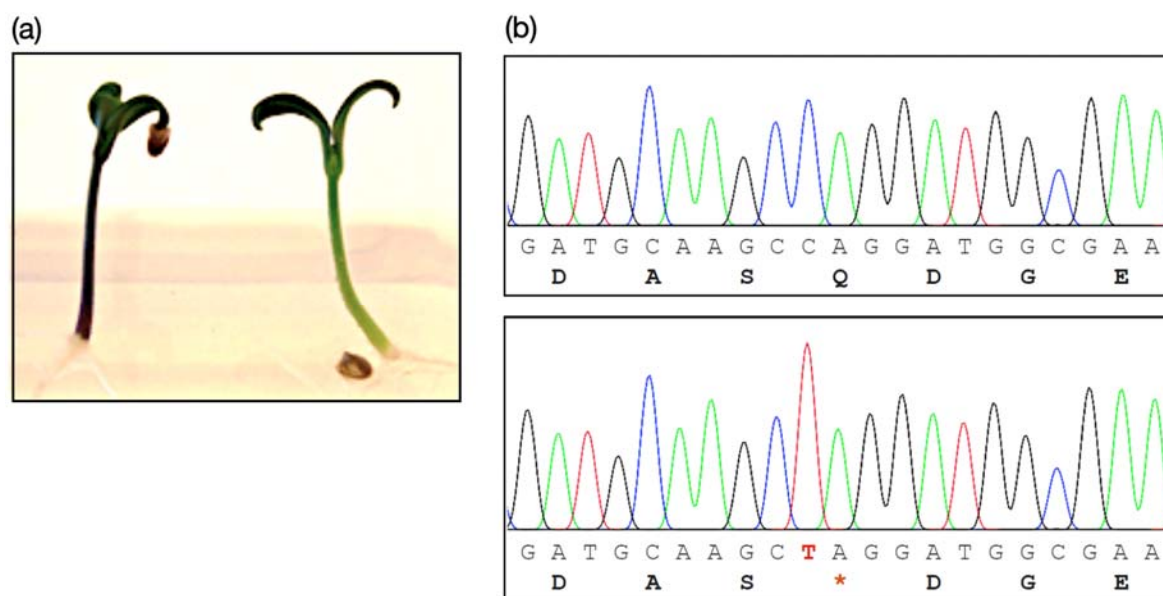


Figure S1. Phenotypic and genetic analysis of tomato seedlings harbouring the $\beta 3'5'h$ mutation. (a) F2 seedlings after self-pollination of F1 hybrids obtained crossing the *anthocyaninless* (*a*) mutant with the 'Purple' *Del/Ros1* tomato showing presence (left) or absence (right) of anthocyanins in hypocotyl and cotyledons. (b) Chromatograms, nucleotide, and amino acid sequences showing the presence of a premature stop codon in the gene encoding F3'5'H in anthocyaninless (bottom panel) but not in cyanic seedlings (top panel).

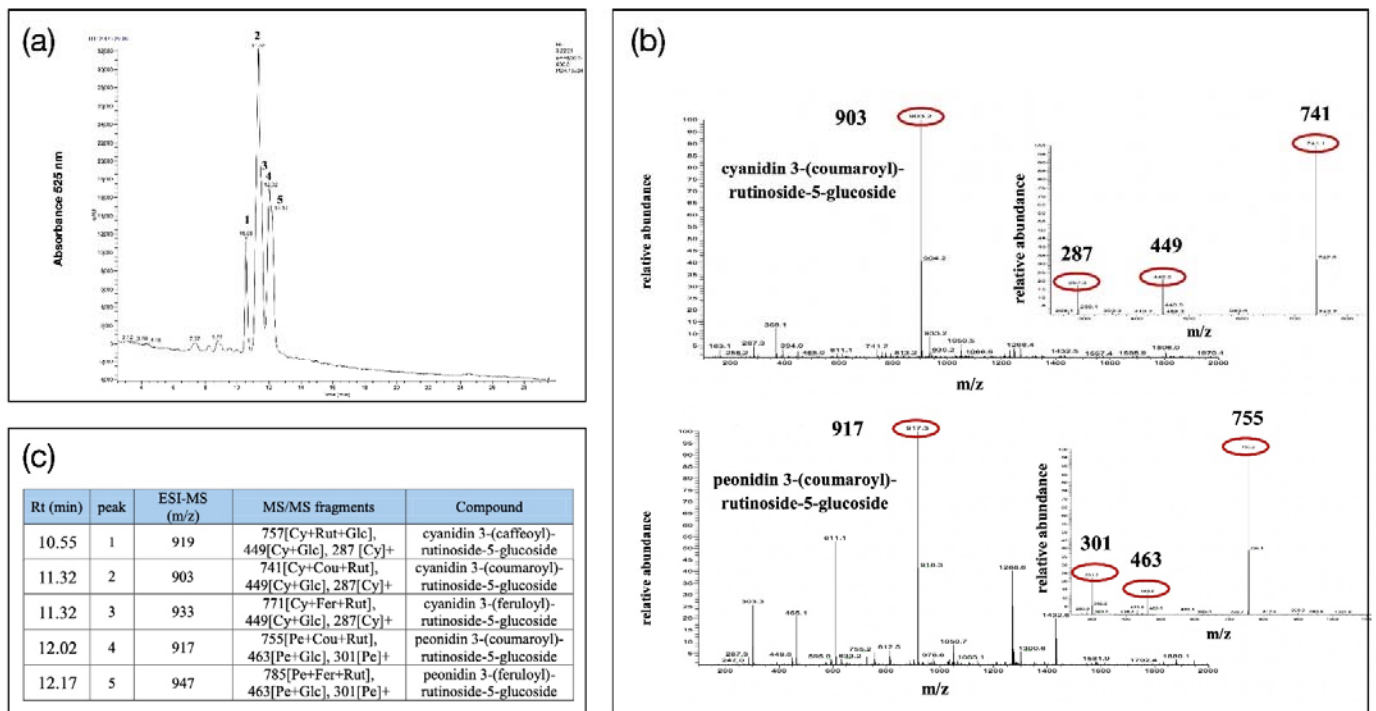


Figure S2. Analysis of anthocyanins extracted from the fruit of 'Pink' tomatoes (*Del/Ros1; f3'5'h*). (a) HPLC analysis showing the presence of five major compounds. (b) Mass analysis (MS1 and MS2) of the two most abundant compounds indicated as '2' and '4'. (c) Table showing the identity of all the five major peaks, all corresponding to C2 anthocyanins.

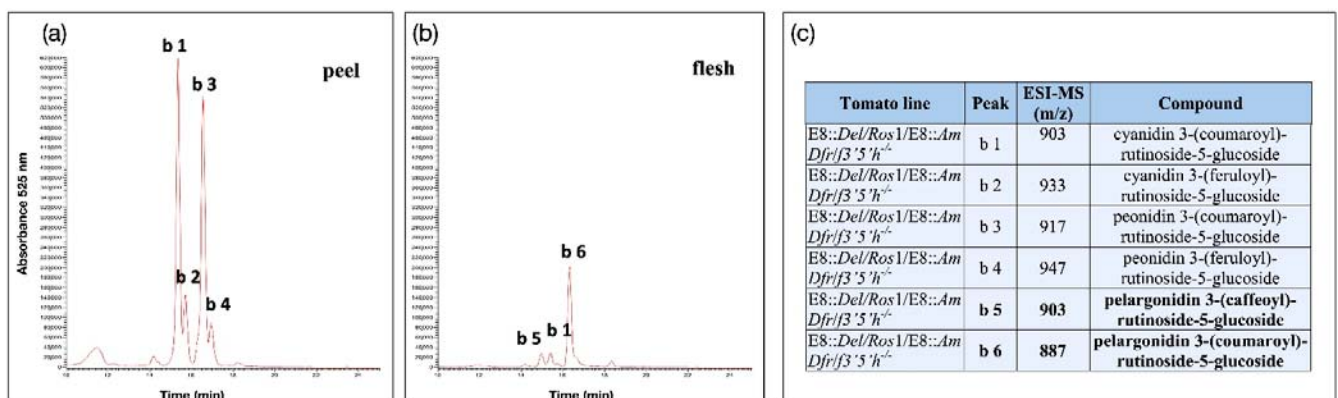


Figure S3. Analysis of anthocyanins from the fruit of 'Crimson' tomatoes (*Del/Ros1; AmDFR; f3'5'h*). (a) HPLC analysis in fruit peel. (b) HPLC analysis in fruit flesh. (c) Table showing the identity of all the major peaks based on mass spectrometry analysis.

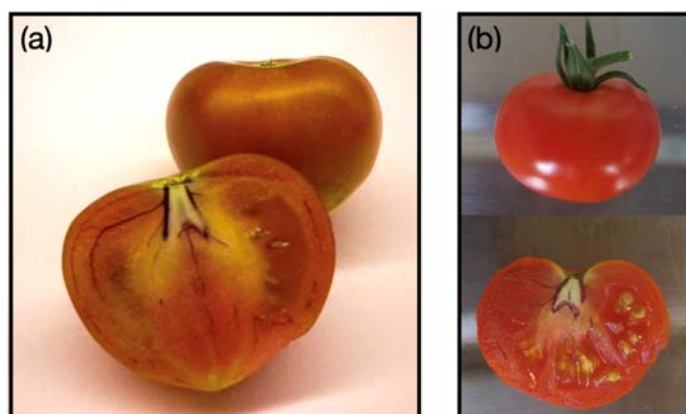


Figure S4. Fruit phenotypes of tomato lines expressing *Del/Ros1* in the presence of homozygous mutations affecting *F3'5'H* (a) or *DFR* (b). Both types of fruit display a vascular tissue visibly pigmented with anthocyanins.

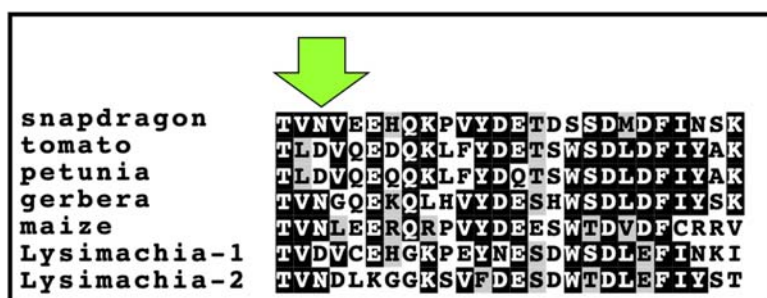


Figure S5. Alignment of a region of 26 amino acids in DFR identified as the substrate binding site for dihydroflavonols. The arrow indicates a key residue associated with the ability to recognise dihydrokaempferol (P1 precursor). Asparagine (N) is conserved in dihydrokaempferol-accepting DFRs but not in tomato or petunia, where it is replaced by aspartic acid (D). The GenBank accession numbers are: P14721 (snapdragon); NP_001234408.2 (tomato); P14720.2 (petunia); P51105.1 (gerbera); P51108.1 (maize); BioProject PRJNA701383 (*Lysimachia arvensis*).

Table S1. Sequences of the primers used in the study.

Name	Sequence	Description
DfrBglII_FA	CGAGAAGATCTATGAGTCCCACTTCACTAAATACGAG	Amplification and cloning of full-length <i>AmDFR</i> cDNA
DfrSmaI_RE	CAGTCCCCCGGGCTAGATTCTGCCATCAGTATGATCG	
DFR_F1	ACCACCGTATGTCACAGGA	Sequencing of <i>AmDFR</i> cDNA
DFR_F2	GAGAAAGCTGGAATGGAAGCA	
DFR_F3	AAGGGAGACGAGAAGGAACC	
DFR_R1	GGAAGGCAGACATGACAGTAGA	
DFR_R2	TGGATGATCTGTGTGAGGGTCA	
DFR_R3	CGTGTTAATGGACTAGAGAGCG	
EB-241	GACTGGATGGATGTATTTTGTGTCA	Genotyping of <i>AmDFR</i> transgenic plants
EB-242	AGCAAATGTATCTTCCTTCTGCCT	
EB-655	CAGGGTTGAACCGGTGTAGGA	Genotyping of <i>Del/Ros1</i> transgenic plants (<i>AmRos1</i>)
EB-656	CCATCCTCCGTTGTTTCTAGCA	
EB-430	GTCCAAGGCGATGAGGTCCA	Genotyping of <i>Del/Ros1</i> transgenic plants (<i>AmDel</i>)
EB-431	ACCTCTGCTATTACGGCTCCA	
MYB-F	AGGCAAAACGCAGACTTGGGAGAACGA	Genotyping of <i>AtMYB12</i> transgenic plants
MYB-R	CACTACCTTCTTGCCAAACACAATCCCA	
EB-742	GCCTATAATGCCCAAGACATGGT	Isolation of <i>SIF3'5'H</i> genomic fragment
EB-743	TCCTTCTGAATTATCCCTATTGCCA	
EB-744	ACCCTATGGACCACGTTGGA	Detection of <i>f3'5'h</i> mutation