

Cytological and molecular characterization of carotenoid accumulation in normal and high-lycopene mutant oranges

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Supplementary data

Figure S1. Carotenoid biosynthetic pathway in higher plants with the sites inhibited by CPTA and NFZ indicated.

Figure S2. The mRNA sequences of *phytoene synthase 1 (PSY1)* alleles, *PSY1-a* and *PSY1-b*, in Newhall and Cara Cara oranges.

Figure S3. The mRNA sequences of *lycopene β -cyclase (LCYB)* alleles, *LCYB-a* and *LCYB-b*, in Newhall and Cara Cara oranges.

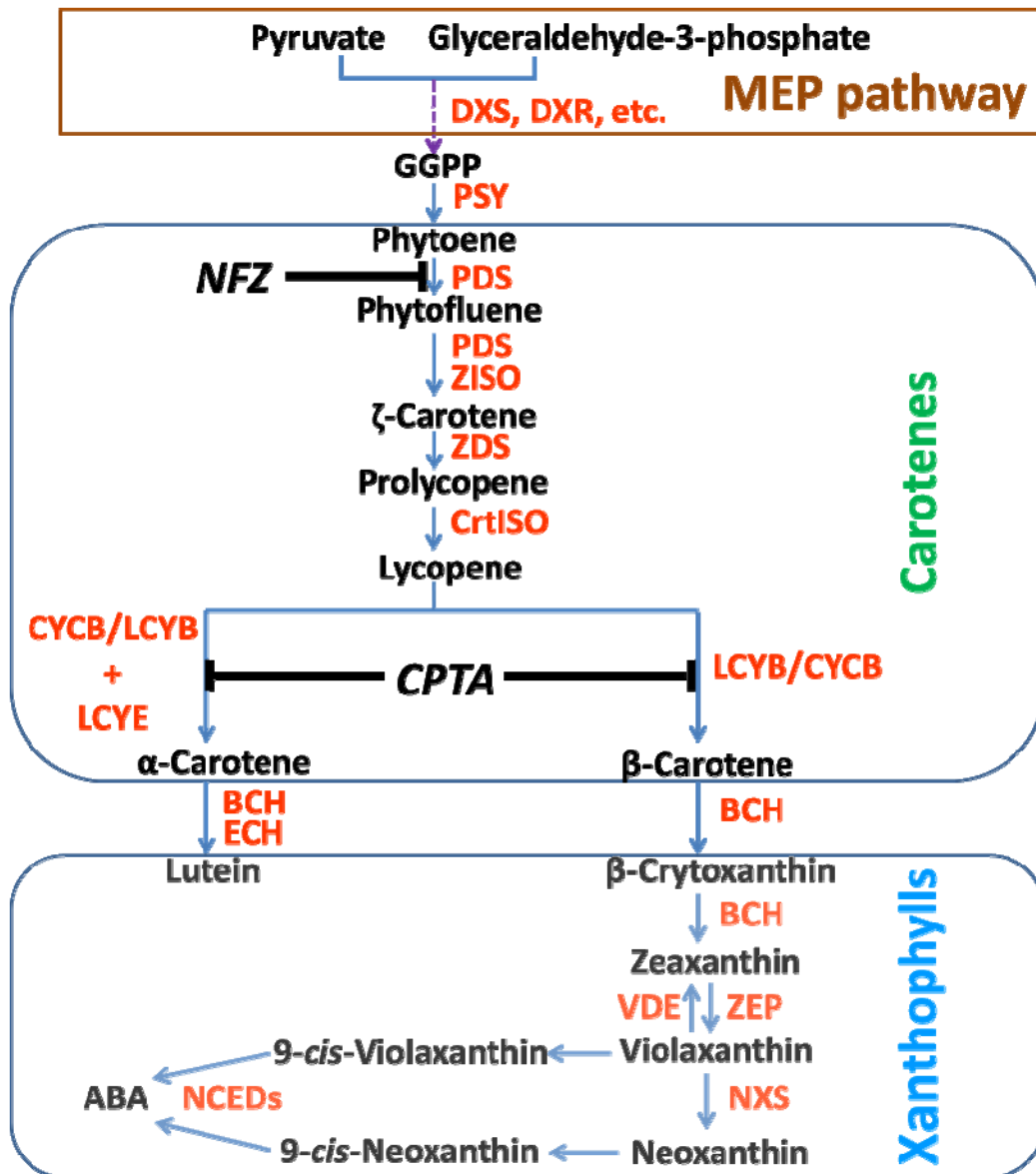
Figure S4. The mRNA sequences of *chromoplast-specific lycopene β -cyclase (CYCB)* alleles, *CYCB-a* and *CYCB-b*, in Newhall and Cara Cara oranges.

Figure S5. The observation of red crystalline chromoplast under the light microscope in 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) treated albedo layers of Newhall, control Newhall and control Cara Cara oranges.

Table S1. List of primers for real-time quantitative PCR.

Table S2. Effects of 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) and norflurazon treatments on accumulation of carotenoids in *in vitro* cultured flesh tissues of Newhall and Cara Cara.

Table S3. List of primers for full-length gene cloning.



Supplementary Fig. S1. Carotenoid biosynthetic pathway in higher plants with the sites inhibited by CPTA and NFZ indicated. BCH, β -carotene hydroxylase; CPTA, 2-(4-chlorophenylthio)-triethylamine hydrochloride; CRTISO, carotene isomerase; CYCB, chromoplast-specific lycopene β -cyclase; DXS, 1-deoxy-D-xylulose 5-phosphate-synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; ECH, ϵ -carotene hydroxylase; GGPP, geranylgeranyl diphosphate; LCYB, lycopene β -cyclase; LCYE, lycopene ϵ -cyclase; MEP, 2-C-methyl-D-erythritol 4-phosphate; NCED, nine-*cis*-epoxycarotenoid dioxygenase; NFZ, norflurazon; NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin de-epoxidase; ZDS, ζ -carotene desaturase; ZEP, zeaxanthin epoxidase; ZISO, 15-*cis*- ζ -carotene isomerase

PSY1-a : CTACAGAGAGTTGAAGTTACAGGGGCCTCAA TTTTCTTTTATCCAAAAAATAATTTTACAGCATGCTCTGTT CATT : 82
 PSY1-b : CTACAGAGAGTTGAAGTTACAGGGGCCTCAA TTTTCTTTTATCCAAAAAATAATTTTACAGCATGCTCTGTT CATT : 82

PSY1-a : GCTGTGGGTTGTATCACCTAACTCACAATTTGCCAATGCTTCGGGTTTCGTCGATT CAGTTCGAGAGGAAAACAGGCTGTTT : 164
 PSY1-b : GCTGTGGGTTGTATCACCTAACTCACAATTTGCCAATGCTTCGGGTTTCGTCGATT CAGTTCGAGAGGAAAACAGGCTGTTT : 164

PSY1-a : TATTCATCAAGATTTCTTACCAACATCAAACCCGGACTGCTGTGTTAATTTAGACCTAAGCAGTTTAAATAAT TAATA : 246
 PSY1-b : TATTCATCAAGATTTCTTACCAACATCAAACCCGGACTGCTGTGTTAATTTAGACCTAAGCAGTTTAAATAAT TAATA : 246

PSY1-a : A-----GCAGAGACGGGAATTCCTATCCCTTAGATACAGATTTGAGGCATCCTTGCTCATCTGGAATCGACTTGCCTGAAAT : 322
 PSY1-b : ATAAATAACGGGAATTCCTATCCCTTAGATACAGATTTGAGGCATCCTTGCTCATCTGGAATCGACTTGCCTGAAAT : 328

PSY1-a : ATCATGTATGGTTGCTAGCACTGCTGGAGAAGTGGCCATGCTTCAGAAGAAATGGTTTACAATGTTGTGCTCAAGCAGGCA : 404
 PSY1-b : ATCATGTATGGTTGCTAGCACTGCTGGAGAAGTGGCCATGCTTCAGAAGAAATGGTTTACAATGTTGTGCTCAAGCAGGCA : 410

PSY1-a : GCCTTGGTTAATAAGCAACCAAGTGGGGTACTCGTGATCTTGATGTGAACCCAGATATTGCTTTACCCGGAACCTTAAAGTC : 486
 PSY1-b : GCCTTGGTTAATAAGCAACCAAGTGGGGTACTCGTGATCTTGATGTGAACCCAGATATTGCTTTACCCGGAACCTTAAAGTC : 492

PSY1-a : TGCTCAGTGAAGCTTATGATCGTGTGGAGAAGTTTGCGCCGAGTATGCTAAGACATTTACTTGGGAACCTTGTGCTGATGAC : 568
 PSY1-b : TGCTCAGTGAAGCTTATGATCGTGTGGAGAAGTTTGCGCCGAGTATGCTAAGACATTTACTTGGGAACCTTGTGCTGATGAC : 574

PSY1-a : CTCTGAAGGGCAAGGGCTATATGGGCTATATGTGTGGTGTAGGAGGACAGATGAGCTCGTTGATGGGCTTAATGCTTCA : 650
 PSY1-b : CTCTGAAGGGCAAGGGCTATATGGGCTATATGTGTGGTGTAGGAGGACAGATGAGCTCGTTGATGGGCTTAATGCTTCA : 656

PSY1-a : CACATAACTCCAACAGCTTTAGACAGGTGGGAGTCCAGGTTGGAAGACCTTTCCGGGGTTCGTCATTGATATGCTTGATG : 732
 PSY1-b : CACATAACTCCAACAGCTTTAGACAGGTGGGAGTCCAGGTTGGAAGACCTTTCCGGGGTTCGTCATTGATATGCTTGATG : 738

PSY1-a : CTGCATTATCAGATACAGTAAACAAATTTCTGTGACATTCAGCCATTCAGAGATATGATAGAAGGAAATGAGGATGGACCT : 814
 PSY1-b : CTGCATTATCAGATACAGTAAACAAATTTCTGTGACATTCAGCCATTCAGAGATATGATAGAAGGAAATGAGGATGGACCT : 820

PSY1-a : TAGGAAGTCAAGATACAAAACTTTGATGAATTATACTTGTATTGTTATTATGTTGCTGGGACCGTAGGGCTAATGAGTGT : 896
 PSY1-b : TAGGAAGTCAAGATACAAAACTTTGATGAATTATACTTGTATTGTTATTATGTTGCTGGGACCGTAGGGCTAATGAGTGT : 902

PSY1-a : CCAGTTATGGGCATAGCACCTGACTCACAGGCAACAACAGAGAGCGTCTACAATGCAGCATTGGCACTAGGGATTGCTAATC : 978
 PSY1-b : CCAGTTATGGGCATAGCACCTGACTCACAGGCAACAACAGAGAGCGTCTACAATGCAGCATTGGCACTAGGGATTGCTAATC : 984

PSY1-a : AGCTCACTAACATACTCAGAGATGTTGGAGAGGATGCCCAAGAGGAAGGGTTTATCTACCACAAGATGAGTTGGCACAGGC : 1060
 PSY1-b : AGCTCACTAACATACTCAGAGATGTTGGAGAGGATGCCCAAGAGGAAGGGTTTATCTACCACAAGATGAGTTGGCACAGGC : 1066

PSY1-a : AGGGCTTTAGATGATGACATATTTGCTGGAGAGGTGACCTTAAATGGGAAACTTCATGAAGAACCAATTAAGAGGGCA : 1142
 PSY1-b : AGGGCTTTAGATGATGACATATTTGCTGGAGAGGTGACCTTAAATGGGAAACTTCATGAAGAACCAATTAAGAGGGCA : 1148

PSY1-a : AGGATGTTCTTTGATATGGCTGAGAACCGTGTGACCGAGCTGAGTGAAGCTAGT GATGGCCGATGGGCTTCATTGCTGT : 1224
 PSY1-b : AGGATGTTCTTTGATATGGCTGAGAACCGTGTGACCGAGCTGAGTGAAGCTAGT GATGGCCGATGGGCTTCATTGCTGT : 1230

PSY1-a : TGTACCGGCAAAATACTGGATGAGATTGAGGCCAATGATTACAACAACCTTCACAAGAGAGCTTATGTTGAGTAAAGCCAAGAA : 1306
 PSY1-b : TGTACCGGCAAAATACTGGATGAGATTGAGGCCAATGATTACAACAACCTTCACAAGAGAGCTTATGTTGAGTAAAGCCAAGAA : 1312

PSY1-a : GATAGCTGCACCTACCAATTGCATATGCAAAATCCCTCTTACGCCCGTCAAGAAATATATACCAGTAAGGCTTAAACTGAACAT : 1388
 PSY1-b : GATAGCTGCACCTACCAATTGCATATGCAAAATCCCTCTTACGCCCGTCAAGAAATATATACCAGTAAGGCTTAAACTGAACAT : 1394

PSY1-a : TTAACATCAAAGTTTAGAAGCATATATAGTTGAAACGGATTCAAAGTGGAAA AAAAATGAAAATGATTGCTTGAATATTAG : 1470
 PSY1-b : TTAACATCAAAGTTTAGAAGCATATATAGTTGAAACGGATTCAAAGTGGAAA AAAAATGAAAATGATTGCTTGAATATTAG : 1476

PSY1-a : GAATTGTTGGTATGCAGCATGTATTTGATGGTAAAGTTAGAATAGTGAATCCAATTCACAATCCAAGGCCGATGCCCTA : 1549
 PSY1-b : GAATTGTTGGTATGCAGCATGTATTTGATGGTAAAGTTAGAATAGTGAATCCAATTCACAATCCAAGGCCGATGCCCTA : 1555

Supplementary Fig. S2. The mRNA sequences of *phytoene synthase 1 (PSY1)* alleles, *PSY1-a* and *PSY1-b*, in Newhall and Cara Cara oranges. Note that, no difference in sequence was observed between the two oranges. Start codon and stop codon are underlined by red.

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*          20          *          40          *          60          *          80
LCYB-a : ACCAGGAGCTTAGGTTTGGTCTCAAGAAGTCTCGTCAAAGAGGAATACGAGTTGTTTCATTAAGGCTAGTAGTAGTCTCT : 82
LCYB-b : ACCAGGAGCTTAGGTTTGGTCTCAAGAAGTCTCGTCAAAGAGGAATAGAGTTGTTTCATTAAGGCTAGTAGTAGTCTCT : 82
ACCAGGAGCTTAGGTTTGGTCTCAAGAAGTCTCGTCAAAGAGGAATA GAGTTGTTTCATTAAGGCTAGTAGTAGTCTCT

*          100         *          120         *          140         *          160
LCYB-a : TTTGGACTAGTTCCTGAAACCAAGAAGGAAAATCTTGAATTTGAGCTTCCCATGTATGACCCATCAAAGGGCCTTGTGTGA : 164
LCYB-b : TTTGGACTAGTTCCTGAAACCAAGAAGGAAAATCTTGAATTTGAGCTTCCCATGTATGACCCATCAAAGGGCCTTGTGTGA : 164
TTTGGACTAGTTCCTGAAACCAAGAAGGAAAATCTTGAATTTGAGCTTCCCATGTATGACCCATCAAAGGGCCTTGTGTGA

*          180         *          200         *          220         *          240
LCYB-a : GACCTAGCAGTTGTCTGGTGGCGGCCCGCTGGGCTTGCTGTGCTCAGCAAGTTTCAGAGGCGGGGCTTTCAGTTTGCTCGA : 246
LCYB-b : GACCTAGCAGTTGTCTGGTGGCGGCCCGCTGGGCTTGCTGTGCTCAGCAAGTTTCAGAGGCGGGGCTTTCAGTTTGCTCGA : 246
GACCTAGCAGTTGTCTGGTGGCGGCCCGCTGGGCTTGCTGTGCTCAGCAAGTTTCAGAGGCGGGGCTTTCAGTTTGCTCGA

*          260         *          280         *          300         *          320
LCYB-a : TTGATCCATCTCCCAAATTGATTTGGCCAAATAAATATGGTGTTTGGTGGATGAATTTGAGGCCATGGATTTGCTTGATTG : 328
LCYB-b : TTGATCCATCTCCCAAATTGATTTGGCCAAATAAATATGGTGTTTGGTGGATGAATTTGAGGCCATGGATTTGCTTGATTG : 328
TTGATCCATCTCCCAAATTGATTTGGCCAAATAAATATGGTGTTTGGTGGATGAATTTGAGGCCATGGATTTGCTTGATTG

*          340         *          360         *          380         *          400         *
LCYB-a : CCTTGATACTACTTGGTCTGGTGTGTTGTGCACATGTATGATAAATACAAAGAAGGATCTTATAGACCTTATGGCAGAGTT : 410
LCYB-b : CCTTGATACTACTTGGTCTGGTGTGTTGTGCACATGTATGATAAATACAAAGAAGGATCTTATAGACCTTATGGCAGAGTT : 410
CCTTGATACTACTTGGTCTGGTGTGTTGTGCACATGTATGATAAATACAAAGAAGGATCTTATAGACCTTATGGCAGAGTT

*          420         *          440         *          460         *          480         *
LCYB-a : AATAGGAAGTTGCTGAAGTCGAAAATGCTGCAAAAATGCATAACCAATGGTGTAAAGTTTCACCAAGCTAAAGTTATTAAGG : 492
LCYB-b : AATAGGAAGTTGCTGAAGTCGAAAATGCTGCAAAAATGCATAACCAATGGTGTAAAGTTTCACCAAGCTAAAGTTATTAAGG : 492
AATAGGAAGTTGCTGAAGTCGAAAATGCTGCAAAAATGCATAACCAATGGTGTAAAGTTTCACCAAGCTAAAGTTATTAAGG

*          500         *          520         *          540         *          560         *
LCYB-a : TTATTCATGAAGAGTCCAAATCTTTGTTGATTTGCAATGATGGTGTGACAATTCAGGCTGCCGTGGTCTTGATGCTACGGG : 574
LCYB-b : TTATTCATGAAGAGTCCAAATCTTTGTTGATTTGCAATGATGGTGTGACAATTCAGGCTGCCGTGGTCTTGATGCTACGGG : 574
TTATTCATGAAGAGTCCAAATCTTTGTTGATTTGCAATGATGGTGTGACAATTCAGGCTGCCGTGGTCTTGATGCTACGGG

*          580         *          600         *          620         *          640         *
LCYB-a : CTTCTCTAGGTTGCTTTGTTCAGTATGATAAACCCTATAATCCAGGTTACCAAGTGGCATATGGAATACTAGCTGAGGTAGAA : 656
LCYB-b : CTTCTCTAGGTTGCTTTGTTCAGTATGATAAACCCTATAATCCAGGTTACCAAGTGGCATATGGAATACTAGCTGAGGTAGAA : 656
TTCTCTAGGTTGCTTTGTTCAGTATGATAAACCCTATAATCCAGGTTACCAAGTGGCATATGGAATACTAGCTGAGGTAGAA

*          660         *          680         *          700         *          720         *          7
LCYB-a : GAGCACCCGTTTGTATTTAGACAAGATGGTTTTTCATGGATTGGAGAGATTCGCATCTGAACAACAATTCGGAGCTCAAAGAGG : 738
LCYB-b : GAGCACCCGTTTGTATTTAGACAAGATGGTTTTTCATGGATTGGAGAGATTCGCATCTGAACAACAATTCGGAGCTCAAAGAGG : 738
GAGCACCCGTTTGTATTTAGACAAGATGGTTTTTCATGGATTGGAGAGATTCGCATCTGAACAACAATTCGGAGCTCAAAGAGG

*          740         *          760         *          780         *          800         *          820
LCYB-a : CAAAATAGCAAAATTCCTACTTTTCTTTATGCCATGCCCTTTTCGTCAAACAGGATATTTCTTGAAGAGACTTCGCTAGTGGC : 820
LCYB-b : CAAAATAGCAAAATTCCTACTTTTCTTTATGCCATGCCCTTTTCGTCAAACAGGATATTTCTTGAAGAGACTTCGCTAGTGGC : 820
CAAAATAGCAAAATTCCTACTTTTCTTTATGCCATGCCCTTTTCGTCAAACAGGATATTTCTTGAAGAGACTTCGCTAGTGGC

*          840         *          860         *          880         *          900
LCYB-a : GCGGCCCTGGAGTGCCAAATGAAAGATATCCAGGAAAGAAATGGTGGCTAGATTAAAGCACTTAGGCATAAAAAGTTAAAGCATT : 902
LCYB-b : GCGGCCCTGGAGTGCCAAATGAAAGATATCCAGGAAAGAAATGGTGGCTAGATTAAAGCACTTAGGCATAAAAAGTTAAAGCATT : 902
GCGGCCCTGGAGTGCCAAATGAAAGATATCCAGGAAAGAAATGGTGGCTAGATTAAAGCACTTAGGCATAAAAAGTTAAAGCATT

*          920         *          940         *          960         *          980
LCYB-a : GAAGAGGATGACCATTTGTCTCATTCCGATGGGTGGGCCCTTCCAGTGTCTCCCAAAAGAGTTGTTGGAATAGGTTGGTACCC : 984
LCYB-b : GAAGAGGATGACCATTTGTCTCATTCCGATGGGTGGGCCCTTCCAGTGTCTCCCAAAAGAGTTGTTGGAATAGGTTGGTACCC : 984
GAAGAGGATGACCATTTGTCTCATTCCGATGGGTGGGCCCTTCCAGTGTCTCCCAAAAGAGTTGTTGGAATAGGTTGGTACCC

*          1000        *          1020        *          1040        *          1060
LCYB-a : CTGGGATGGTGCACCCTTCAACTGGCTATATGGTGGCAAGGACTTTAGCTGCCGCTCCTATTGTTGCAAAATGCAATCGTTCC : 1066
LCYB-b : CTGGGATGGTGCACCCTTCAACTGGCTATATGGTGGCAAGGACTTTAGCTGCCGCTCCTATTGTTGCAAAATGCAATCGTTCC : 1066
CTGGGATGGTGCACCCTTCAACTGGCTATATGGTGGCAAGGACTTTAGCTGCCGCTCCTATTGTTGCAAAATGCAATCGTTCC

*          1080        *          1100        *          1120        *          1140
LCYB-a : AAGCCTCAGTTCTGACAGAAGCATTTCAGGACACAAATTTGCTGCTGAAGTTTGGAAAGATTGTTGGCCCATAGAAAGGAGA : 1148
LCYB-b : AAGCCTCAGTTCTGACAGAAGCATTTCAGGACACAAATTTGCTGCTGAAGTTTGGAAAGATTGTTGGCCCATAGAAAGGAGA : 1148
AAGCCTCAGTTCTGACAGAAGCATTTCAGGACACAAATTTGCTGCTGAAGTTTGGAAAGATTGTTGGCCCATAGAAAGGAGA

*          1160        *          1180        *          1200        *          1220        *
LCYB-a : AGGCAAAGGGAGTTCTTCTGTTTTGGTATGGATATCCTGCTCAAACCTTGACTTACCTGCCACTAGAAGGTTTTTCGATGCTT : 1230
LCYB-b : AGGCAAAGGGAGTTCTTCTGTTTTGGTATGGATATCCTGCTCAAACCTTGACTTACCTGCCACTAGAAGGTTTTTCGATGCTT : 1230
AGGCAAAGGGAGTTCTTCTGTTTTGGTATGGATATCCTGCTCAAACCTTGACTTACCTGCCACTAGAAGGTTTTTCGATGCTT

*          1240        *          1260        *          1280        *          1300        *
LCYB-a : TTTTTGTACTGGAGCCCGTTATTTGGCATGGTTTCTTATCATCGAGATTGTTTCTCCCGAGCTTTTAGTTTTTGGGCTTTC : 1312
LCYB-b : TTTTTGTACTGGAGCCCGTTATTTGGCATGGTTTCTTATCATCGAGATTGTTTCTCCCGAGCTTTTAGTTTTTGGGCTTTC : 1312
TTTTTGTACTGGAGCCCGTTATTTGGCATGGTTTCTTATCATCGAGATTGTTTCTCCCGAGCTTTTAGTTTTTGGGCTTTC

*          1320        *          1340        *          1360        *          1380        *
LCYB-a : TCTATTCTCACATGCCTCTAATACTTCTAGGCTAGAGATCATGGCAAAGGGACTCTTCTCTTGGTTAACAATGATCAACAAC : 1394
LCYB-b : TCTATTCTCACATGCCTCTAATACTTCTAGGCTAGAGATCATGGCAAAGGGACTCTTCTCTTGGTTAACAATGATCAACAAC : 1394
TCTATTCTCACATGCCTCTAATACTTCTAGGCTAGAGATCATGGCAAAGGGACTCTTCTCTTGGTTAACAATGATCAACAAC

*          1400        *          1420        *
LCYB-a : TTGGTACAAGATACAGATTAAGGTGACCAAGGATATTATAAT : 1436
LCYB-b : TTGGTACAAGATACAGATTAAGGTGACCAAGGATATTATAAT : 1436
TTGGTACAAGATACAGATTAAGGTGACCAAGGATATTATAAT

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Supplementary Fig. S3. The mRNA sequences of *lycopene β-cyclase (LCYB)* alleles, *LCYB-a* and *LCYB-b*, in Newhall and Cara Cara oranges. Note that, no difference in sequence was observed between the two oranges. Start codon and stop codon are underlined by red.

CYCB-a : CTTTCTTTTGTCTTTCTCATTTCCTCTG GCTCTGGCATGGCAACTCTTCTTAGCCCGTTTTCTCCTTCTCCTTTAGCTAAAG : 82
 CYCB-b : CTTTCTTTTGTCTTTCTCATTTCCTCTG GCTCTGGCATGGCAACTCTTCTTAGCCCGTTTTCTCCTTCTCCTTTAGCTAAAG : 82
 CTTTCTTTTGTCTTTCTCATTTCCTCTG GCTCTGGCATGGCAACTCTTCTTAGCCCGTTTTCTCCTTCTCCTTTAGCTAAAG

CYCB-a : TTTCCGAAATAATTGATTCAACATCATCAG CTTTCAATTTTC CTATTTCCATTAGGCCGCAAAATGCATGTTCAAGAAAGGC : 164
 CYCB-b : TTTCCGAAATAATTGATTCAACATCATCAG CTTTCAATTTTC CTATTTCCATTAGGCCGCAAAATGCATGTTCAAGAAAGGC : 164
 TTTCCGAAATAATTGATTCAACATCATC TTCATTTTC CTATTTCCATTAGGCCGCAAAATGCATGTTCAAGAAAGGC

CYCB-a : GGATCATCATCATCATCACAGGATCCGGACAAGCAAGTTTGGTAACTTCTTAGAGTTGACACCCGGAGTCGG ACCTGAATTC : 246
 CYCB-b : GGATCATCATCATCATCACAGGATCCGGACAAGCAAGTTTGGTAACTTCTTAGAGTTGACACCCGGAGTCGG ACCTGAATTC : 246
 GGATCATCATCATCATCACAGGATCCGGACAAGCAAGTTTGGTAACTTCTTAGAGTTGACACCCGGAGTCGG ACCTGAATTC

CYCB-a : TTAGA CTTTGTATCTCCCTGGTTTCATCCGTCGGATTCGATTCGATATGACGTGATCATCATTTGGCACTGGACCGCCGCC : 328
 CYCB-b : TTAGA CTTTGTATCTCCCTGGTTTCATCCGTCGGATTCGATTCGATATGACGTGATCATCATTTGGCACTGGACCGCCGCC : 328
 TTAG CTTTGTATCTCCCTGGTTTCATCCGTCGGATTCGATTCGATATGACGTGATCATCATTTGGCACTGGACCGCCGCC

CYCB-a : FCCGCTAGCTGAGCAAGTCTCATCGCGTCATAGT TCAAGGTATGTTGTTGTTGATCCTTCACCTCTTTCTACGTGGCCTAA : 410
 CYCB-b : FCCGCTAGCTGAGCAAGTCTCATCGCGTCATAGT TCAAGGTATGTTGTTGTTGATCCTTCACCTCTTTCTACGTGGCCTAA : 410
 FCCGCTAGCTGAGCAAGTCTCATCGCGTCAT GT TCAAGGTATGTTGTTGTTGATCCTTCACCTCTTTCTACGTGGCCTAA

CYCB-a : CAACTATGGAGTTTGGGTTGATGAGTTTGAAGACATAGGACTTCTAGACTGTTTGGACAAAACCTTGGCCGATGACTTGTGTT : 492
 CYCB-b : CAACTATGGAGTTTGGGTTGATGAGTTTGAAGACATAGGACTTCTAGACTGTTTGGACAAAACCTTGGCCGATGACTTGTGTT : 492
 CAACTATGGAGTTTGGGTTGATGAGTTTGAAGACATAGGACTT TAGACTGTTTGGACAAAACCTTGGCCGATGACTTGTGTT

CYCB-a : TTTATTAATGATCACAAGACCAAGTATCTAGACAGGCCCTACGGTCGTTGTTAGTAGAAATATTTTGAAGACAAAGTTATTAG : 574
 CYCB-b : TTTATTAATGATCACAAGACCAAGTATCTAGACAGGCCCTACGGTCGTTGTTAGTAGAAATATTTTGAAGACAAAGTTATTAG : 574
 TTTATTAATGATCACAAGACCAAGTATCTAGACAGGCCCTACGGTCGTTGTTAGTAGAAATATTTTGAAGACAAAGTTATTAG

CYCB-a : AGAATTTGTTTCAAATGCTGTTAAGTTTCATAAGGCTAAAGTTTGGCA CTTGATCATCAGGAGTTCGAGTCTTCGATTGT : 656
 CYCB-b : AGAATTTGTTTCAAATGCTGTTAAGTTTCATAAGGCTAAAGTTTGGCA CTTGATCATCAGGAGTTCGAGTCTTCGATTGT : 656
 AGAATTTGTTTCAAATGCTGTTAAGTTTCATAAGGCTAAAGTTTGGCA GTGATCATCAGGAGTTCGAGTCTTCGATTGT

CYCB-a : TTGTGATGATGGCAATGAGATTAAGGCTAGCTTGTGTTGATGCTAGTGGCTTGTGCTAGTACTTTGTTGATGATAAG : 738
 CYCB-b : TTGTGATGATGGCAATGAGATTAAGGCTAGCTTGTGTTGATGCTAGTGGCTTGTGCTAGTACTTTGTTGATGATAAG : 738
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 CYCB-b : CCAAGAAACCATGGATACCAAATTTGCTCATGGGATTTAGCTGAGGTTGAGAGTACCCCTTTTGATTAGAAAATGGTTTC : 820
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CYCB-a : TCATGGATTGGAGAGATTCCTCATTAGGGAAATGAGCCTTACTTGGCAGCTAGCAATTTGAAGCTCCCAACTTTCTCTATGC : 902
 CYCB-b : TCATGGATTGGAGAGATTCCTCATTAGGGAAATGAGCCTTACTTGGCAGCTAGCAATTTGAAGCTCCCAACTTTCTCTATGC : 902
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CYCB-a : AATGCCATTTGATTCAAAATTTGGTATTTTTAGAAGAAACATCTTTGGTTAGTAGGCCAGTTTGTCTATATAAAGAGGTTAAG : 984
 CYCB-b : AATGCCATTTGATTCAAAATTTGGTATTTTTAGAAGAAACATCTTTGGTTAGTAGGCCAGTTTGTCTATATAAAGAGGTTAAG : 984
 AATGCCATTTGATTCAAAATTTGGTATTTTTAGAAGAAACATCTTTGGTTAGTAGGCCAGTTTGTCTATATAAAGAGGTTAAG

CYCB-a : AGCAGAAATGGCAGCGAGTTAAGGCATATGGGAATTAGAGTTAAAAGAGTGATTAAGATGAAAAATGTTTGATTCCAAATGG : 1066
 CYCB-b : AGCAGAAATGGCAGCGAGTTAAGGCATATGGGAATTAGAGTTAAAAGAGTGATTAAGATGAAAAATGTTTGATTCCAAATGG : 1066
 AGCAGAAATGGCAGCGAGTTAAGGCATATGGGAATTAGAGTTAAAAGAGTGATTAAGATGAAAAATGTTTGATTCCAAATGG

CYCB-a : GAGGTCCTCTGCTGTGATCCC CAAAGTGTGATGGCTATTGGC GCACGCTCTGGTTTATCCATCCTGCAACTGGGTATAT : 1148
 CYCB-b : GAGGTCCTCTGCTGTGATCCC CAAAGTGTGATGGCTATTGGC GCACGCTCTGGTTTATCCATCCTGCAACTGGGTATAT : 1148
 GAGGTCCTCTGCTGTGATCCC CAAAGTGTGATGGCTATTGGC GCACGCTCTGGTTTATCCATCCTGCAACTGGGTATAT

CYCB-a : GGTGGCTCGGACCATGGCTCTGGCCCTGCTTGGCTGATGCAATAGCTGATGCCTTGGCTCAACCAGGATGATCAGAGGC : 1230
 CYCB-b : GGTGGCTCGGACCATGGCTCTGGCCCTGCTTGGCTGATGCAATAGCTGATGCCTTGGCTCAACCAGGATGATCAGAGGC : 1230
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CYCB-a : AGGCCACTTCATCAGAAAGTGTGGAATGGGTTGTGGCCAATTGACAGAAGATGCAATAGGGAGTTTTATTCAATTTGGTATGG : 1312
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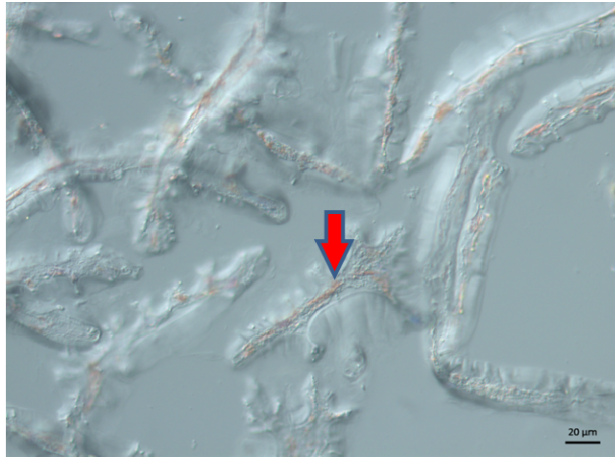
CYCB-a : AGACTTTGTTGAAGCTGGATTTGAAGGGGACTAGGAGATTCCTTTGATGCTTTCTTTGATTGAATCCTCACTACTGGCATGG : 1394
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CYCB-a : GTTTCCTCCTCAAGGTTGCTCTTGCAGAGCTTGGCCTAAGCTTGTCTCTCTTTGGACACGGCTCGAATTTCTCCAGG : 1476
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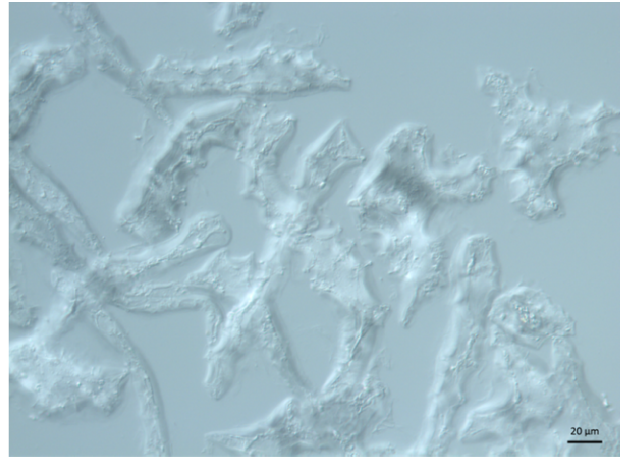
CYCB-a : TTGGATATTGTTACCAAGTGCCCTCTTCCTCTGGTTAAAATGATGGGGAATCTTGGCCTTGAACCATTGAAGATTAATG : 1558
 CYCB-b : TTGGATATTGTTACCAAGTGCCCTCTTCCTCTGGTTAAAATGATGGGGAATCTTGGCCTTGAACCATTGAAGATTAATG : 1558
 TTGGATATTGTTACCAAGTGCCCTCTTCCTCTGGTTAAAATGATGGGGAATCTTGGCCTTGAACCATTGAAGATTAATG

CYCB-a : TTCTTGAATAATAGCGTCTC : 1579
 CYCB-b : TTCTTGAATAATAGCGTCTC : 1579
 TTCTTGAATAATAGCGTCTC

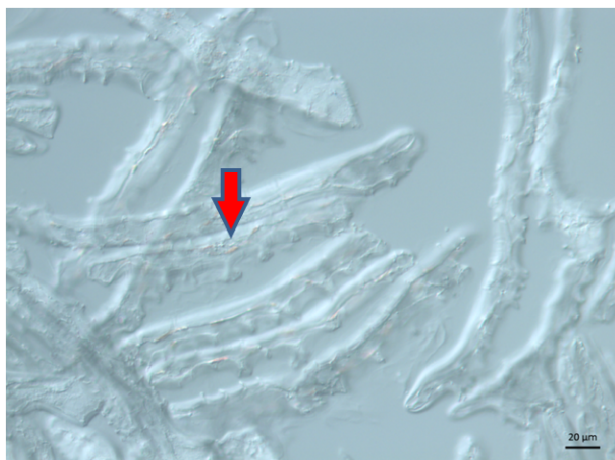
Supplementary Fig. S4. The mRNA sequences of *chromoplast-specific lycopene β-cyclase (CYCB)* alleles, *CYCB-a* and *CYCB-b*, in Newhall and Cara Cara oranges. Note that, no difference in sequence was observed between the two oranges. Start codon and stop codon are underlined by red.



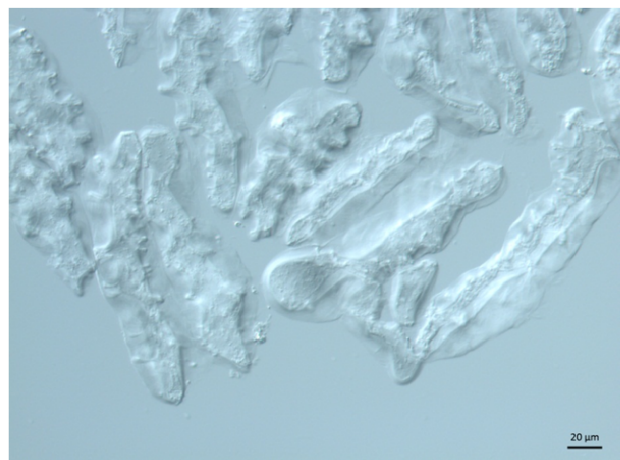
**CPTA treated Newhall
albedo layers**



**Control Newhall
albedo layers**



**Control Cara Cara
albedo layers
with red chromoplasts**



**Control Cara Cara
albedo layers
without red chromoplasts**

Supplementary Fig. S5. The observation of red crystalline chromoplast under the light microscope in 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) treated albedo layers of Newhall, control Newhall and control Cara Cara oranges. Bar, 20µm.

Supplementary Table S1. List of primers for real-time quantitative PCR

Gene	Primers (Forward; Reverse)	Genome accession
<i>Actin</i>	5'-CATCCCTCAGCACCTTCC-3'; 5'-CCAACCTTAGCACTTCTCC-3'	Cs1g05000
<i>BCH</i>	5'-GTTTGCCATAATCAACGC-3'; 5'-CTCTCCGAAATAAGGCA-3'	Cs9g19270
<i>CCD1</i>	5'-GCACGAAATGGAATAGTGGC-3'; 5'-CTTTAGTAGGAGGAGTCTCG-3'	Cs7g01710
<i>CRTISO</i>	5'-ATTTGATAACCCAAGCACTG-3'; 5'-TGAGGCAGATAATAGGCAAG-3'	Cs6g13340
<i>CYCB</i>	5'-CCCTATTTCATTAGGCCGC-3'; 5'-CACGTCATATCGAATACGATC-3'	Cs1g04120
<i>CYCB-a</i>	5'-GAGCAAGTCTCATCGCGTCATA-3' 5'-ACTTTAGCCTTATGAAACTTAACTCCATTTG-3'	
<i>CYCB-b</i>	5'-GCAAGTCTCATCGCGTCATGGTA-3' 5'-ACTTTAGC CTTATGAAACCTAACGCCATTTA-3	
<i>DXR</i>	5'-GAAGTTAAAGTGGCTGATGC-3'; 5'-GAAGAATCCTGTGTTTCGAC-3'	Cs5g05440
<i>DXS1</i>	5'-CCGCATTCTCATTCCCGACT-3'; 5'-TTGTGAGTGATACTCTCCTCT-3'	Cs9g05150
<i>DXS2</i>	5'-TTCCGGGATTGCTATTGGAG-3'; 5'- AAATCAATTTCCAGCCGCC-3'	Cs1g20530
<i>LCYB</i>	5'-GAACCAGGAGCTTAGGTCTG-3'; 5'-GCTAGGTCTACAACAAGGCC-3'	orange1.1t00772
<i>LCYE</i>	5'-AACCCATCTTGATTGGTCGT-3'; 5'-AGAAGCAACAGTAGCAAGCCT-3'	Cs4g14850
<i>NCED2</i>	5'-CCATGGCCTTGGACATGGCG-3'; 5'-TGATGCATTTCGGGGATGGAG-3'	Cs2g03270
<i>NCED3</i>	5'-CTTTAGCCTTGGACGCAGTC-3'; 5'-GAATGCAGTCGGGGACCTTT-3'	Cs5g14370
<i>PDS</i>	5'-TTCAGCCGATTTGATTTTCC-3'; 5'-ACACCCTGCTTTCTCATCCA-3'	orange1.1t02361
<i>PSY1</i>	5'-CTACAATGCAGCATTGGCAC-3'; 5'-CATGAAGTTTCTCCATTTAATGG-3'	Cs6g15910
<i>PSY1-a</i>	5'-CAAGATGAGTTGGCACAGGCA-3' 5'-AGCCCATAACCGGCCATCG-3'	
<i>PSY1-b</i>	5'-CAAGATGAGTTGGCACAGGCG-3' 5'-GAAGCCCATAACCGGCCATCT-3'	
<i>PSY2</i>	5'-TTACAATGCAGCATTGGCCT-3'; 5'-CATGAAGTTTCTCCATTTATTAAT-3'	orange1.1t00657
<i>VDE</i>	5'-ACCGAATGCCAGATCAAATGTGG-3'; 5'-CCACCATATCCATCCCATGC(A/G)TC-3'	Cs5g26080
<i>ZDS</i>	5'-TCATCCCAAGGTTTAGAAG-3'; 5'-TACCAGACAAAGTTGCTCC-3'	orange1.1t06069
<i>ZEP</i>	5'-ACTTGTTACTGGAATTGCAGA-3'; 5'-CCCATATTTGGCTGCATAGCATG-3'	orange1.1t04051
<i>ZISO</i>	5'-ACTCCGTCTCCTCCCTTCA-3'; 5'-TTACCTGCCCAACCATCTGT-3'	Cs5g24730

Supplementary Table S2. Effects of 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) and norflurazon treatments on accumulation of carotenoids in *in vitro* cultured flesh tissues of Newhall and Cara Cara. The 0 week tissues were separated from fruit at S5 stage.

Treatment	Contents ($\mu\text{g g}^{-1}$ FW)								
	Phytoene	Phytofluene	β -Carotene	Lycopene	Violaxanthin	Luteoxanthin	<i>cis</i> -Violaxanthin	Others	Total carotenoids
Control (2 weeks) Newhall					0.20 ± 0.05	0.20 ± 0.02	0.56 ± 0.11	0.12 ± 0.02	1.08 ± 0.13
Control (2 weeks) Cara Cara	24.07 ± 0.20	3.87 ± 0.17	0.14 ± 0.08	5.85 ± 0.30	0.12 ± 0.03	0.11 ± 0.02	0.36 ± 0.05	0.57 ± 0.05	35.08 ± 0.70
CPTA (2 weeks) Newhall	1.60 ± 0.07	0.39 ± 0.03		13.91 ± 0.89			0.14 ± 0.01	1.41 ± 0.11	17.44 ± 1.02
CPTA (2 weeks) Cara Cara	10.64 ± 0.49	1.91 ± 0.11	0.24 ± 0.03	7.39 ± 0.45			0.03 ± 0.02	0.82 ± 0.01	21.04 ± 0.98
Norflurazon (2 weeks) Newhall	32.22 ± 0.53	1.77 ± 0.03			0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.00		34.07 ± 0.55
Norflurazon (2 weeks) Cara Cara	46.60 ± 1.34	3.04 ± 0.08		2.62 ± 0.17			0.02 ± 0.01	0.53 ± 0.03	52.81 ± 1.48
Control (4 weeks) Newhall	0.03 ± 0.03				0.17 ± 0.01	0.27 ± 0.02	0.60 ± 0.05	0.41 ± 0.03	1.48 ± 0.04
Control (4 weeks) Cara Cara	33.44 ± 1.44	5.33 ± 0.24		5.37 ± 0.46	0.10 ± 0.02	0.19 ± 0.05	0.37 ± 0.04	0.85 ± 0.09	45.64 ± 2.24
CPTA (4 weeks) Newhall	2.52 ± 0.03	0.83 ± 0.02		18.13 ± 0.69	0.05 ± 0.00	0.03 ± 0.00	0.24 ± 0.00	1.40 ± 0.06	23.20 ± 0.80
CPTA (4 weeks) Cara Cara	31.48 ± 1.28	6.25 ± 0.23		16.64 ± 0.74	0.02 ± 0.00		0.08 ± 0.02	1.47 ± 0.05	55.95 ± 2.29
Norflurazon (4 weeks) Newhall	60.09 ± 3.37	4.13 ± 0.20			0.04 ± 0.01	0.07 ± 0.00	0.13 ± 0.02	0.08 ± 0.01	64.54 ± 3.59
Norflurazon (4 weeks) Cara Cara	82.76 ± 2.15	5.10 ± 0.17		0.64 ± 0.00		0.03 ± 0.00	0.05 ± 0.02	0.24 ± 0.04	88.82 ± 2.31

Supplementary Table S3. List of primers for full-length gene cloning

Gene	Primers (Forward; Reverse)
<i>CYCB</i>	5'- CTTTCTTTTGTCTTTCTCATTTC-3' 5'- GAGACGCTAATTATTCAAGAAC-3'
<i>LCYB</i>	5'-TGAAGATTCAGAACCAGGAG-3' 5'- GCATGAGTTATTAAGCACATTA-3'
<i>PSYI</i>	5'-CTACAGAGAGTTGAAGTTACAGGG-3' 5'-TAGGGCATCGGCCTTGGATTGTG-3'
