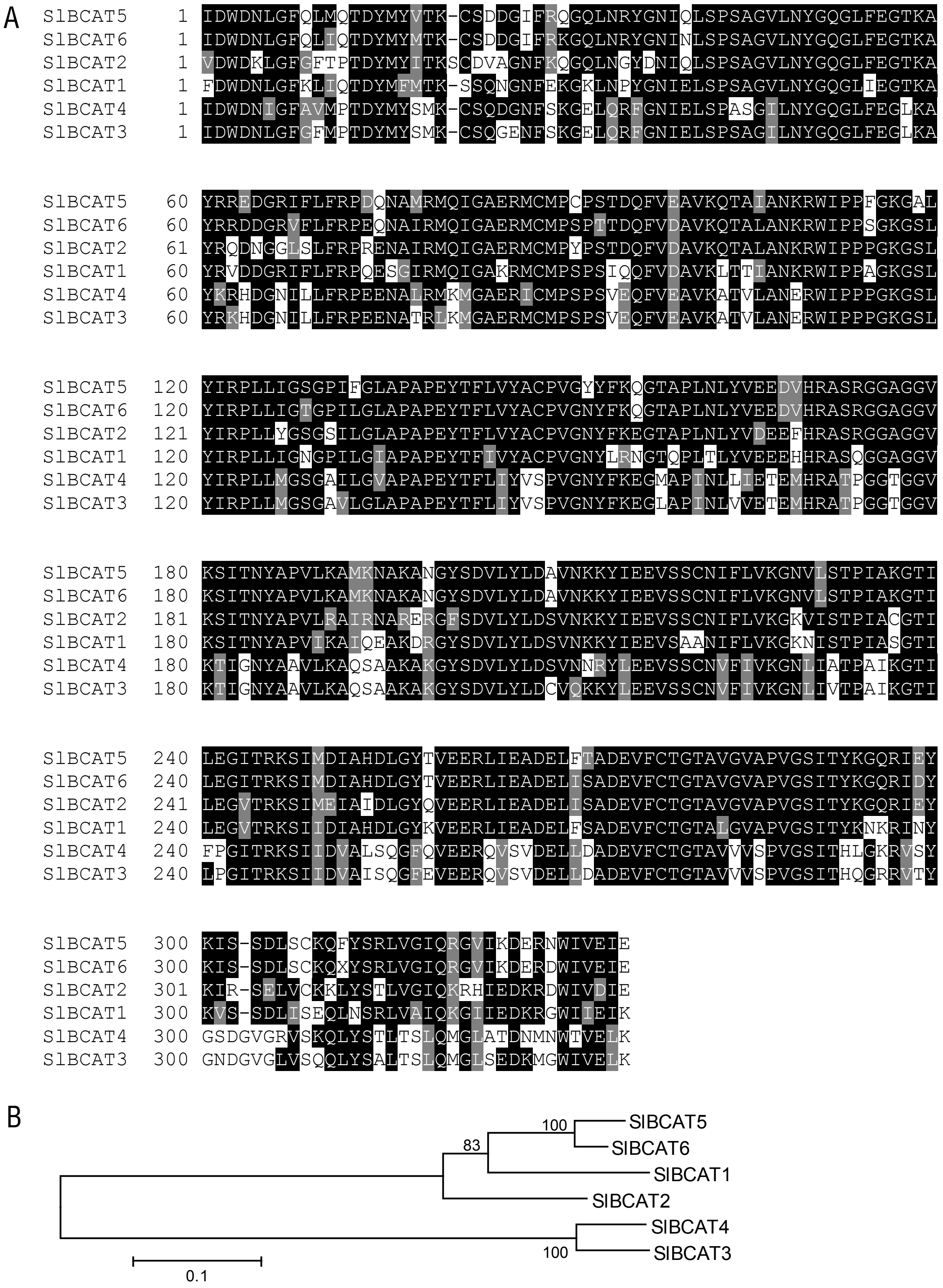

A

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SlBCAT2     1 VDWDKLGFGFTPTDYMYITKSCDVAGNFKQGQLNGYDNIQLSPSAGVLNYGQGLFEGTKA
SlBCAT1     1 FDWDNLGFKLIQTDYMFMTK-SSQNGNFEKGKLNPYGNIELSPSAGVLNYGQGLIEGTKA
SlBCAT4     1 IDWDNIGFAVMPTDYMYSMK-CSQDGNFSKGELQRFGNIELSPASGILNYGQGLFEGLKA
SlBCAT3     1 IDWDNLGFGFMPTDYMYSMK-CSQGENFSKGELQRFGNIELSPSAGILNYGQGLFEGLKA

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SlBCAT6    60 YRRDDGRVFLFRPEQNAIRMQIGAERMCMPSPTTDQFVDAVKQTALANKRWIPPSGKGSL
SlBCAT2    61 YRQDNGGLSLFRPRENAIRMQIGAERMCMPYPSTDQFVDAVKQTALANKRWIPPPGKGSL
SlBCAT1    60 YRVDDGRIFLFRPQESGIRMQIGAKRMCMPSPSIQQFVDAVKLTTIANKRWIPPAGKGSL
SlBCAT4    60 YKRHDGNILLFRPEENALRMKMGAERICMPSPSVEQFVEAVKATVLANERWIPPPGKGSL
SlBCAT3    60 YRKHDGNILLFRPEENATRLKMGAERMCMPSPSVEQFVEAVKATVLANERWIPPPGKGSL

SlBCAT5   120 YIRPLLIGSGPIFGLAPAPEYTFLVYACPVGYYFKQGTAPLNLYVEEDVHRASRGGAGGV
SlBCAT6   120 YIRPLLIGTGPILGLAPAPEYTFLVYACPVGNYFKQGTAPLNLYVEEDVHRASRGGAGGV
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SlBCAT1   120 YIRPLLIGNGPILGIAPAPEYTFIVYACPVGNYLRNGTQPLTLYVEEEHHRASQGGAGGV
SlBCAT4   120 YIRPLLMGSGAILGVAPAPEYTFLIYVSPVGNYFKEGMAPINLLIETEMHRATPGGTGGV
SlBCAT3   120 YIRPLLMGSGAVLGLAPAPEYTFLIYVSPVGNYFKEGLAPINLVVETEMHRATPGGTGGV

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SlBCAT6   180 KSITNYAPVLKAMKNAKANGYSDVLYLDAVNKKYIEEVSSCNIFLVKGNVLSTPIAKGTI
SlBCAT2   181 KSITNYAPVLRAIRNARERGFSDVLYLDSVNKKYIEEVSSCNIFLVKGKVISTPIACGTI
SlBCAT1   180 KSITNYAPVIKAIQEAKDRGYSDVLYLDSVNKKYIEEVSAANIFLVKGKNISTPIASGTI
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SlBCAT6   240 LEGITRKSIMDIAHDLGYTVEERLIEADELISADEVFCTGTAVGVAPVGSITYKGQRIDY
SlBCAT2   241 LEGVTRKSIMEIAIDLGYQVEERLIEADELISADEVFCTGTAVGVAPVGSITYKGQRIEY
SlBCAT1   240 LEGVTRKSIIDIAHDLGYKVEERLIEADELFSADEVFCTGTALGVAPVGSITYKNKRINY
SlBCAT4   240 FPGITRKSIIDVALSQGFQVEERQVSVDELLDADEVFCTGTAVVVSPVGSITHLGKRVSY
SlBCAT3   240 LPGITRKSIIDVAISQGFEVEERQVSVDELLDADEVFCTGTAVVVSPVGSITHQGRRVTY

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SlBCAT6   300 KIS-SDLSCKQXYSRLVGIQRGVIKDERDWIVEIE
SlBCAT2   301 KIR-SELVCKKLYSTLVGIQKRHIEDKRDWIVDIE
SlBCAT1   300 KVS-SDLISEQLNSRLVAIQKGIIEDKRGWIIEIK
SlBCAT4   300 GSDGVGRVSKQLYSTLTSLQMGLATDNMNWTVELK
SlBCAT3   300 GNDGVGLVSQQLYSALTSLQMGLSEDKMGWIVELK
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B

**Figure S1.** Evolutionary relationships of mature SlBCAT proteins. Mature peptides were predicted by omitting highly divergent N-terminal residues thought to be transit peptides.

A, Alignment of amino acid sequences using ClustalW (Larkin et al., 2007). B, The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 1.21366717 is shown. The percentage of replicate trees in which the proteins clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. There were a total of 332 amino acids in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

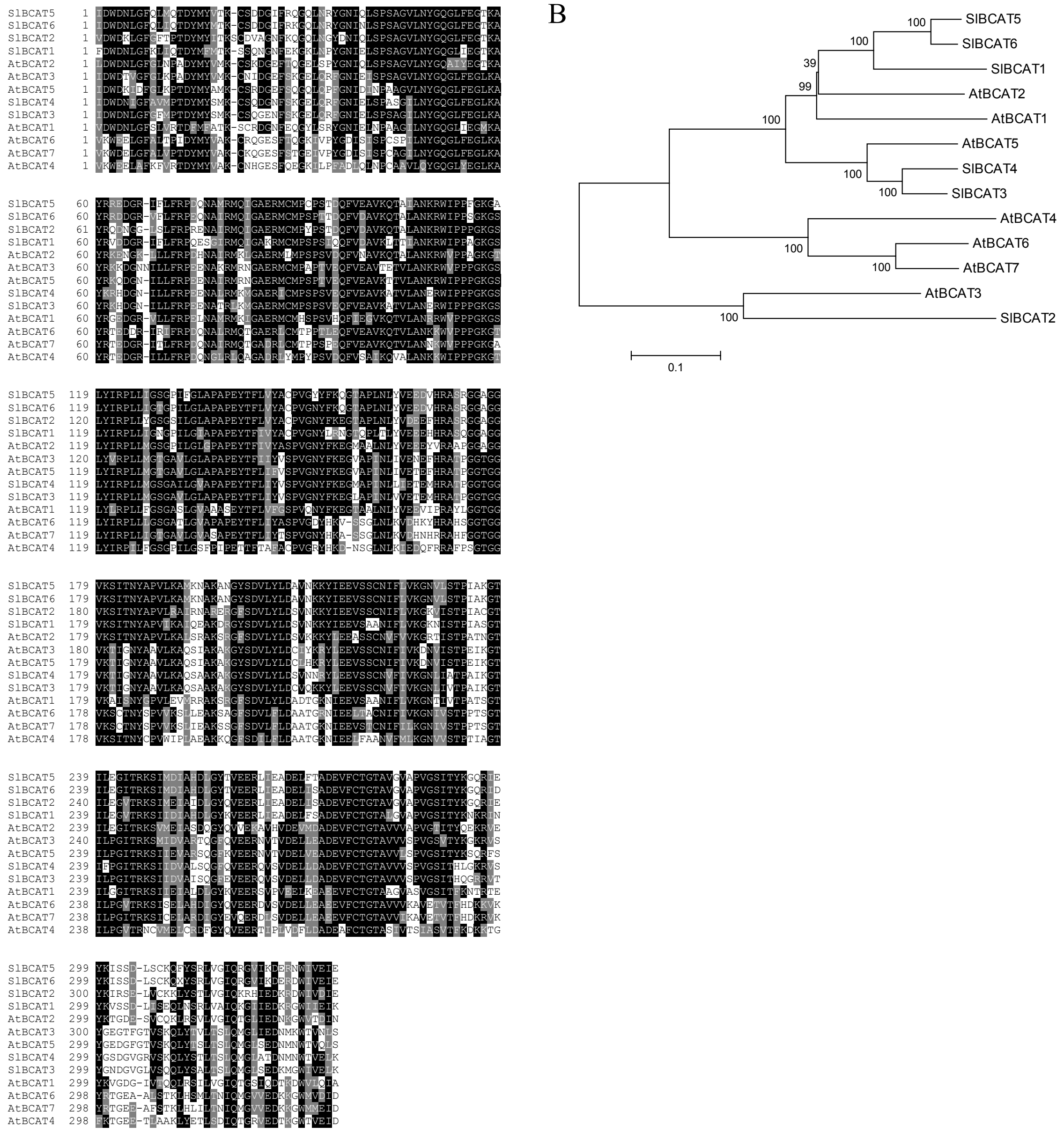
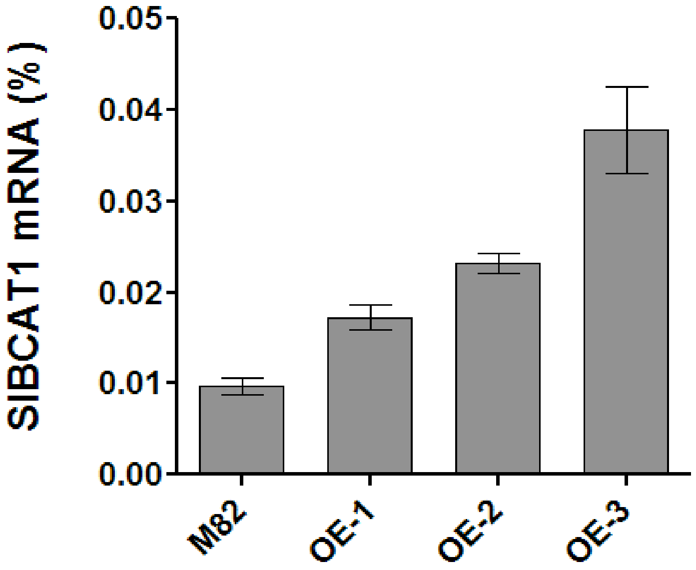


**Figure S2.** Evolutionary relationships of mature SlBCAT and AtBCAT proteins. Mature peptides were predicted by omitting highly divergent N-terminal residues thought to be transit peptides. A, Alignment of amino acid sequences using ClustalW (Larkin et al., 2007). B, The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 2.55921922 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. There were a total of 331 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).



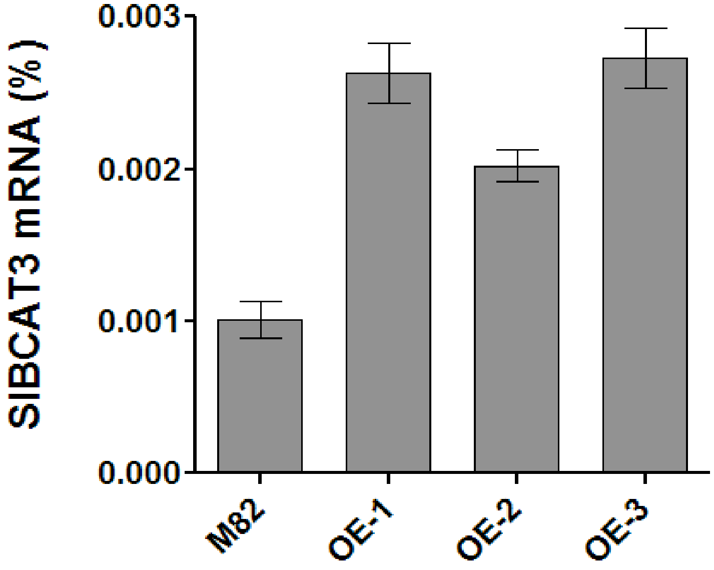
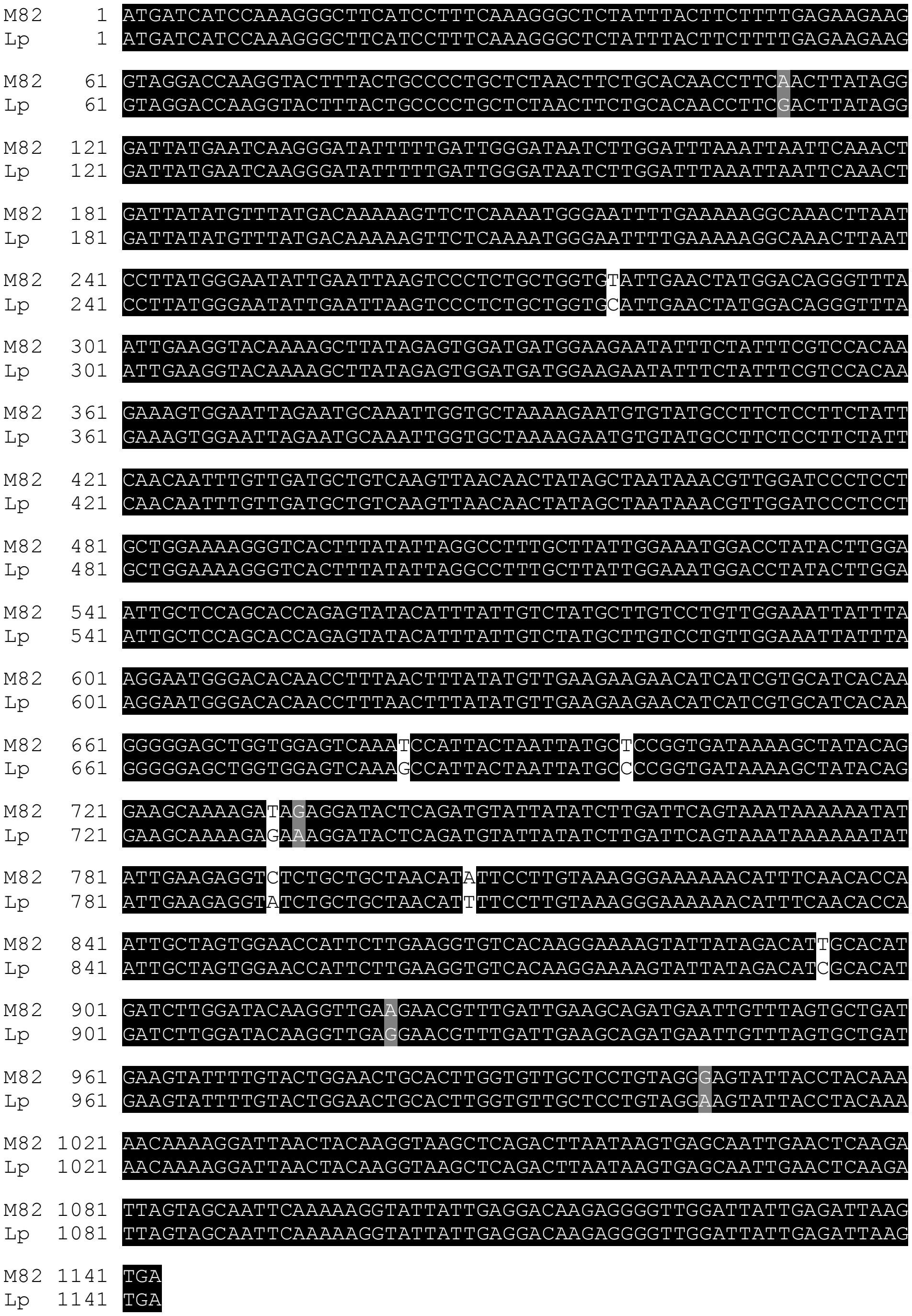


**Figure S3.** Transcript analysis in fruits of transgenic plants overexpressing *SlBCATs*. Analysis was performed on three biological and three technical replicates of ripe fruit for each sample. Values represent percentage of total mRNA per sample ± SD, calculated from a standard curve for each cDNA. Data were analyzed by one-way ANOVA and values of each line are significantly different compared to control as determined by Tukey's test ($p<0.05$).



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M82    1 ATGATCATCCAAAGGGCTTCATCCTTTCAAAGGGCTCTATTTACTTCTTTTGAGAAGAAG
Lp     1 ATGATCATCCAAAGGGCTTCATCCTTTCAAAGGGCTCTATTTACTTCTTTTGAGAAGAAG

M82   61 GTAGGACCAAGGTACTTTACTGCCCCTGCTCTAACTTCTGCACAACCTTCAACTTATAGG
Lp    61 GTAGGACCAAGGTACTTTACTGCCCCTGCTCTAACTTCTGCACAACCTTCGACTTATAGG

M82  121 GATTATGAATCAAGGGATATTTTTGATTGGGATAATCTTGGATTTAAATTAATTCAAACT
Lp   121 GATTATGAATCAAGGGATATTTTTGATTGGGATAATCTTGGATTTAAATTAATTCAAACT

M82  181 GATTATATGTTTATGACAAAAAGTTCTCAAAATGGGAATTTTGAAAAAGGCAAACTTAAT
Lp   181 GATTATATGTTTATGACAAAAAGTTCTCAAAATGGGAATTTTGAAAAAGGCAAACTTAAT

M82  241 CCTTATGGGAATATTGAATTAAGTCCCTCTGCTGGTGTATTGAACTATGGACAGGGTTTA
Lp   241 CCTTATGGGAATATTGAATTAAGTCCCTCTGCTGGTGCATTGAACTATGGACAGGGTTTA

M82  301 ATTGAAGGTACAAAAGCTTATAGAGTGGATGATGGAAGAATATTTCTATTTCGTCCACAA
Lp   301 ATTGAAGGTACAAAAGCTTATAGAGTGGATGATGGAAGAATATTTCTATTTCGTCCACAA

M82  361 GAAAGTGGAATTAGAATGCAAATTGGTGCTAAAAGAATGTGTATGCCTTCTCCTTCTATT
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M82  421 CAACAATTTGTTGATGCTGTCAAGTTAACAACTATAGCTAATAAACGTTGGATCCCTCCT
Lp   421 CAACAATTTGTTGATGCTGTCAAGTTAACAACTATAGCTAATAAACGTTGGATCCCTCCT

M82  481 GCTGGAAAAGGGTCACTTTATATTAGGCCTTTGCTTATTGGAAATGGACCTATACTTGGA
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M82  601 AGGAATGGGACACAACCTTTAACTTTATATGTTGAAGAAGAACATCATCGTGCATCACAA
Lp   601 AGGAATGGGACACAACCTTTAACTTTATATGTTGAAGAAGAACATCATCGTGCATCACAA

M82  661 GGGGGAGCTGGTGGAGTCAAATCCATTACTAATTATGCTCCGGTGATAAAAGCTATACAG
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M82  721 GAAGCAAAAGATAGAGGATACTCAGATGTATTATATCTTGATTCAGTAAATAAAAAATAT
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M82  841 ATTGCTAGTGGAACCATTCTTGAAGGTGTCACAAGGAAAAGTATTATAGACATTGCACAT
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M82  901 GATCTTGGATACAAGGTTGAAGAACGTTTGATTGAAGCAGATGAATTGTTTAGTGCTGAT
Lp   901 GATCTTGGATACAAGGTTGAGGAACGTTTGATTGAAGCAGATGAATTGTTTAGTGCTGAT

M82  961 GAAGTATTTTGTACTGGAACTGCACTTGGTGTTGCTCCTGTAGGGAGTATTACCTACAAA
Lp   961 GAAGTATTTTGTACTGGAACTGCACTTGGTGTTGCTCCTGTAGGAAGTATTACCTACAAA

M82 1021 AACAAAAGGATTAACTACAAGGTAAGCTCAGACTTAATAAGTGAGCAATTGAACTCAAGA
Lp  1021 AACAAAAGGATTAACTACAAGGTAAGCTCAGACTTAATAAGTGAGCAATTGAACTCAAGA

M82 1081 TTAGTAGCAATTCAAAAAGGTATTATTGAGGACAAGAGGGGTTGGATTATTGAGATTAAG
Lp  1081 TTAGTAGCAATTCAAAAAGGTATTATTGAGGACAAGAGGGGTTGGATTATTGAGATTAAG

M82 1141 TGA
Lp  1141 TGA
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**Figure S4.** Comparison of nucleotide sequences of BCAT1 from *S. lycopersicum* cv. M82 and *S. pennellii* (Lp). Alignment of the sequences was carried out using the ClustalW and output processed using the Boxshade program. White boxes indicate nucleotide changes between variants. The black boxes indicate identical base pairs, gray indicates conservative substitutions.

**Table S1.** Levels of free amino acids in red ripe fruit of M82 and *SlBCAT* over-expression lines.

| **Line** | **Amino acid quantity** (ng $gFW^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Val** | **Leu** | **Ile** | **Ala** | **Pro** | **Met** | **Lys** |
| M82 | 0.79 ± 0.06 | 1.12 ± 0.14 | 2.19 ± 0.26 | 6.69 ± 0.48 | 1.91 ± 0.28 | 0.65 ± 0.10 | 3.01 ± 0.22 |
| SlBCAT1-OE-1 | 0.61 ± 0.06 | 0.95 ± 0.09 | 2.22 ± 0.18 | 3.25 ± 0.29 | 3.73 ± 0.35 | 0.60 ± 0.05 | 2.58 ± 0.19 |
| SlBCAT1-OE-2 | 0.70 ± 0.10 | 1.06 ± 0.09 | 2.21 ± 0.18 | 6.92 ± 1.27 | 3.45 ± 0.54 | 0.47 ± 0.05 | 2.04 ± 0.21 |
| SlBCAT1-OE-3 | 0.72 ± 0.17 | 1.13 ± 0.15 | 2.64 ± 0.40 | 6.20 ± 1.65 | 3.32 ± 0.41 | 0.62 ± 0.04 | 2.62 ± 0.45 |
| SlBCAT3-OE-1 | 0.73 ± 0.12 | 1.19 ± 0.20 | 3.38 ± 0.60 | 3.44 ± 0.50 | 2.88 ± 0.06 | 0.74 ± 0.16 | 1.87 ± 0.26 |
| SlBCAT3-OE-2 | 0.64 ± 0.07 | 1.08 ± 0.12 | 3.52 ± 0.42 | 5.02 ± 0.68 | 2.61 ± 0.29 | 0.62 ± 0.06 | 2.37 ± 0.26 |
| SlBCAT3-OE-3 | 1.47 ± 0.32 | 2.61 ± 0.45 | 7.96 ± 1.81 | 7.92 ± 1.00 | 5.57 ± 1.05 | 1.00 ± 0.03 | 4.83 ± 0.72 |

Values are expressed as means ± SD.

**Table S2.** Primer sequences used in present study.

| **Primer name** | **Primer sequence (5'→ 3')** |
|---|---|
| *ilvE*-FlankF | GATGCAACATCAGGTCAATGT |
| *ilvE*-FlankR | CGCAATGGTGTTGAACTCTT |
| *tyrB*-FlankF | CTTATTACGCGCCTGACTTC |
| *tyrB*-FlankR | CACAGGCAATAAGGCAAAGC |
| *SlBCAT3*-pET28bF | GCTAGCGAGAGCGCCGCCGTATTT |
| *SlBCAT3*-pET28bR | GTCGACTTTGAGCTCAACAATCCAACCC |
| *SlBCAT1*-pET28bF | GCTAGCTCTGCACAACCTTCAACTTATAG |
| *SlBCAT1*-pET28bR | GTCGACCTTAATCTCAATAATCCAACCCCT |
| *SlBCAT4*-pET28bF | GCTAGCTTTCAGAAGCAGTCACATTTTGC |
| *SlBCAT4*-pET28bR | GTCGACTCATTTTAGCTCAACAGTCCAATT |
| *SlBCAT2*-pET28bF | GCTAGCTACTACACAGCTCAGGTTG |
| *SlBCAT2*-pET28bR | GTCGACTCATTCAATGTCAACAATCCAATC |
| *SlBCAT5*-pET28bF | GCTAGCGCTTCTTCTCAATCTGTTCTCT |
| *SlBCAT5*-pET28bR | GTCGACTTCGATCTCCACGATCCAATT |
| *SlBCAT6*-pET28bF | GCTAGCTGTTATACAGCTCAGGCGG |
| *SlBCAT6*-pET28bR | GTCGACTCATTCAATCTCCACGATCCAAT |
| *SlBCAT3*-pDONRF | AAAAAGCAGGCTCCATGGAGAGCGCCGCCGTATTT |
| *SlBCAT3*-pDONRR | AGAAAGCTGGGTCTTTGAGCTCAACAATCCAACC |
| *SlBCAT1*-pDONRF | AAAAAGCAGGCTCCATGATCATCCAAAGGGCTTCA |
| *SlBCAT1*-pDONRR | AGAAAGCTGGGTCCTTAATCTCAATAATCCAACCC |
| *SlBCAT4*-pDONRF | AAAAAGCAGGCTCCATGGAGAGCGGCGGCG |
| *SlBCAT4*-pDONRR | AGAAAGCTGGGTCTTTTAGCTCAACAGTCCAATTCA |
| *SlBCAT2*-pDONRF | AAAAAGCAGGCTCCATGATTCAAAGGGCCGCACCT |
| *SlBCAT2*-pDONRR | AGAAAGCTGGGTCTTCAATGTCAACAATCCAATC |
| *SlBCAT5*-pDONRF | AAAAAGCAGGCTCCATGGCTTCTTCTCAATCTGTT |
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| *SlBCAT6*-pDONRR | AGAAAGCTGGGTCGTAAAGTGACCCTTTTCCAGAAG |
| Attb1-pDONR | GGGGACAAGTTTGTACAAAAAAGCAGGCT |
| Attb2-pDONR | GGGGACCACTTTGTACAAGAAAGCTGGGT |
| *SlBCAT1*-pENTR/SD/D-TOPOF | ATGATCATCCAAAGGGCTTCA |
| *SlBCAT1*-pENTR/SD/D-TOPOR | AATCTCAATAATCCAACCCCTCTT |
| *SlBCAT3*-qRT-PCRF | GTCACCATAACCACCTTCTGG |
| *SlBCAT3*-qRT-PCRR | GGACTCAACTCAATGTTACCG |
| *SlBCAT1*-qRT-PCRF | AGGGCTCTATTTACTTCTTTTGAG |
| *SlBCAT1*-qRT-PCRR | CATACACATTCTTTTAGCACCAATT |
| *SlBCAT4*-qRT-PCRF | TACTTGCCACCACCTTCCC |
| *SlBCAT4*-qRT-PCRR | TCCATAATTCAATATCCCAGAAGC |
| *SlBCAT2*-qRT-PCRF | AATTGTTTGAATTTTCATCTCTGCG |
| *SlBCAT2*-qRT-PCRR | ATACACATTCTTTCAGCTCCAATC |
| *SlBCAT5*-qRT-PCRF | CTCACCTCTTCTCTACACCAC |
| *SlBCAT5*-qRT-PCRR | AATAGCCGTTTGCTTAACAGCC |
| *SlBCAT6*-qRT-PCRF | GGTGTTATACAGCTCAGGCG |
| *SlBCAT6*-qRT-PCRR | CAGGACGAAATAAAAATACTCTCC |
| *UBI3F* | GGTTAAGCTCGCTGTGTTGCA |
| *UBI3R* | CGAAGCCTCTGAACCTTTCCA |

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**Tamura K, Dudley J, Nei M, Kumar S** (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution **24:** 1596-1599