Molecular identification of a further branched-chain aminotransferase 7 (BCAT7) in tomato plants

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Abstract

Although the branched-chain amino acids (BCAAs) are essential components of the mammalian diet, our current understanding of their metabolism in plants is still limited. It is however well known that the branched-chain amino acid transaminases (BCATs) play a crucial role in both the synthesis and degradation of the BCAAs leucine, isoleucine and valine. We previously characterized the BCAT gene family in tomato, revealing it to be highly diverse in subcellular localization, substrate preference, and expression. Here we performed further characterization of this family and provide evidence for the presence of another member, BCAT7. On mapping the chromosomal location of this enzyme, it was possible to define the exact chromosome map position of the gene. Although in Arabidopsis thaliana the AtBCAT7 has been considered a pseudo-gene, quantitative evaluation of the expression levels of this gene revealed that the expression profile of the BCAT7 in different tissues of tomato (Solanum lycopersicum cv. MB2) plants is highly variable with the highest expression found in developed flowers. By using a C-terminal E-GFP gene fusion we demonstrate that the BCAT7 is extraplasmidial and in combination with the kinetic characterization of BCAT7 our results suggest that it most likely operates in BCAA degradation in vivo and support our hypothesis of another functional member of BCAT family. The combined data presented are discussed within the context of BCAA metabolism and its functions in higher plants.

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Introduction

The amino acids valine, leucine and isoleucine are commonly referred to as branched-chain amino acids (BCAAs) due to the nature of their carbon skeletons. As a result of their aliphatic nature, BCAAs are mostly found in membrane-spanning protein domains (Binder, 2010). Plant synthesizes all amino acids commonly found in proteins. In contrast, humans and most animals cannot synthesize these amino acids and must obtain these so-called essential amino acids from their diet. It has been extensively demonstrated that these amino acids play important roles in many aspects of animal growth and development (Harris et al., 2005; Nair and Short, 2005; Brosnan and Brosnan, 2006; Avruch et al., 2009; Fuji et al., 2011). When taken together with the lack the ability of humans and many animals to synthesize BCAAs it is not surprisingly that there is a tremendous interest in increasing their levels in crop plants (Ufaz and Galli, 2008).

One distinguishing characteristic related to biosynthesis of the BCAAs is that valine and isoleucine are synthesized in parallel pathways. To this end, a particular set of four enzymes that catalyze the reactions towards the formation of these amino acids from different substrates are required (Binder, 2010). Briefly these enzymes are: acetoacetylhydrazide synthase (AHAS, EC 4.1.3.18), ketolactic reductoisomerase (KARI, EC 1.1.1.86), dihydroxyacyl dehydratase (DHAD, EC 4.2.1.9) and branched-chain aminotransferase (BCAT, EC 2.6.1.42) (Singh and Shaner, 1995; Singh, 1999). The last step in biosynthesis of BCAA is a transamination step converting the 2-oxo acids into the corresponding BCAA (Singh, 1999; Diebold et al., 2002; Schuster and Binder, 2005). This last reaction is catalyzed by BCATs, also known as branched-chain amino acid transaminase, which also initiate degradation of BCAA.

Thus, the BCATs are at the interface of BCAA synthesis, catabolism and further metabolism (Binder et al., 2007; Binder, 2010). Despite their obvious importance, the functions of the individual members of the family are not well understood. This fact notwithstanding, BCATs have been partially characterized in a number of species, including spinach, potato, barley, Nicotiana

Abbreviations: BCAAs, branched-chain amino acids; BCAT, branched-chain aminotransferase; ILS, introgression lines; QTL, quantitative trait loci; TCA, tricarboxylic acid.
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sylvestris, and melon (Hagelstein et al., 1997; Campbell et al., 2001; Malatras et al., 2006; Gao et al., 2009; Gonda et al., 2010). In Arabidopsis thaliana, seven BCAT genes have been identified, six of them being transcribed and found in either plastids or mitochondria (Diebold et al., 2002). These six genes have been designated BCAT1 (AT1G00600), BCAT2 (AT1G00700), BCAT3 (AT3G49680), BCAT4 (AT3G19710), BCAT5 (AT5G65780) and BCAT6 (AT1G50110) but so far no evidence has been obtained for the transcription of BCAT7 (AT1G50090) (Binder, 2010). This gene is therefore considered to be a pseudogene despite the fact that it encodes an intact open reading frame (Binder, 2010). As in Arabidopsis, six BCAT genes from the cultivated tomato (Solanum lycopersicum) have been identified and characterized to date (Maloney et al., 2010). SIBCAT1, -2, -3, and -4 are expressed in multiple plant tissues, whilst the expression of SIBCAT5 and -6 was undetectable. SIBCAT1 and -2 are located in the mitochondria, SIBCAT3 and -4 are located in chloroplasts, whilst SIBCAT5 and -6 are located in the cytosol and vacuole, respectively. SIBCAT1, -2, -3, and -4 were able to restore growth of Escherichia coli BCAT auxotrophic cells, but SIBCAT1 and -2 were less effective than SIBCAT3 and -4 in growth restoration. Thus the tomato BCAT family is highly diverse in subcellular location, substrate preference, and expression (Maloney et al., 2010). Additionally we previously suggested that a catabolic mitochondrial housekeeping gene is likely to exist, since BCAAs are precursors to the tricarboxylic acid (TCA) cycle intermediates succinyl-CoA and acetyl-CoA as well as direct electron donors of the mitochondrial electron transport chain (Ishizaki et al., 2005, 2006; Araújo et al., 2010, 2011).

BCAA catabolism likely has many functions in plants (Däschner et al., 2001; Taylor et al., 2004; Engqvist et al., 2009, 2011; Gonda et al., 2010; Araújo et al., 2011; Witt et al., in press) and it is clear that BCAAs are synthesized in chloroplasts and catabolized in mitochondria (Aubert et al., 1996; Hagelstein et al., 1997; Anderson et al., 1998; Däschner et al., 1999, 2001; Fujiki et al., 2000, 2001, 2002). Towards a better understanding of BCAA metabolism in higher plants, we here identify and characterize another, as yet unknown, putative BCAT from tomato which we designate SIBCAT7. We focus on its bioinformatics identification and characterization, as well as performing genetic, localization and biochemical analyses. Our results provide further insights into the specific functions of BCATs in plants and indicate the presence of another extraplasmoidal BCAT family member strongly expressed in tissues bearing a high respiratory demand.

Materials and methods

All chemicals and reagents used were purchased from Sigma–Aldrich (St. Louis, MO), unless otherwise noted. All supplies were purchased from Fisher Scientific (Pittsburgh, PA), unless otherwise noted.

Protein production and purification

Protein expression constructs were transformed into BL21(DE3) competent cells (Invitrogen). Expression of protein was induced with isopropylthio-β-galactoside according to the BL21(DE3) manufacturer’s instructions. Cells were pelleted and lysed by sonication in phosphate-buffered saline and then treated with Protease Inhibitor Cocktail (Sigma–Aldrich) according to the manufacturer’s directions. Proteins were purified from cell lysates using gravity flow with TALON Affinity Purification Resin or Glutathione-Superflow Resin (Clontech) according to the manufacturer’s instructions. Protein elutions were quantified using the Bradford method (Bradford, 1976). Protein purity was determined to be at least 95% by analysis with SDS-PAGE and staining with Coomassie Brilliant Blue Safestain (Invitrogen).

Microscopy and subcellular localization

Agrobacterium cultures transformed with SIBCAT7-GFP constructs were grown overnight in Luria broth, then pelleted and resuspended in infiltration solution (10 mm MgCl2 and 10 mm MES) to an optical density at 600 nm (OD600) of 0.4. Agrobacterium solutions were injected into the underside of young fully expanded Nicotiana benthamiana leaves with a 2-μL syringe. Plants were grown for 4 d after infection. Protoplasts were released from N. benthamiana leaves as previously described (Yoo et al., 2007). Protoplasts transformed with SIBCAT7-GFP construct were stained with 500 nm MitoTracker Orange, as directed by the manufacturer (Invitrogen). Cells were visualized using a Zeiss Pascal LSM5 Confo- cal Laser Scanning Microscope (Carl Zeiss Microimaging) with a 40× objective. GFP was visualized with an argon laser exciting at 488 nm and detected between 500 and 530 nm. A helium–neon laser, exciting at 543 nm, was used to visualize chlorophyll autofluorescence, detected at 633, and MitoTracker Orange, detected at 576 nm.

Expression analysis

RNA was isolated from different tomato tissues using the RNeasy Plant RNA Extraction Kit (Qiagen) followed by DNase treatment to rid samples of contaminating DNA. RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific). Omniscript reverse transcriptase (Qiagen) was used with 1 μg of each RNA sample to synthesize oligo(dT)-primed cDNA. 1 × SYBR Green Master Mix (Applied Biosystems) was used with 1 μL of each cDNA sample and 500 nm gene-specific primers for qRT-PCR on the Applied Biosystems StepOnePlus real-time PCR machine. For RT-PCR of SIBCAT7 in different plant tissues, five-point standard curves were made for each BCAT to calculate the ratio of transcript. ΔCt values were determined by comparison with the ubiquitin control gene. Amplification conditions were as follows: 2 min at 50 °C; 10 min at 95 °C; 40 cycles each of 15 s at 95 °C followed by 1 min at 60 °C; 15 s at 95 °C; 20 s at 60 °C; 15 s at 95 °C. Primer specificity was confirmed with melting curve analysis on the StepOnePlus real-time PCR machine.

The map positions of SIBCAT7 scoring were determined by genomic DNA–blot analysis exactly as previously described (Maloney et al., 2010).

Enzyme assays

Forward and reverse assays were performed essentially as described previously (Cooper et al., 2002; Maloney et al., 2010). One microgram of purified SIBCAT was used in each reaction, which was carried out at 25 °C for 5 min. Forward assays were recorded at 340 nm in quartz cuvettes and reverse assays at 440 nm in plastic cuvettes. Samples with heat-denatured enzymes were used to obtain blank readings. For both assays, reactions lacking substrate or enzyme or containing boiled enzyme were used as controls. Kinetic data were calculated using GraphPad Prism5 software (Graphpad Software).

Results

As part of our ongoing characterization of BCAA metabolism in higher plants, we previously identified six unique tomato sequences potentially encoding BCAT enzymes in the SOL Genomics Network tomato EST database (Maloney et al., 2010). Further phylogenetic analyses of all putative SIBCATs revealed that the most similar protein are SIBCAT3 and SIBCAT4 with less than 100 nucleotide substitutions (Fig. 1). Additionally SIBCAT2 and SIBCAT6 are also relatively similar with around 200 nucleotide substitutions and they also have a relatively high similarity with SIBCAT1. By
using a similar approach we here identified a seventh putative BCAT gene in the tomato genome, the unigene SGN-U656681 (24 members). The BCAT7 gene was identified as a result of search in the SOL Genomics Network tomato EST database for the sequences homologous to those encoding previously described BCATs of cultivated tomato. However, it should be noted that this predicted protein was overlooked previously (Maloney et al., 2010) not only due to its limited amino acid sequence identity to the other expressed SIBCATs in tomato (Table 1) but also due to the similar size of the identified gene families in both Arabidopsis and tomato plants (Diebold et al., 2002; Maloney et al., 2010). Despite the relatively limited similarity to the other BCATs, we here denominated this gene BCAT7.

To gain a better understanding of the potential role of this SIBCAT family member, expression analysis was performed by quantitative reverse transcription (qRT)-PCR on SIBCAT cDNAs. Tissues tested were shoots, young leaves, inflorescences at 1 d after anthesis (dpa), as well as fruit harvested 10, 20, 30, and 40 dpa (Fig. 2). Interestingly SIBCAT7 is expressed in all tissues at relatively high levels except for inflorescences, where it is much more highly expressed. Accordingly, there is a strong decrease in the levels of BCAAs during fruit development with their levels at 40 dpa between 25% and 10% of those determined in fruits of 10 dpa (Carrari et al., 2006; Maloney et al., 2010). Taken together these results suggest that the BCAAs may act as respiratory substrate and that BCAT7 might be involved in mediating this process.

Since plant organelles have specific functions that are variable during cell and organ development (see for example Lytvchenko et al., 2011), it is reasonable to assume that the subcellular localization of metabolic enzymes is of high functional importance. Additionally previous analyses have already demonstrated that the BCAAs are synthesized in chloroplasts, whereas the degradation pathways are commonly accommodated inside of the mitochondria (Binder et al., 2007; Binder, 2010). With that in mind SIBCAT cDNA was cloned with a C-terminal E-GFP gene fusion, expressed in Nicotiana benthamiana leaf protoplasts, and analyzed by confocal microscopy (Fig. 3). Although we could not observe a clear subcellular localization, SIBCAT7 was definitely not located in the chloroplast with the GFP fluorescence being highly distinct from chlorophyll autofluorescence. Indeed the punctate nature of expression is consistent with that of mitochondria, however, given that there was little overlap with the mitochondrial marker dye it is possible that the expression is alternatively in a similar sized non-mitochondrial compartment such as the peroxisomes. That said to date BCATs have not been reported in peroxisomal proteomics experiments (Reumann et al., 2004) and localization prediction software outputs indicate a mitochondrial localization (Heazlewood et al., 2007). Despite a lack of precision in the exact site of operation of this protein the extraplastidal localization of SIBCAT7 nevertheless suggests that this isomerase is likely to mainly function in BCAA catabolism.

We have previously identified quantitative trait loci (QTL) for the BCAAs in tomato fruit pericarp of introgression lines (ILs) resulting from the interspecific cross of S. lycopersicum and its wild relative Solanum pennellii (Schauer et al., 2006, 2008) and shown that two of these are likely controlled by the branched-chain amino-transferases BCAT1 and 4 (Maloney et al., 2010). Given that BCAT enzymes participate both in the biosynthesis and degradation of all three BCAAs, we determined whether the SIBCAT7 gene colocalized with the seven QTLs simultaneously affecting all three BCAAs. We were able to precisely map the SIBCAT7 gene by its proximities to previously mapped markers within scaffolds (Fig. 4; see map position on the chromosome and the DNA blot hybridized with S. lycopersicum BCAT7). SIBCAT7 mapped to IL bin 1–3 (Fig. 4B). However, none of the coordinate QTL for BCAA content were found

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>BCAT1</th>
<th>BCAT2</th>
<th>BCAT3</th>
<th>BCAT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>% amino acid identity</td>
<td>14.5</td>
<td>14.2</td>
<td>16.4</td>
<td>15.3</td>
</tr>
</tbody>
</table>

Note that SIBCAT1, -2, -3, and -4 are expressed in multiple plant tissues, whilst the expression of SIBCAT5 and -6 was undetectable.
to co-localize with this SIBCAT7 gene. Consistent with the lack of an associated QTL, comparative analysis of BCAT7 expression in S. lycopersicum and its wild species S. pennellii revealed that the expression of this gene was invariant. We evaluated the nucleotide sequences of the translated regions of BCAT7 amplified from M82 and IL1–3. These analyses revealed the presence of ten single nucleotide polymorphisms on nucleic acid level between the S. lycopersicum and S. pennellii alleles resulting in four amino acid changes (Fig. 5).

In order to determine whether differences in these four residues confer altered enzymatic function, both S. lycopersicum and S. pennellii were expressed and purified from E. coli cells and further assayed. Since mitochondrially located BCAT members are thought to function primarily in amino acid catabolism, our analyses were concentrated on BCAA substrates (Table 2). The enzyme was active in the forward (BCAA synthesis) and reverse (branched-chain keto acid synthesis) reactions exhibiting a clear preference for the reverse reaction. This preference supports a primarily catabolic function. The S. pennellii $K_m$ values were significantly ($P<0.05$) higher than the S. lycopersicum enzyme. Consistent with the $K_m$ values obtained, the S. pennellii exhibited a lower catalytic efficiencies independently of the BCAA used as substrate. These small but significantly different values indicated that the S. lycopersicum enzyme is likely more catabolically efficient.

**Discussion**

The biosynthetic pathways of BCAAs in plants have been extensively investigated and a number of genes have been characterized to date, mainly in the context of targets of commercially important herbicides (Singh, 1999; Binder, 2010). Whilst the biosynthesis of BCAAs is relatively well characterized (Singh and Shaner, 1995; Aubert et al., 1997; Halgand et al., 1998; Gaston et al., 2003), our current understanding of the catabolic pathways remains incomplete. We report here the identification and molecular characterization of another individual member of this highly diverse BCAT multigenic family in tomato plants. We demonstrate that SIBCAT7, like SIBCAT1 and SIBCAT2 is extraplastidially localized (Fig. 3). Since SIBCAT7 has its highest expression in both flowers and ripening fruit (Fig. 2), it is reasonable to assume that BCAT7 might have a key role for recycling BCAAs generated by protein degradation in such tissues.

From a previous study conducted in Arabidopsis (Diebold et al., 2002), it could be assumed that AtBCAT7, which is located directly downstream of AtBCAT6, is not an actively transcribed gene but...
S.lyc 1 MGEEIEVHWSAPRSLSTSMLYSFQRSNDEIVDLEPLYANFLRVTGVDRPYEKLRSEL
S.pen 1 MGEEIEVHWSAPRSLSTSMLYSFQRSNDEIVDLEPLYANFLRVTGVDRPYEKLRSEL

S.lyc 61 ENDGKVVKEFAGGVKVYCKHIQAKVRGPTNLKMKFRKLHFLNLPLDLFSLF
S.pen 61 ENDGKVVKEFAGGVKVYCKHIQAKVRGPTNLKMKFRKLHFLNLPLDLFSLF

S.lyc 121 IVPSFLEGLGVSVSYSELSEGRFVVIDALRENPENLGLCDELDDIFPDML
S.pen 121 IVPSFLEGLGVSVSYSELSEGRFVVIDALRENPENLGLCDELDDIFPDML

S.lyc 181 RWEAGPKPFDGIAFVYKSHFVHSTGAPAKKYPTPFPTSLYDLEQSLPYNMLKRHAK
S.pen 181 RWEAGPKPFDGIAFVYKSHFVHSTGAPAKKYPTPFPTSLYDLEQSLPYNMLKRHAK

S.lyc 241 RSSSNYLNSTLPHPSPVAKKLLAWVGDEIVPRETAKYVSVDSIVQGGVDEGLRVY
S.pen 241 RSSSNYLNSTLPHPSPVAKKLLAWVGDEIVPRETAKYVSVDSIVQGGVDEGLRVY

S.lyc 301 GGKVFKLEEHLDRMFDSAKALAFSNVPTREEVKEAHFRTLLNNMDNAHILRILTRGK
S.pen 301 GGKVFKLEEHLDRMFDSAKALAFSNVPTREEVKEAHFRTLLNNMDNAHILRILTRGK

S.lyc 361 VTSGMSPAFNRYGCTLIVLAEWKPPVYDNEKGLMLVATRRNNPSNLKIHNNNLNN
S.pen 361 VTSGMSPAFNRYGCTLIVLAEWKPPVYDNEKGLMLVATRRNNPSNLKIHNNNLNN

S.lyc 421 ILAKIEGNNAGADDAIMLDKDGYVSETNATNIFLVKKGRVVTPHMAYCLPGITRATVMEL
S.pen 421 ILAKIEGNNAGADDAIMLDKDGYVSETNATNIFLVKKGRVVTPHMAYCLPGITRATVMEL

S.lyc 481 VIKESALEERNSLSEFHTAEVTTGTMGEQPVAKIDRGIVGDRVGPILRLQNA
S.pen 481 VIKESALEERNSLSEFHTAEVTTGTMGEQPVAKIDRGIVGDRVGPILRLQNA

S.lyc 541 RNLSKDSGVPPIPETYK
S.pen 541 RNLSKDSGVPPIPETYK

Fig. 5. Nucleotide sequences comparison of BCAT7 between S. lycopersicum and S. pennellii. 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. S. lycopersicum cv. M82 (S.lyc) and S. pennellii (S.pen).

Table 2
Kinetic parameters of BCAT7 enzymes in two alleles. Activities of purified recombinant SBCAT7 and SpBCAT7 proteins using branched-chain amino acids and branched-chain keto acids as substrates. K_m is presented as average ± SE. Kinetic data were obtained using GraphPad Prism5 software. Enzymes contained an N-terminal glutathione S-transferase tag. K_m values of S. pennellii are significantly higher than values obtained for M82 when using the same substrate.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Substrate</th>
<th>K_m (mM)</th>
<th>V_max (nkat mg^{-1})</th>
<th>K_cat (s^{-1})</th>
<th>K_cat/K_m (μM^{-1} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leucine</td>
<td>0.24 ± 0.06</td>
<td>0.3</td>
<td>8.1</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>0.31 ± 0.08</td>
<td>0.3</td>
<td>7.6</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>0.35 ± 0.06</td>
<td>1.1</td>
<td>28.1</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>KIC</td>
<td>4.92 ± 0.91</td>
<td>6.8</td>
<td>173.8</td>
<td>0.038</td>
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<tr>
<td></td>
<td>KMV</td>
<td>5.52 ± 0.63</td>
<td>3.4</td>
<td>83.9</td>
<td>0.017</td>
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<tr>
<td></td>
<td>KIV</td>
<td>5.58 ± 0.74</td>
<td>1.1</td>
<td>23.4</td>
<td>0.005</td>
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<tr>
<td></td>
<td>Leucine</td>
<td>0.67 ± 0.09</td>
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<td>40.8</td>
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<tr>
<td></td>
<td>Isoleucine</td>
<td>0.65 ± 0.07</td>
<td>1.9</td>
<td>46.5</td>
<td>0.070</td>
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<tr>
<td></td>
<td>Valine</td>
<td>0.56 ± 0.04</td>
<td>1.6</td>
<td>39.1</td>
<td>0.070</td>
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<tr>
<td></td>
<td>KIC</td>
<td>11.65 ± 1.67</td>
<td>0.7</td>
<td>16.3</td>
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<tr>
<td></td>
<td>KMV</td>
<td>7.90 ± 0.83</td>
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<td>84.8</td>
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<tr>
<td></td>
<td>KIV</td>
<td>7.09 ± 0.92</td>
<td>0.5</td>
<td>11.7</td>
<td>0.002</td>
</tr>
</tbody>
</table>

KIC, 4-methyl-2-oxopentanoic acid; KMV, 3-methyl-2-oxopentanoic acid; and KIV, 3-methyl-2-oxobutanoic acid.

* Significance as determined by Student’s t test (P<0.05).
rather a pseudogene (Binder, 2010). In contrast, our studies suggest that the tomato SIBCA7 is actively transcribed and is extraplastidically localized and thus most likely involved in BCAA degradation. Considering that some enzymes involved in valine turnover are located in the peroxisomes (Binder, 2010) and that a full set of enzymes required for a complete breakdown of this amino acid is not present in this organelle it is reasonable to assume that the BCA7 is not a peroxisomal enzyme. It should be noted however that the pathways for degradation of BCAAs are distributed over several subcellular compartments with both mitochondria and peroxisomes being important for leucine and valine turnover (Binder, 2010).

Whilst BCAAT activities have been detected in a wide range of eukaryotic organisms, including yeasts, plants, rats and humans (Singh, 1999) the experimental assays for subcellular localization of the individual enzymes have only been performed in a few instances. Although our results does not support a clear cellular localization for BCA7 (Fig. 3), here we provide compelling evidence for the existence of another putative BCAAT and after assessing its activity, it seems reasonable to suggest that BCA7 is also involved in the degradation of BCAs. Since it has recently been demonstrated that the degradation of both BCAs and lysine can provide electrons to the mitochondrial electron transport chain (Araújo et al., 2010) it is thus tempting to speculate a potential role of BCA7 in this process. Additionally in Arabidopsis, branched–chain keto acid dehydrogenase subunits E1β and E2, isovaleryl-CoA dehydrogenase, and both methylcrotonyl-CoA carboxylase subunits, all involved in BCAA degradation, are highly expressed in darkness or under carbohydrate starvation (Binder, 2010). This observation clearly indicates an important role for BCAA catabolism under such environmental conditions. This conclusion is further supported by analysis of the electron transfer flavoprotein/electron-transfer flavoprotein:ubiquinone oxidoreductase (ETF/ETFQ) complex and more recently of isovaleryl-CoA dehydrogenase knockout mutants, which show an accelerated senescence phenotype under cultivation in extended darkness (Ishizaki et al., 2005, 2006; Araújo et al., 2010). However, direct evidence for the involvement of BCATs in feeding electrons into the mitochondrial electron transport chain is still lacking. Although experiments to date go a long way towards describing the structure of the pathways of BCAA metabolism, knowledge concerning its regulation is very limited. By analogy to mammalian systems the results obtained under dark induced senescence (Araújo et al., 2010), alongside expression profiling during plant development (Faivre-Nitschke et al., 2001), implicates isovaleryl-CoA dehydrogenase to be also highly important in the breakdown of leucine. However, as yet, direct evidence in support of this theory is lacking and more work is needed to full understand the role of BCAs on plant metabolism. However due to the multiplicity of mitochondrial and extraplastidially localized BCATs in tomato it is reasonable to suggest that consider further study will be required to comprehensively understand the relative important and regulation of these isoforms as well as their interactions with diverse branches of primary metabolism.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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