Abstract

The amino acids found in plants play important roles in protein biosynthesis, signaling processes, and stress responses, and as components in other biosynthesis pathways. Amino acid degradation helps maintain plant cells' energy states under certain carbon starvation conditions. Branched-chain amino acid transferases (BCATs) play an essential role in the metabolism of branched-chain amino acids (BCAAs) such as isoleucine, leucine and valine. In this paper, we performed genome-wide RNA-seq analysis using CsBCAT7-overexpressing Arabidopsis plants. We observed significant changes in genes related to flowering time and genes that are germination-responsive in transgenic plants. RNA-seq and RT-qPCR analyses revealed that the expression levels of some BCAA catabolic genes were upregulated in these same transgenic plants, and that this correlated with a delay in their senescence phenotype when the plants were placed in extended darkness conditions. These results suggest a connection between BCAT and the genes implicated in BCAA catabolism.

Keywords
Branched-chain amino acid, Branched-chain amino acid transferase, Catabolism, CsBCAT7, RNA-Seq

Introduction

In plant cells, amino acids as the proteins’ constituents play a number of essential roles in the central metabolism. Amino acids, for example, act as intermediates of final metabolites or regulators in certain metabolic pathways (Amir et al. 2018; Yang et al. 2018). They are also involved in a number of cellular reactions, through which they affect a variety of physiological processes including plant growth and development, production of metabolic energy or redox potential, and tolerance to both abiotic and biotic stressors (Fagard et al. 2014; Galli et al. 2014; Moe 2013; Pratelli and Pilot 2014; Watanabe et al. 2013; Zeier 2013). Furthermore, recent reports have shown that certain amino acids function as signaling molecules (Hausler et al. 2014; Szabados and Savoure 2010).

Leucine (Leu), isoleucine (Ile) and valine (Val) are generally referred to as the branched-chain amino acids (BCAAs) because of their branched carbon skeletal structures (Neinast et al. 2019). The metabolic pathway of BCAAs are well analyzed in plants, as some enzymes within the pathway are known herbicide targets (Singh and Shaner 1995). The biosynthesis of Leu and Val occurs using pyruvate, while the biosynthesis of Ile occurs with oxaloacetate as a substrate. All BCAAs share four conserved enzymatic steps catalyzed by acetolactate synthase/acetohydroxyacid synthase (ALS/AHAS), ketol-acid reductoisomerase (KARI), dihydroxyacid dehydratase (DHAD), and the branched-chain aminotransferase (BCAT) (Binder et al. 2007). Notably, BCATs acting during the final step in the biosynthetic pathway also degrade the BCAAs into...
branched-chain keto acids (BCKAs). These BCAT enzyme bifunctional activities have been observed in Arabidopsis thaliana, tomato (Solanum lycopersicum), and cucumber (Cucumis sativus) using complementation and enzyme activity assays (Diebold et al. 2002; Knill et al. 2008; Lee et al. 2019; Liepman and Olsen 2004; Maloney et al. 2010; Schuster and Binder 2005; Schuster et al. 2010). All six SlBCAT enzymes in tomato are active in both forward (BCAA synthesis) and reverse (BCKA synthesis) reactions, whereas only two cucumber BCATs [CsBCAT2 and CsBCAT3] among three CsBCATs perform bifunctional activities, suggesting that different BCAT isoforms found in various plant species have different substrate preferences and therefore function in either one or both reactions.

While BCAAs are essential nutrients in humans and animals (Chen et al. 2010), they and their derivatives also make a significant contribution to plant growth, and play a role in the stress response and the production of volatile components (Xing and Last 2017). Their catabolism pathways have been conferred to drought tolerance during dehydration conditions in Arabidopsis (Pires et al. 2016). They also affect plant resistance to distinct pathogens by regulating the crosstalk between salicylic acid- and jasmonic acid-dependent pathways (Zeier 2013). BCAA catabolism also serves an alternative energy source for plants under conditions of extended darkness (Araujo et al. 2010; Ishizaki et al. 2005) (Daschner et al. 2001). In sum, these findings suggest the importance of BCAA catabolism to various aspects of plant stress and growth conditions.

We recently identified several effects of CsBCATs on both flowering time under normal conditions and seed germination under abiotic stress conditions (Lee et al. 2019a; Lee et al. 2019b). Among three CsBCAT genes, CsBCAT7 targeting to mitochondria functioned in a reverse reaction for three BCAAs. In this study, we profiled the genome-wide transcriptome analysis of p35S::CsBCAT7 transgenic plants. We also observed significant expression changes in some BCAA catabolic genes in these same transgenic plants, a result that suggests a relationship between CsBCAT7 and BCAA catabolism.

Materials and Methods

Plant materials and growth conditions

We used p35S::CsBCAT7 transgenic plants (Lee et al. 2019b; Lee et al. 2019b) and wild-type Arabidopsis (Col-0) plants for transcriptome deep sequencing (RNA-Seq). Seeds from both were sown on 1/2 murashige and skoog (MS) medium that contained 1% sucrose, and the MS medium was maintained at 4°C for 2 days in darkness and then transferred to a normal growth chamber maintained at 23°C under long-day (LD) conditions at a light intensity of 120 μmol m⁻² s⁻¹. The seedlings were harvested at zeitgeber time (ZT) 8 for 7 days, then immediately frozen in liquid nitrogen and stored at −70°C.

RNA isolation and RNA-seq analysis

Total RNA was isolated from 7-d-Arabidopsis seedlings using Plant RNA Purification Reagent (Thermo Fisher Scientific, Waltham, USA) consistent with the manufacturer’s instructions. RNA quality was evaluated with an Agilent 2100 BioAnalyzer (Agilent, CA, USA). The isolated total RNA was used for messenger RNA (mRNA) isolation and subsequent library construction, purified, end-repaired, poly A-tailed, and ligated to index adapters using the RNA sample preparation protocol associated with an Illumina HiSeq2500 sequencer (Illumina, San Diego, CA, USA). A 100 bp paired-end sequencing protocol was employed.

Transcriptome analysis

Analysis of differentially expressed genes (DEGs) was performed using Cuffdiff (Trapnell et al. 2012). To enhance the accuracy of this analysis, the multi-read-correction and frag-bias-correct options were applied. All other options were set to default values. DEGs were identified based on a q-value threshold lower than 0.05 to correct errors caused by multiple-testing (Reiner et al. 2003). For gene ontology (GO) analysis, GO term annotation was performed using GO classification including biological process (BP), cellular component (CC), and molecular function (MF). To characterize the identified genes from DEG analysis, a GO based trend test was carried out using the quantile method (Bolstad et al. 2003). Selected genes of P value < 0.001 were regarded as statistically significant.

RNA expression analysis

For the real-time quantitative polymerase chain reaction (RT-qPCR) analysis, complementary DNA (cDNA) was synthesized from 5 μg of RNA following the protocol associated with the ReverTra Ace qPCR RT Master Mix kit (Toyobo, Osaka, Japan). The RT-qPCR analysis was
conducted in 96-well plates which used a CFX real-time system (Bio-Rad, Hercules, CA, USA) and a THUNDER-BIRD SYBR qPCR mix (Toyobo). PP2AA3 was used as a stably expressed reference gene in the Arabidopsis (Lee et al. 2019b). Two biological replicates were analyzed for each RT-qPCR analysis to ensure the reliability of quantitative assay. The primer sequences used for the RT-qPCR analysis were as follows: for MCCA, 5'- AGAGGAATGAAGATGGAGCAC-3' and 5' AGGTTCCTGTATGCTTCCAGAGG-3'; for AIM1, 5'- ATATCATGGCTTGGGGAAGCC-3' and 5'- GACTGTGTTCTTGCCAACCTTTC-3'; for HCDH, 5'- ATTCGCCGAGTGCAGATACCTCA-G-3' and 5'- GAACC AACATAATTCGTTGCTG-3'. To determine the relative abundance of the transcripts, the data was analyzed using Bio-Rad CFX Manager software (Bio-Rad), and expression values were presented as a bar graph.

Results and Discussion

To comprehensively examine the effect of CsBCAT7 on plant growth and development, we prepared cDNA libraries from the samples of p35S::CsBCAT7 and wild-type (Col-0) plants collected 8 days after seeding for transcriptome deep-sequencing (RNA-Seq). We sequenced two libraries on an Illumina HiSeq2500 platform and generated short raw reads for each genotype: 34.2 million (CsBCAT7 OX) and 36.2 million (Col-0). After filtering out adaptor sequences, contaminating sequences, and low-quality reads, we retained 33.2 and 35.9 million high-quality processed reads for further analysis. More than 97.6% of the RNA-Seq reads mapped to the Arabidopsis reference genome. Overall, we detected 23,055 annotated transcripts in the reference genome with at least one sequencing read.

Based on the criteria [adjusted $P$ value (FDR) $\leq 0.05$ and log2 fold change ($\log_2$ FC $\geq 1$ or $\log_2$ FC $\leq 1$)], we selected differentially expressed genes (DEGs) in p35S::CsBCAT7 and wild-type (Col-0) plants. Of those 23,055 transcripts, we identified 67 DEGs in p35S::CsBCAT7 plants compared to wild-type (Col-0) plants. Thirty-nine up-regulated genes were identified, while 28 were down-regulated genes in the p35S::CsBCAT7 plants (Fig. 1a and Table 1). Interestingly, we found that twelve genes involved in BCAA catabolism were up-regulated, whereas 3 genes were down-regulated in the same transgenic plants (Table 2).

To elucidate the potential biological functions of these DEGs, we performed Gene Ontology (GO) enrichment analysis. We annotated and categorized the 67 DEGs using three GO input classes from a public database (https://www.ebi.ac.uk/QuickGO): biological progress (12 functional groups), molecular function (10 functional groups), and cellular component (3 functional groups) (Fig. 1b). The major categories in the biological progress class were “cell wall organization” (15 genes, 22.4%) and “response to abiotic and biotic stimulus” (20 genes, 29.9%). In the molecular function class, “binding activity” (15 genes, 22.4%) and “structural molecular activity” (14 genes, 20.9%) were the two main categories. Finally, cellular

![Fig. 1](attachment:image.png) Comparison of differentially expressed genes (DEGs) between p35S::CsBCAT7 and wild-type (Col-0) plants. (a) The number of DEGs between p35S::CsBCAT7 and wild-type (Col-0) plants grown over eight days under long-day (LD) conditions. The following criteria were used: adjusted $P$ value (FDR) $\leq 0.05$ and log2 fold change ($\log_2$ FC $\geq 1$ or $\log_2$ FC $\leq 1$). (b) Gene ontology (GO) enrichment analysis of up- and down-regulated genes between p35S::CsBCAT7 and wild-type (Col-0) plants. CC, MF and BP indicate cellular component, molecular function and biological process, respectively.
| Gene Locus | Description | Symbol | Log.FC | p-value | Change |
|------------|-------------|--------|--------|---------|--------|
| AT2G01422 | Unknown protein | -9.37  | 1.05E-48 | Up      |
| AT4G03590 | Cystatin/monellin superfamily protein | -4.53  | 1.33E-03 | Up      |
| AT1G34440 | Unknown protein | -3.55  | 1.71E-03 | Up      |
| AT2G14610 | Pathogenesis-related gene 1 | PR1    | -2.97  | 7.58E-03 | Up      |
| AT5G44440 | FAD-binding Berberine family protein | BBE28  | -2.48  | 5.14E-06 | Up      |
| AT1G23720 | Proline-rich extensin-like family protein | EXT15  | -2.05  | 2.77E-13 | Up      |
| AT3G28550 | Proline-rich extensin-like family protein | EXT16  | -1.95  | 1.47E-12 | Up      |
| AT2G43150 | Proline-rich extensin-like family protein | EXT21  | -1.88  | 6.18E-13 | Up      |
| AT3G22142 | Protease inhibitor/seed storage/LTP family protein | -1.86  | 3.23E-11 | Up      |
| AT1G69140 | Unknown protein | -1.78  | 5.37E-03 | Up      |
| AT3G54580 | Proline-rich extensin-like family protein | EXT17  | -1.78  | 2.23E-10 | Up      |
| AT2G24850 | Tyrosine aminotransferase 3 | TAT3   | -1.71  | 5.42E-04 | Up      |
| AT3G54590 | Hydroxyproline-rich glycoprotein | HRGP1  | -1.64  | 1.15E-07 | Up      |
| AT5G06640 | Proline-rich extensin-like family protein | -1.64  | 1.36E-06 | Up      |
| AT3G57260 | Beta-1,3-glucanase 2 | BGL2   | -1.53  | 1.72E-04 | Up      |
| AT5G49080 | Transposable element gene | EXT11  | -1.48  | 7.75E-04 | Up      |
| AT5G06630 | Proline-rich extensin-like family protein | EXT9   | -1.47  | 2.63E-04 | Up      |
| AT1G21310 | Extensin 3 | EXT3   | -1.44  | 1.50E-08 | Up      |
| AT4G15160 | Protease inhibitor/seed storage/LTP family protein | PAC2   | -1.42  | 7.07E-05 | Up      |
| AT4G22505 | Protease inhibitor/seed storage/LTP family protein | -1.37  | 8.04E-07 | Up      |
| AT2G14560 | Protein of unknown function (DUF567) | LURP1  | -1.36  | 2.83E-03 | Up      |
| AT2G24980 | Proline-rich extensin-like family protein | -1.36  | 1.21E-03 | Up      |
| AT3G24480 | Leucine-rich repeat (LRR) family protein | LRX4   | -1.29  | 3.03E-06 | Up      |
| AT1G12040 | Leucine-rich repeat/extensin 1 | LRX1   | -1.27  | 1.41E-03 | Up      |
| AT4G18670 | Leucine-rich repeat (LRR) family protein | LRX5   | -1.26  | 2.92E-06 | Up      |
| AT1G36180 | Acetyl-CoA carboxylase 2 | ACC2   | -1.24  | 7.90E-05 | Up      |
| AT4G33610 | Glycine-rich protein | -1.24  | 4.20E-03 | Up      |
| AT5G35190 | Proline-rich extensin-like family protein | -1.2   | 1.11E-03 | Up      |
| AT4G13420 | High affinity K+ transporter 5 | HAK5   | -1.19  | 9.91E-03 | Up      |
| AT4G22485 | Protease inhibitor/seed storage/LTP family protein | -1.17  | 3.16E-05 | Up      |
| AT5G07740 | Actin binding protein | FH20   | -1.14  | 3.67E-05 | Up      |
| AT4G13390 | Proline-rich extensin-like family protein | -1.13  | 3.35E-03 | Up      |
| AT5G43500 | Actin-related protein 9 | ARP9   | -1.12  | 1.12E-04 | Up      |
| AT4G13340 | Leucine-rich repeat (LRR) family protein | LRX3   | -1.08  | 1.51E-04 | Up      |
| AT3G02260 | Auxin transport protein (BIG) | BIG    | -1.04  | 4.89E-05 | Up      |
| AT1G67120 | ATPases;nucleotide binding | -1     | 2.06E-04 | Up      |
| AT4G12550 | Auxin-Induced in Root cultures 1 | AIR1   | -1     | 6.83E-04 | Up      |
| AT3G01345 | Unknown protein | -7.8   | 2.79E-22 | Up      |
| AT5G24770 | Vegetative storage protein 2 | VSP2   | -1     | 2.72E-03 | Up      |
| AT1G15405 | Other RNA | -5.37  | 2.46E-66 | Down    |
| AT5G24240 | Phosphatidylinositol 3- and 4-kinase | PI4Kc3 | 4.86   | 7.78E-31 | Down    |
| AT4G28520 | Cruciferin 3 | CRU3   | 4.72   | 1.48E-07 | Down    |
| AT1G68250 | Unknown protein | 4.51   | 1.98E-03 | Down    |
| AT3G22640 | Cupin family protein | PAP85  | 4.44   | 2.69E-03 | Down    |
Table 1 List of differentially expressed genes (DEGs) between p35S::CsBCAT7 and Col-0 plants (continued)

| Gene Locus   | Description                      | Symbol  | LogFC  | p-value | Change |
|--------------|----------------------------------|---------|--------|---------|--------|
| AT3G22640    | Cupin family protein             | PAP85   | 4.44   | 2.69E-03 | Down   |
| AT5G54740    | Seed storage albumin 5           | SEAS5   | 4.44   | 2.69E-03 | Down   |
| AT2G31141    | Unknown protein                  |         | 4.24   | 7.77E-31 | Down   |
| AT3G42658    | SADHU Non-coding Retro Transposon 3-2 | SADHU3-2 | 4.18   | 3.38E-17 | Down   |
| AT3G30720    | Qua-quine starch                 | DEG6    | 3.9    | 5.33E-13 | Down   |
| AT1G27565    | Unknown protein                  |         | 3.48   | 3.25E-03 | Down   |
| AT3G41768    | rRNA                             |         | 3.39   | 9.08E-36 | Down   |
| AT5G53902    | U3 small nucleolar RNA           | U3B     | 3.24   | 2.59E-03 | Down   |
| AT3G41762    | Unknown protein                  |         | 3.23   | 3.30E-12 | Down   |
| AT5G44120    | RmlC-like cupins superfamily protein | CRA1   | 2.17   | 1.70E-04 | Down   |
| AT1G71000    | Chaperone DnaJ-domain superfamily protein |         | 1.98   | 5.99E-04 | Down   |
| AT1G71000    | Chaperone DnaJ-domain superfamily protein |         | 1.98   | 5.99E-04 | Down   |
| AT2G01008    | Maternal effect embryo arrest protein; |         | 1.87   | 3.93E-04 | Down   |
| AT2G41240    | Basic helix-loop-helix protein 100 | BHLH100 | 1.73   | 1.76E-04 | Down   |
| AT3G56970    | Basic helix-loop-helix (bHLH) DNA-binding protein | ORG2   | 1.71   | 1.40E-03 | Down   |
| AT2G07698    | ATPase                           |         | 1.56   | 6.42E-03 | Down   |
| AT1G3609     | Defensin-like (DEFL) family protein |         | 1.44   | 1.09E-05 | Down   |
| AT4G26200    | 1-Amino-cyclopropane-1-carboxylate synthase 7 | ACS7   | 1.31   | 2.56E-03 | Down   |
| AT1G49700    | Unknown protein                  |         | 1.29   | 1.36E-03 | Down   |
| AT2G35750    | Transmembrane protein            |         | 1.29   | 1.55E-03 | Down   |
| AT1G47395    | Unknown protein                  |         | 1.15   | 4.33E-03 | Down   |
| AT1G77940    | Ribosomal protein                | RPL30B  | 1.14   | 1.28E-05 | Down   |
| AT2G30230    | 6,7-Dimethyl-8-ribityllumazine synthase |         | 1.13   | 6.37E-03 | Down   |
| AT2G42540    | Cold-regulated 15a               | COR15A  | 1.1    | 1.45E-04 | Down   |
| AT5G59320    | Lipid transfer protein 3         | LTP3    | 1.08   | 6.38E-03 | Down   |

Table 2 List of DEGs related to BCAA catabolism between p35S::CsBCAT7 and Col-0 plants

| Gene Locus   | Description                                      | Symbol  | LogFC  | p-value | Change |
|--------------|--------------------------------------------------|---------|--------|---------|--------|
| AT1G03090    | Methylcrotonyl-CoA carboxylase, alpha chain      | MCCA    | 0.40   | 1.52E-01 | Up     |
| AT4G34030    | 3-Methylcrotonyl-CoA carboxylase, beta chain     | MCCB    | 0.11   | 6.77E-01 | Up     |
| AT3G15290    | 3-Hydroxyacetyl-CoA dehydrogenase                | HCDH    | 0.02   | 9.73E-01 | Up     |
| AT4G29010    | Enoyl-CoA hydratase/isomerase family             | AIM1    | 0.12   | 6.36E-01 | Up     |
| AT3G06860    | Multifunctional protein 2                        | MFP2    | 0.17   | 5.07E-01 | Up     |
| AT4G16760    | Acyl-CoA oxidase 1                               | ACX1    | 0.29   | 2.65E-01 | Up     |
| AT5G65110    | Acyl-CoA oxidase 2                               | ACX2    | 0.30   | 2.69E-01 | Up     |
| AT2G33150    | Peroxisomal 3-ketoacyl-CoA thiolase 3            | PKT3    | 0.042  | 0.86785 | Up     |
| AT1G06290    | Acyl-CoA oxidase 3                               | ACX3    | 0.18   | 4.98E-01 | Up     |
| AT3G51840    | Acyl-CoA oxidase 4                               | ACX4    | 0.03   | 9.18E-01 | Up     |
| AT2G35690    | Acyl-CoA oxidase 5                               | ACX5    | 0.12   | 7.15E-01 | Up     |
| AT2G35390    | Phosphoribosyltransferase                        | PRS1    | 0.02   | 9.35E-01 | Up     |
| AT5G48880    | Peroxisomal 3-keto-acyl-CoA thiolase 2           | PKT1    | -0.222 | 0.40552 | Down   |
| AT1G04710    | Peroxisomal 3-ketoacyl-CoA thiolase 4            | PKT4    | -0.215 | 19.91  | Down   |
| AT1G06310    | Acyl-CoA oxidase 6                               | ACX6    | -0.239 | 9.21E-01 | Down   |
components were divided into inner and outer parts of the cell. The proportions of the two functional groups were similar (31 genes and 28 genes, respectively). This annotation data proved to be a valuable resource that allowed greater inquiry into specific biological processes associated with, and functional differences between, p35S::CsBCAT7 and wild-type (Col-0) plants.

In previous research, we demonstrated that CsBCAT7 overexpression in Arabidopsis significantly affects flowering time (Lee et al. 2019b). To evaluate our RNA-Seq data, we first analyzed differences between p35S::CsBCAT7 and wild-type (Col-0) plant gene expression levels associated with flowering time. The expression levels of known floral activator genes [CONSTANS (CO), GIGANTEA (GI), and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)] (Boss et al. 2004) were slightly upregulated in p35S::CsBCAT7 plants (Fig. 2a). In contrast, the expression of flower repressors [FLOWERING LOCUS M (FLM) and TERMINAL FLOWER 1 (TFL1)] was downregulated in these same plants. These results were consistent with those from our previous study (Lee et al. 2019b). We further investigated the expression of other genes related to flowering time (Fig. 2a). The expression of known floral activator MOTHER OF FT AND TFL1 (MFT) (Yoo et al. 2004) was increased in p35S::CsBCAT7 plants, while expression levels of three floral repressors (MADS AFFECTING

![Fig. 2 Expression levels of genes related to flowering time (a) and germination (b) in RNA-Seq data. To validate our RNA-Seq data, we confirmed the expression levels of some previously reported genes (Lee et al. 2019a; Lee et al. 2019b).](image)

![Fig. 3 Analysis of DEGs related to branched chain amino acid (BCAA) catabolism. (a) Phenotype of four-week short-day grown p35S::CsBCAT7 and wild-type (Col-0) plants after 15 d of further growth in extended darkness. (b) Expression levels of genes related to BCAA catabolism. The genes analyzed in this study were described in Hildebrandt et al. (2015). (c) RT-qPCR validation of some BCAA catabolism genes identified from RNA-Seq analysis. The PP2AA3 gene was an internal control.](image)
FLOWERING4 (MAF4), MAF5 and BROTHER OF FT AND TFL1 (BFT) (Gu et al. 2013; Ryu et al. 2011; Yoo et al. 2010) were lower.

Because CsBCAT7 overexpression in Arabidopsis also promotes germination under abiotic stress conditions by affecting expression levels of germination-responsive genes (Lee et al. 2019a), we next confirmed their gene expression in our RNA-Seq data (Fig. 2b). Expression of germination-responsive genes such as isocitrate lyase (ISO), myrosinase (MFR)-binding protein 1 (MBP1), MBP2, PHYTOCHROME B (PHYB), S-adenosylmethionine synthetase 1 (SAM1), and SAM52 were higher in p35S::CsBCAT7 plants compared to wild-type (Col-0) plants. Meanwhile, Cruciferin (CRU3), CRU5, RnlC-like cupins 1 (CRA1), and Late Embryogenesis Abundant 4-5 (LEA4-5) had decreased in p35S::CsBCAT7 plants. These results were consistent with those of our previous study (Lee et al. 2019a).

Recalling that carbon starvation caused by extended darkness induces high ATP production from BCAA catabolism, and thereby delays the senescence phenotype (Hildebrandt et al. 2015), we examined the phenotype of p35S::CsBCAT7 plants grown for 5 weeks under SD conditions and subsequently transferred to dark conditions (Fig. 3a). Wild-type (Col-0) plants began to show signs of senescence after 10 d of continuous darkness and were apparently dead after 15 d of continuous darkness. In contrast, p35S::CsBCAT7 plants were still alive after 15 d of continuous darkness and showed only limited senescence signs. To further investigate the observed delay in senescence in p35S::CsBCAT7 plants, we analyzed the expression of the genes involved in BCAA catabolism in our RNA-Seq data (Fig. 3b and Table 2). We found that expression levels of the genes that encode the alpha and beta chain of methylcrotonyl-CoA carboxylase (MCCA), 3-hydroxyacyl-CoA dehydrogenase (HCDH), Enoyl-CoA hydratase/isomerase (AIM1) family, and acyl-CoA oxidase (ACX) family protein had increased in the p35S::CsBCAT7 plants. These RT-qPCR expression patterns were consistent with the RNA-Seq analysis (Fig. 3c), and suggest that overexpression of CsBCAT7 causes an increase in the expression of other BCAA catabolism genes, thereby delaying senescence phenotype in p35S::CsBCAT7 plants.

These results, viewed in their entirety, indicate that overexpression of CsBCAT7 in transgenic Arabidopsis plants placed in darkness for an extended period of time delays senescence by upregulating some of the genes involved in BCAA catabolism. This suggests that CsBCAT7 may be involved in the regulation of BCAA catabolism under certain carbohydrate starvation conditions. As the mitochondria is a known important site of BCAA catabolism in plant cells (Binder et al. 2007) and CsBCAT7 protein with BCAA catabolism activity is known to target the mitochondria (Lee et al. 2019b), it is probable that CsBCAT7 produces ATP as an energy source from BCAAs in conditions of extended darkness. This hypothesis is supported by our observation that the mutations in the genes that encode electron-transfer flavoprotein:ubiquinone oxidoreductase (ETFQO), isovaleryl-CoA dehydrogenase (IVDH), and 2-hydroxyglutarate dehydrogenase (D2HGDH), which are involved in BCAA catabolism, showed accelerated senescence phenotypes during dark-induced starvation (Araujo et al. 2010; Daschner et al. 2001; Ishizaki et al. 2005). As CsBCAT7 had a high amino acid similarity with AT3G05190 (Lee et al. 2019b), further investigation into the effect of mutations in AT3G05190 on senescence during periods of extended darkness and the genetic interaction between known BCAA catabolism mutants is warranted, and would provide a better understanding of the role of BCAT genes during dark-induced starvation.

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