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Research Article

Transcriptome analysis of a transgenic *Arabidopsis* plant overexpressing *CsBCAT7* reveals the relationship between *CsBCAT7* and branched-chain amino acid catabolism

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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

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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; Galili 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 *Sl*BCAT enzymes in tomato are active in both forward (BCAA synthesis) and reverse (BCKA synthesis) reactions, whereas only two cucumber BCATs [*Cs*BCATs (*Cs*BCAT2 and *Cs*BCAT3)] among three *Cs*BCATs 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 THUNDERBIRD 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'- AGAGGC AATGAAGATGGAGCAC-3' and 5'- AGGTCCTGTATG CTTCCAGAGG-3'; for *AIM1*, 5'- ATATCATGCCTGGT GGGAAGCC-3' and 5'- AGGCTCGGATCACAACTCCT TC-3'; for *HCDH*, 5'- ATTCGCGGTGCAGATACCTCA G-3' and 5'- GACTGTTGTCTTGCCAAACCTTTC-3'; for *ACX1*, 5'- TGATTTGGTTGAGGCCGCTATTG-3' and 5'-TGCTCCAGTTTCGCTATGAACTTG-3'; for *PP2AA3*, 5'-GCGGTTGTGGAGAACATGATACG-3' and 5'-GAACC AAACACAATTCGTTGCTG-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 (log2 FC $\geq$ 1 or log2 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

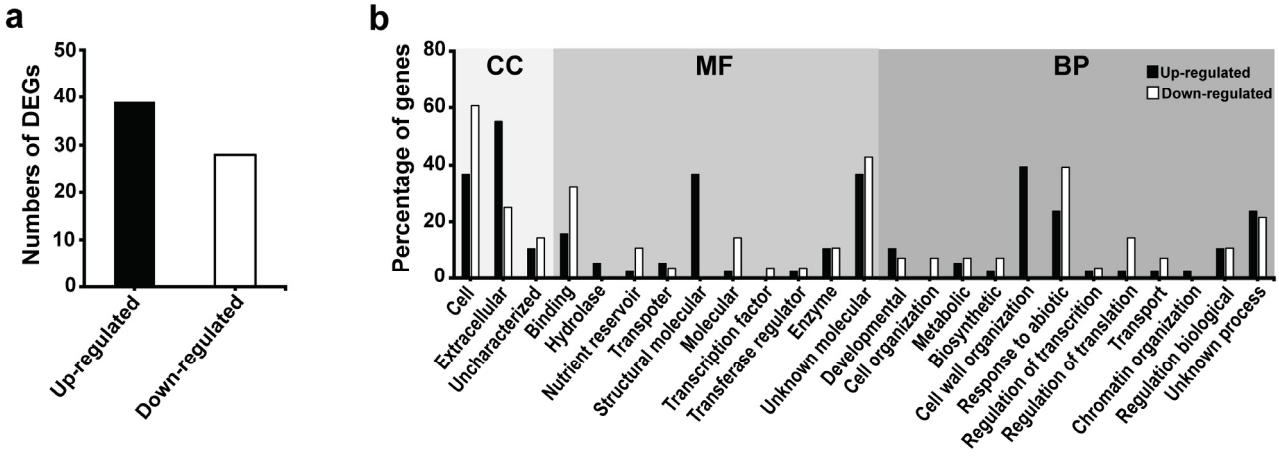

Fig. 1 Comparison of differentially expressed genes (DEGs) between *p35S::CsBCAT7* and wild-type (Col-0) plants. (a) The number of DEGs between *p35S::CsBCAT7* and wild-type (Col-0) plants grown over eight days under long-day (LD) conditions. The following criteria were used: adjusted *P* value (FDR) $\leq$ 0.05 and $\log_2$ 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

Table 1 List of differentially expressed genes (DEGs) between *p35S::CsBCAT7* and Col-0 plants

Gene Locus	Description	Symbol	Log_2FC	*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	Log_2FC	*p*-value	Change
AT3G22640	Cupin family protein	PAP85	4.44	2.69E-03	Down
AT5G54740	Seed storage albumin 5	SESA5	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
AT1G13609	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	FEP2	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	Log_2FC	*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-Hydroxyacyl-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 *p35::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 *p35::CsBCAT7* plants, while expression levels of three floral repressors (MADS AFFECTING

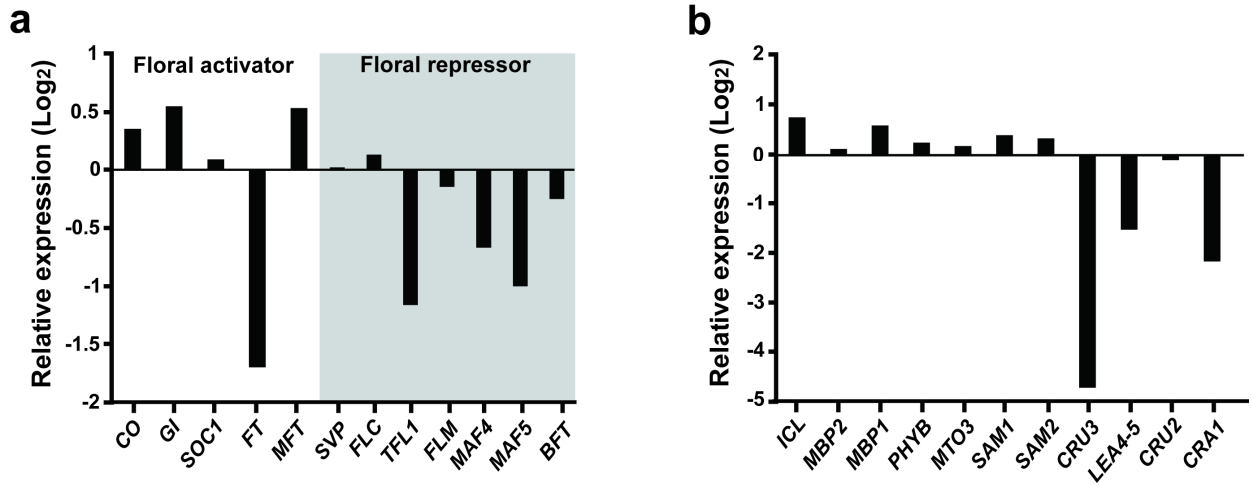

Fig. 2 Expression levels of genes related to flowering time (a) and germination (b) in RNA-Seq data. To validate our RNA-Seq data, we confirmed the expression levels of some previously reported genes (Lee, et al. 2019a; Lee, et al. 2019b)

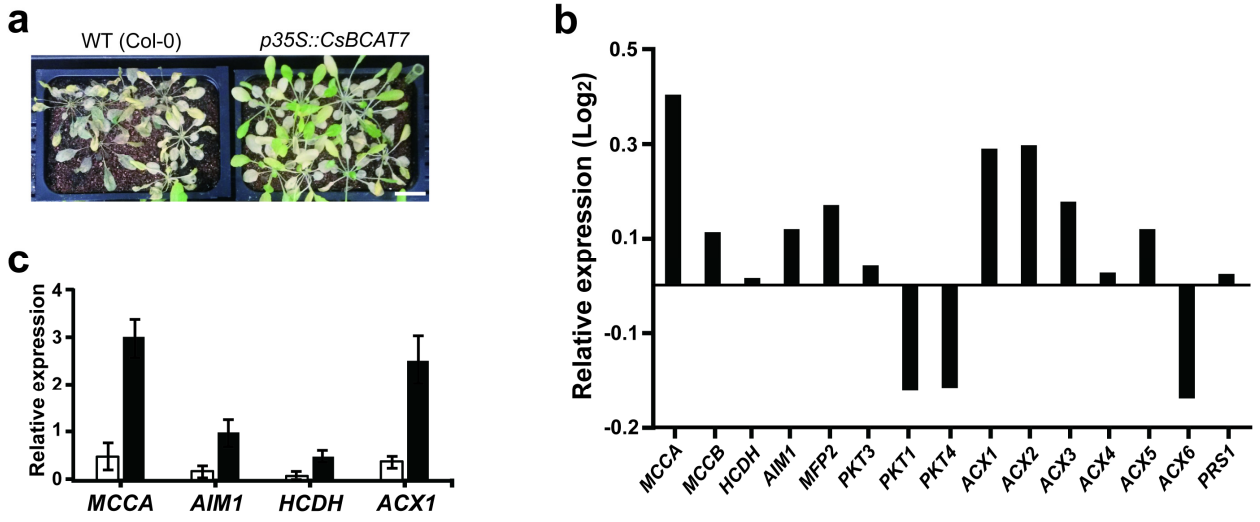

Fig. 3 Analysis of DEGs related to branched chain amino acid (BCAA) catabolism. (a) Phenotype of four-week short-day grown *p35S::CsBCAT7* and wild-type (Col-0) plants after 15 d of further growth in extended darkness. (b) Expression levels of genes related to BCAA catabolism. The genes analyzed in this 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

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* (*MYR*)*-binding protein1* (*MBP1*)*, MBP2*, *PHYTOCHROME B* (*PHYB*), *S-adenosylmethionine synthetase1* (*SAMS1*), and *SAMS2* were higher in *p35S::CsBCAT7* plants compared to wild-type (Col-0) plants. Meanwhile, *Cruciferin* (*CRU3*)*, CRU5*, *RmlC-like cupins1* (*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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