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Research Article

Induction of a bZIP Type Transcription Factor and Amino Acid Catabolism-Related Genes in Soybean Seedling in Response to Starvation Stress

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To address roles of bZIP transcription factors on regulation of amino acid catabolism under autophagy-induced plant cells, we examined the effect of nutrient starvation on the expression of low energy stress-related transcription factor homologs, *GmbZIP53A and GmbZIP53B*, and amino acid catabolism-related genes in soybean (*Glycine max* (L.) Merr.). Sucrose starvation treatment significantly enhanced the expressions of *GmbZIP53A*, but not *GmbZIP53B asparagine synthase* (*GmASN1*), *proline dehydrogenase1* (*GmProDH*), and *branched chain amino acid transaminase 3* (*GmBCAT3*). GmbZIP53-related immunoreactive signals were upregulated under severe starvation with sucrose starvation and protease inhibitors, while 3% sucrose and sucrose starvation had no or marginal effects on the signal. Profiles of induction of *GmASN1, GmProDH* and *GmBCAT3* under various nutrient conditions were consistent with the profiles of GmbZIP53 protein levels but not with those of GmbZIP mRNA levels. These results indicate that GmbZIP53 proteins levels are regulated by posttranslational mechanism in response to severe starvation stress and that the increased protein of GmbZIP53 under severe starvation accelerates transcriptional induction of *GmASN1*, *GmProDH*, and *GmBCAT3*. Furthermore, it is conceivable that decrease of branched chain amino acid level by the BCAT-mediated degradation eventually enhances autophagy under severe starvation.

1. Introduction

The perception and management of nutrient and energy levels in organisms are crucial for survival by adjusting metabolism to available resources. Recent studies revealed that sugar signals in higher plants activate various biological modules such as sugar sensor, transcription factors, sugar transporters, and metabolic enzymes of sugar and amino acids [1, 2]. In higher plants, sugar deprivation and/or low energy stress by decreased photosynthesis have appeared to induce protein degradation via autophagy and amino acid metabolism, leading to translocation of nutrients and senescence [3, 4]. Recently accumulated studies on bZIP type transcription factors of *Arabidopsis* concerning nutrient signal and amino acid metabolism have unveiled that a set of bZIP transcription factors, bZIP1 and bZIP53, classified to S-type subgroup among the bZIP superfamily, are master regulatory components in transcriptional induction of amino acid catabolism-related enzymes involved in low energy stress, sucrose starvation, and senescence-induced nutrient translocation [5–7]. Sucrose starvation and/or low energy stress activate *bZIP1* and *bZIP53a* by transcriptional and translational regulation mechanisms. Resultantly, increased proteins of the bZIP1 and bZIP53 forming heterodimers with

other bZIP members initiate the transcriptional activation of amino acid metabolism-related genes by binding to ACGT- or ACTCAT-like cis-elements within the promoters of the target genes [8].

Autophagy is a nonspecific and bulk protein degradation system of intracellular components and is induced under nutrient starvation. Molecular genetic studies using mutants of autophagy-related genes (*ATG*) unveiled that autophagy plays essential roles in growth, senescence, nutrient translocation, and stress responses of higher plants [9–11]. The expressions of *ATG8-* and *ATG4*-related genes are enhanced under sucrose starvation in *Arabidopsis* suspension cells, possibly generating intracellular amino acid pools and bioenergetic resources [12]. It is well known that status of nutrient conditions has pleiotropic effects on stress-related phytohormonal signals [13]. Our previous studies indicated that the expression of *GmATG8c* and *GmATG8i* in soybean leaf transiently increased at the senescence stage and that combination of sucrose starvation and protease inhibitors significantly enhanced the induction of *GmATG8c,i* and *GmATG4* in soybean seedling [14, 15]. One of the most important roles of autophagy is regulation of intracellular amino acid pool which tends to be fluctuated by starvation and senescence via amino acid metabolism and translocation. Furthermore, intracellular amino acid pool, particularly branched chain amino acid (BCAA), which appeared to activate target of rapamycin (TOR) which negatively regulates autophagy [2]. Therefore, the regulation mechanism of amino acid catabolism plays an important role in autophagy induction under sucrose starvation.

By starvation-induced autophagy experiment and specific antibodies against GmbZIP53, BCAT, and ATG8i, we demonstrate that the nutrient starvation activates a sucrose-induced transcription factor *GmbZIP53A* at transcription and protein levels, which possibly play a key role in the regulatory network of amino acid catabolism and autophagy of soybean.

2. Materials and Methods

2.1. Plant Materials. Seeds of soybean (*Glycine max* (L.) Merr. cv. Fukuyutaka) were sterilized and sown on 1% agarose in plastic box (10 × 10 × 15 cm) inside shading incubator set at 25°C. After 5 days of seeding, cotyledon was removed from soybean seedlings (about 8–10 cm length), before nutrient treatments.

2.2. Treatments of Sucrose Starvation and Sucrose-Rich Medium. Soybean seedlings were incubated at 25°C in a nutrient-rich, starvation, or starvation-protease inhibitor medium and harvested at indicated intervals as previously described [14]. The nutrient-rich medium contained 0.5 × Murashige Skoog (MS) medium with 3% sucrose. Sucrose starvation medium contained 0.5 × MS medium. Severe starvation medium was supplemented with protease inhibitors, 1 mM phenylmethylsulfonyl fluoride, 10 μg mL^{-1} leupeptin, 100 μM E64-d, and 10 mM quinacrine. The medium was adjusted to pH 7.0 by adding KOH. The resultant samples were frozen in liquid nitrogen and then were stored at −80°C.

2.3. RNA Preparation and RT-PCR. Soybean homologs of *AtbZIP1, AtbZIP53*, and amino acid catabolism-related genes were identified by searching at Phytozome V 9 with tBlastx program (http://www.phytozome.net/). Gene specific primers are shown in Table 1. *GmActin* was used as positive control. RNA preparation and RT-PCR were carried out by using Rever TraACE kit (Toyobo Co. Ltd. Osaka, Japan), GoTaq Kit (Promega Bioscience Inc., Madison, USA), as previously described [16]. PCR was performed with thermal cycler PC-816 (ASTEC Inc., Fukuoka, Japan) under a following thermal cycle condition: initial denaturing at 94°C for 2 min, followed by 3 steps of 24–29 cycles (as indicated) denaturing at 94°C for 10 sec, annealing at 58°C for 10 sec, extending at 72°C for 40 sec, and final extention at 72°C for 30 sec. PCR products were subjected to 1.5% agarose electrophoresis and ethidium bromide staining and then visualized by FluorChem (Cell Bioscience, Santa Clara, CA).

2.4. Raising Antibodies, Protein Preparation, and Immunoblot. Synthetic antigen epitopes (H_2N-VEIPEIPDPLLKPWQIPHP-COOH and H_2N-LANKRWVPPPGKGSLYLRP-COOH) were designed for soybean bZIP53 and plant BCAT shown in Figures 1(c) and 2(b), respectively. The antipeptide specific polyclonal antibodies were raised in a rabbit with those synthetic antigen oligopeptides (Sigma-Aldrich Co., St. Louis, USA) [16]. Protein extracts and immunoblot were carried out as previously described with minor modifications [14]. To detect *GmATG8i*, each 50 μg protein per lane was subjected to SDS-PAGE with a gel of 15% acrylamide containing 6 M urea to separate phosphatidylethanolamine-modified ATG8i. After electroblotting polypeptides onto PVDF membranes (Millipore, Billerica, USA) and blocking the PVDF membrane with TBS-Milk, the PVDF membrane was incubated in TBS-Milk-Tween with anti-*GmAtg8i* antibody, anti-bZIP53 antibody, or anti-BCAT antibody (dilution, 1/1,000 [v/v]) for 2 h at 4°C. The resultant PVDF membranes were incubated in TBS-Milk-Tween containing horse raddish peroxidase-conjugated anti-rabbit antibody (dilution, 1/5,000 [v/v], GE Healthcare Bio-Sciences LtD., Piscataway, NJ) for 1 h. Immunoreactive signals were visualized by ECL Plus Kit (GE Healthcare Bio-Sciences Ltd.) and FluorChem.

3. Results

3.1. Nutrient Starvation Stress Induces a bZIP53 Ortholog in Soybean. Heterodimers of *bZIP1* (At5g49450) and *bZIP53* (At3g62420) have been identified to be crucial transcriptional regulators in Asn, Pro, and BCAA under sucrose starvation [7]. Among bZIP homolog genes in soybean genome, *GmbZIP53A* (Glyma03g37790) and *GmbZIP53B* (Glyma19g40390) were identified to have the highest similarities of 51% and 26% in amino acid sequences to *bZIP53* (At3g62420), respectively. Difference of only 14 amino acids among total 150 amino acids between GmbZIP53A and

Table 1: Gene-specific oligo DNA primers used for RT-PCR and cloning.

Gene	Accession number/gene index	Primer sequence
GmbZIP53A	Glyma03g37790	F-AAAAGGATCCTCCTTCTCGGAAATGGCATCACC
		R-TAAAGTCGACAGGAATGATCAACGCAGAAACAT
GmbZIP53B	Glyma19g40390	F-CTCCGGATCCGAAATGGCGTCGTCAATCCAACA
		R-AAGAGTCGACTAACGCAGAAACATGTTTGCGGT
GmBCAT3	Glyma01g40420	F-AATGGATCCCATCGTGCAACTCCTGGTGGC
		R-AGTCCATGGATCTCAGCTCAACAGTCCAATTCA
GmProDH	Glyma13g07110	F-GAGGGGATCCTACATGTCTACAGAGAG
		R-GAACACTGCAGCTTTTAGTCTCCTTCC
GmASN1	Glyma18g06840	F-TCTAGGATCCAGAGTACCATTTTTGGA
		R-GCTCTATATGTCGACTCCTAGTGGAGA
GmASP3	Glyma4g08560	F-ACGTGTTGGATCCTTAAGCATTGTCTG
		R-CTGCGGTCGACAACATGTTTTAGGC
GmATG8i	AB453309/Glyma02g01180	F-CCGGAATTCGTTGAGCTGCACAACAACCTA
		R-GATAGTCGACACTTCACAAAGTGTTGGATA
GmATG4	Glyma18g48380	F-GGCAAACCAGGTGCTTCAACATAC
		R-ATGCCAGGGATCAACCAAACTTGC
GmActin	V00450/Glyma08g15480	F-GCGTGATCTCACTGATGCCCTTAT
		R-AGCCTTCGCAATCCACATCTGTTG

GmbZIP53B was identified in their amino terminal regions. A phylogenic analysis of deduced amino acid sequences of *bZIP53* homologs of *Arabidopsis thaliana*, soybean (*Glycine max* (L.) Merr.), and cowpea (*Vigna unguiculata* L.) showed that GmbZIP53A, GmbZIP53B, and VubZIP53 (accession number AB77966) are classified into one clade containing bZIP1 and bZIP53 (Figure 1(a)) [17].

RT-PCR revealed that the expression profile of *GmbZIP53A* and *GmbZIP53B* is quite different under sucrose starvation treatment, while the two genes share high similarity. Sucrose starvation had significantly enhanced the expression of *GmbZIP53A* but not *GmbZIP53B*. The expression levels of *GmbZIP53A* in soybean seedling were significantly induced at 24 h after response to sucrose starvation treatment, while 3% sucrose had little or marginal effect on the expression of those genes (Figure 1(b)). Furthermore, sucrose starvation with inhibitors (leupeptin and E-64d) of proteases resulted in slightly higher induction of *GmbZIP53A* at 24 and 48 h, compared to those in sucrose starvation treatment. The expression of *GmbZIP53B* was relatively high level compared to *GmbZIP53A* in control and sucrose treatment. The expression levels of *GmbZIP53B* were constant in conditions of control, sucrose treatment, starvation, and starvation with inhibitors.

Synthetic antigen epitopes designed for bZIP53 are shown with alignments of bZIP type transcription factors (Figure 1(c)). A highly conserved motif of soybean bZIP53 was identified as 19 amino acids (VEIPEIPDPLLKPWQIPHP) at the carboxyl region following the conventional bZIP domain [5]. The immunoreactive signal at 24 h with molecular masses of about 16 kD is significantly higher in the presence of the inhibitors of proteases than in the absence, when soybean seedlings were subjected to sucrose starvation treatment. It suggests that a reduction of amino acid pool by suppressed proteolysis with the inhibitor is involved in induction mechanism of *GmbZIP53*-related protein. Treatments of 3% sucrose had no or marginal effects on the signals of bZIP53-related proteins, compared to those in control. In the presence of bZIP53 antigen competitor peptides, those immunoreactive signals disappeared, suggesting that the antibodies are highly specific to bZIP53-related polypeptides.

3.2. Induction of Amino Acid Catabolism-Related Genes. Recently, low energy stresses such as reduced photosynthesis and/or sucrose starvation appeared to induce dramatic change of amino acid catabolism for asparagine biosynthesis by inducing the expression of *asparagine synthase (AtASN1), aspartate aminotransferase (AtASP3), proline dehydrogenase 1* (*AtProDH*), and *branched chain amino acid transaminase* (*AtBCAT2*) [18–20]. Among soybean homolog genes amino acid catabolic-related enzymes, *GmASN1* (Glyma18g06840), *GmProDH* (Glyma13g07110), and *GmBCAT3* (Glyma01g40420) were identified to have the highest similarites of 82%, 62%, and 62% in deduced amino acid sequences to *AtASN1* (At3g47340), *AtProDH* (At3g30775), and *AtBCAT2* (At1g10070), respectively.

Sucrose starvation induced *GmbZIP53A, GmASN1, and GmProDH* but not or marginally *GmASP3* nor *GmBCAT*. On the other hand, sucrose starvation with the protease inhibitors significantly upregulated *GmProDH, GmASP3,* and *GmBCAT*, compared to those in the absence of inhibitors, while there is no difference of the expression of *GmbZIP53A* and *GmASN1* between the presence or the absence of the inhibitor. Sucrose treatment had no or marginal effects on these genes (Figure 2(a)).

The synthetic antigen epitope designed for plant BCAT is shown with alignments of amino acid transaminase-related enzymes (Figure 2(c)). A highly conserved motif of plant BCATs was also identified as 19 amino acids (LANKRWVPPPGKGSLYLRP). Open arrowheads indicated with R (arginine) and E (glutamate) are conserved amino acid

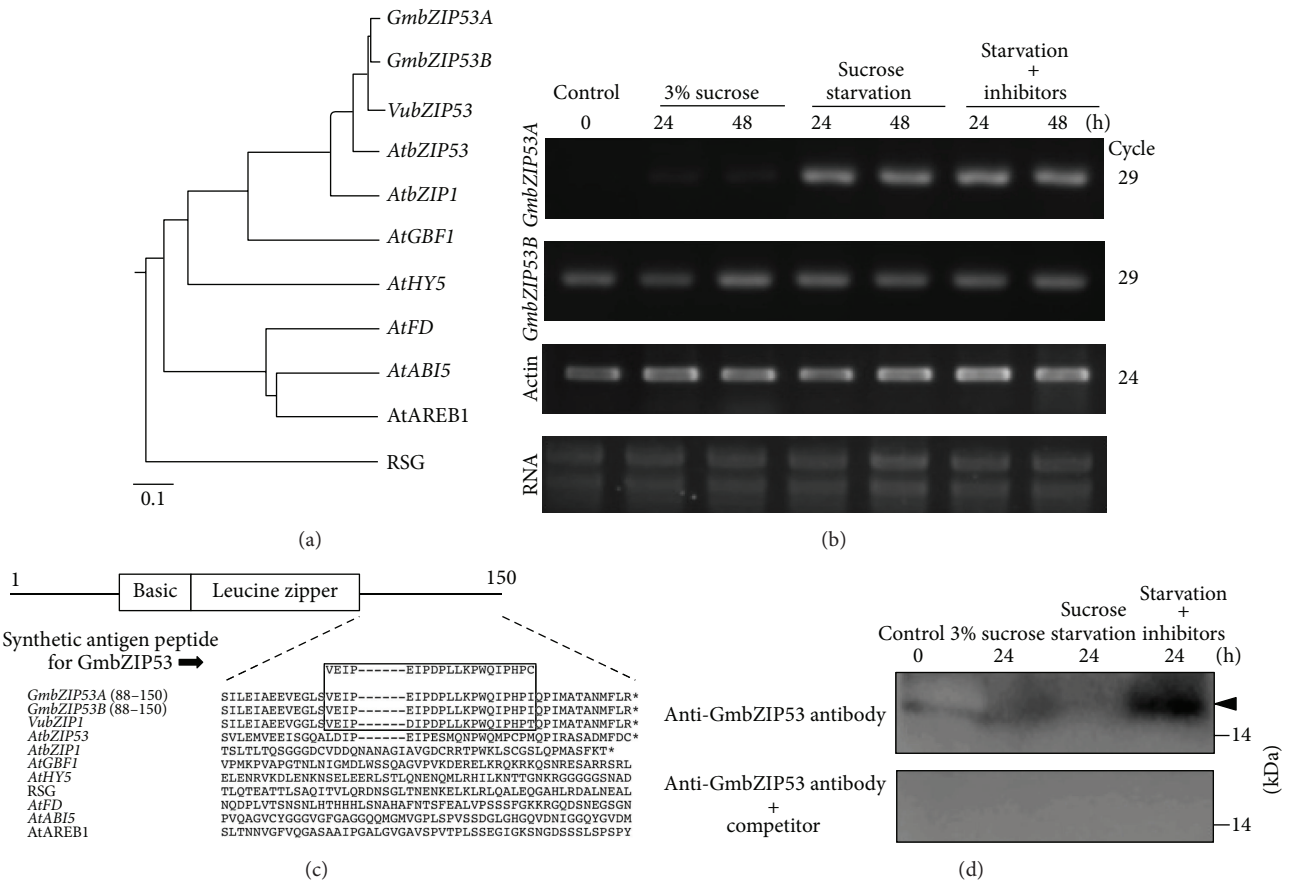

Figure 1: Effects of nutrient starvation on the expression and bZIP53 protein levels of soybean bZIP53 orthologs. (a) A phylogenic tree of bZIP type transcription factors from soybean, cowpea, *Arabidopsis,* and tobacco was constructed by ClustalW (UPGMA). The genes used were follows: *GmbZIP53A* and *GmbZIP53B* (Table 1), *VubZIP53* (AB779667), *AtbZIP53* (At3g62420), *AtbZIP1* (At5g49450), *AtGBF1* (At4g36730), *AtHY5* (At5g11260), *AtFD* (At4g35900), *AtABI5* (At2g36270), AtAREB1 (At1g45249), and RSG (AB040471). Gene indics of soybean and *Arabidopsis* were referred to Phytozome V 9.0 (http://www.phytozome.net/) and TGI database (http://compbio.dfci.harvard.edu/tgi/plant.html), respectively. (b) The expression of *GmbZIP53A* and *GmbZIP53B* was analyzed by RT-PCR. Actin was used as a standard. (c) Alignments of deduced amino acid sequences of bZIP53 orthologs and other bZIP type transcription factors. A highly conserved amino acid motif (H_2N-VEIPEIPDPLLKPWQIPHP-COOH) present in the carboxyl terminal region following bZIP domain was used as an epitope for raising anti-GmbZIP53-specific antibody. (d) Immunoblot of soybean seedlings treated with various nutrient conditions was carried out by anti-GmbZIP53 antibody. Each 50 μg protein per lane was subjected to SDS-PAGE (15% acrylamide gels). Nutrient treatments, semiquantitative RT-PCR, and immunoblot were carried out as described in Section 2.

residues critical for interaction with pyridoxal phosphate and substrate, respectively [2]. Immunoreactive signals of BCAT-related proteins were detected at significant levels in severe starvation treatment with molecular masses of about 50 kDa (Figure 2(c)). On the other hand, treatments of 3% sucrose had little or marginal effects on the signals of BCAT-related proteins. Notably, the profile of the immunoreactive signals of the BCAT-related proteins in nutrient conditions was similar to that of the soybean bZIP53. Immunoblot with BCAT antigen competitor peptides indicates that the antibody cross-reacted to plant BCAT-related proteins specifically. In control, two immunoreactive signals at molecular masses close to each other were detected weakly in control. Accordingly, there are possibilities that the two immunoreactive signals in control indicate the presence of multiple polypeptides derived from other BCAT orthologs, or alternatively that the difference among two BCAT-related signals in control was derived by posttranslational modification.

3.3. Sucrose Treatment and Sucrose Starvation Oppositely Regulate Autophagy in Soybean. Autophagy status in soybean seedling under various nutrient conditions was examined by analyzing the expressions of *GmATG8i* and *GmATG4* and protein levels of ATG8i-related proteins. The expression levels of *GmATG8i* and *GmATG4* in soybean seedling were significantly induced at 24 h in response to sucrose starvation treatment, while 3% sucrose had little or marginal effect on the expression of those genes (Figure 3(a)). Furthermore, sucrose starvation with protease inhibitors resulted in relatively higher induction of *GmATG8i* and *GmATG4* at 12 h, compared to those in sucrose starvation treatment. In contrast to starvation treatments, 3% sucrose treatment

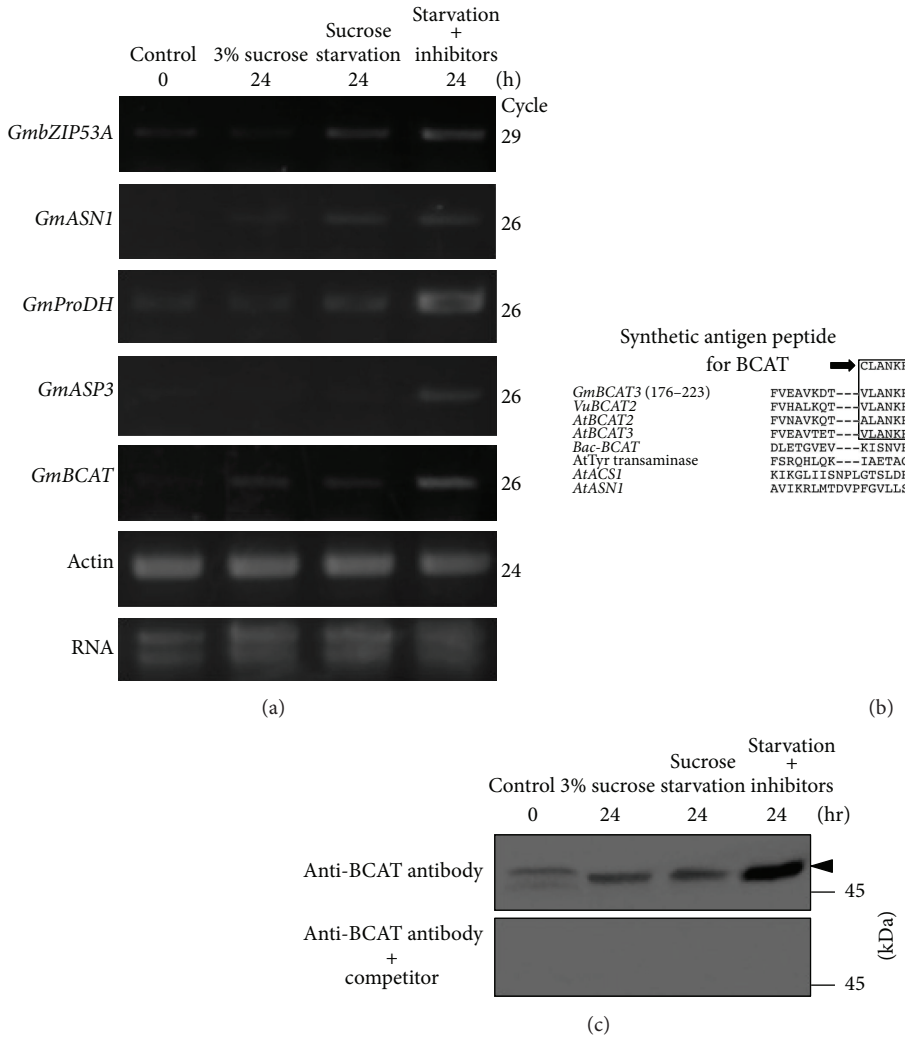

Figure 2: Effects of nutrient starvations on the expression of amino acid catabolism-related genes and BCAT protein levels. (a) The expression of *GmbZIP53A*, *GmASN1*, *GmProDH*, *GmASP3*, and *GmBCAT2* was analyzed. (b) Alignments of deduced amino acid sequences of plant BCAT orthologs and other aminotransferase-related enzymes. A highly conserved amino Acid motif (H_2N-LANKRWVPPPGKGSLYLRP-COOH) present in the catalytic domain was used as an epitope for raising antiplant BCAT-specific antibody. (c) Immunoblot of soybean seedlings treated with various nutrient conditions was carried out by the antiplant BCAT-antibody. Each 50 μg protein per lane was subjected to SDS-PAGE (12.5% acrylamide gels). 14 Nutrient treatments, semiquantitative RT-PCR, and immunoblot were carried out as described in Section 2.

transiently suppressed the expression of *GmATG8i* and *GmATG4i* at 12 h. Previous studies with *Arabidopsis* and soybean revealed that the upregulation of *ATG8s* and *ATG4* mRNA was accompanied with induction of autophagy in response to sucrose starvation stress [12]. The present data that sucrose starvation induced *GmATG8i* and *GmATG4* is consistent with the previous observation on autophagy induction in *Arabidopsis* and soybean [14, 21].

The profiles of ATG8i-related proteins in response to nutrient conditions were analyzed with an anti-ATG8i-specific antibody (Figure 3(b)). Combination of sucrose starvation and protease inhibitors, leupeptin and E-64d, significantly increased immunoreactive signals of ATG8i protein at 12 and 24 h, compared to sucrose starvation in the absence of leupeptin and E-64d (Figure 3(b)). The significant difference of ATG8i levels in sucrose-starved soybean seedlings between the presence and the absence of protease inhibitors indicates that sucrose starvation stimulated autophagy leading to translocation of ATG8-associated autophagosomes to vacuole. Sucrose treatment (3%) increased immunoreactive signals of ATG8i-related protein in soybean seedling at 12 and 24 h, while the treatment had little or marginal effects on the expression of *GmATG8i* (Figures 3(a) and 3(b)). In addition of significant signals of GmATG8i at 14 kDa, upper-shifted signals at 16 kDa of ATG8i-related proteins appeared in 3% sucrose treatment, suggesting that phosphatidylethanolamine-unconjugated GmATG8i was accumulated due to suppression of autophagy.

4. Discussion

Recently, low energy stress on higher plants appeared to induce significant changes of amino acid metabolism via

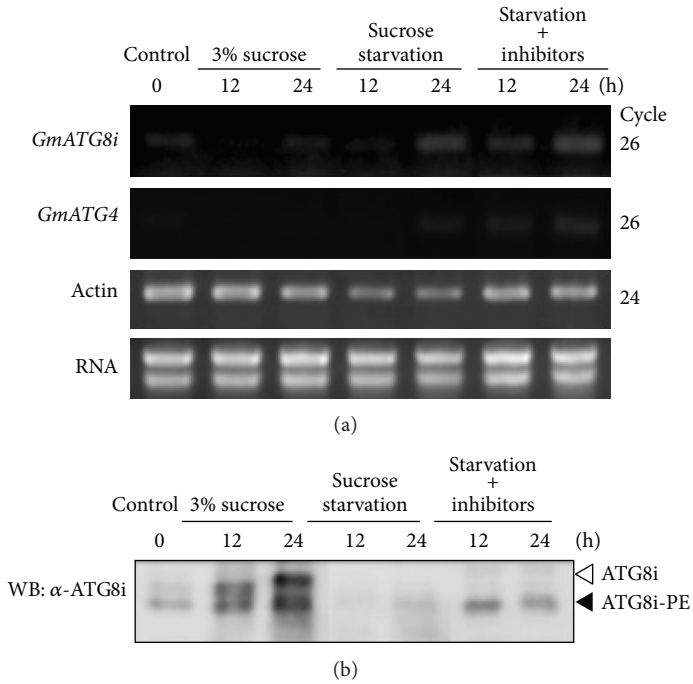

Figure 3: Induction of autophagy of soybean seedlings in response to nutrient starvation. (a) The expression profiles of *GmATG8i* and *GmATG4* under various nutrient condition as indicated. (b) The immunoreactive signals of ATG8i-related polypeptides under various nutrient conditions as indicated. Mobility shift between GmATG8i (open triangle) and phosphatidyl ethanolamine- (PE-) conjugated GmATG8i (closed triangle) is indicated. Nutrient treatments, semiquantitative RT-PCR, and immunoblot were carried out as described in Section 2.

transcriptional and translational regulation mechanisms [7]. The present data indicates that nutrient starvation of soybean seedlings induces the expression of the bZIP53 ortholog and amino acid catabolism-related genes, accompanied with enhanced degradation of intracellular components via autophagy. Previous studies indicated that the intracellular-free amino acid pool generated by autophagy are utilized for amino acid recycle, respiration substrate and/or translocation of nitrogen to other tissues [22]. Among bZIP type transcription factors in *Arabidopsis*, heterodimer bZIP transcription factors, bZIP1 and bZIP53, appeared to be activated, transcriptionally and translationally, in response to low energy stress. Furthermore, it was shown that the bZIP1 and bZIP53 are master transcription factors regulating the expression of *ASN1, ProDH*, and *BCAT2*, which are involved in amino acid catabolism and nitrogen translocation [7]. The expression of GmZIP53A but not GmbZIP53B was significantly induced by sucrose starvation and severe starvation with inhibitors of proteases, while the GmbZIP53-related protein levels were upregulated by severe starvation (Figures 1(b) and 1(d)). Recent studies of plant transcription factors revealed that protein levels and transcriptional activities of Inducer of CBF (C-box Binding Factor) Expression 1 (ICE1) and drought responsive element binding factor 2A (DREB2A) are regulated by the protein stability via ubiquitin-proteasome system [23]. It can be assumed that the severe starvation with protease inhibitors on soybean seedlings suppresses amino acid recycle via autophagy, resulting in decreased amino acid pool. It is conceivable that the a starvation signal of decreased amino acid pool increases the protein stability of GmbZIP53A and/or GmbZIP53B via an independent pathway from sucrose starvation.

Our previous study indicates that leaf senescence accompanied with enhancing of *ATG* expression and nitrogen translocation at the pod filling stage is induced by increased uptake of photoassimilate from source organs [24]. In higher plants, ProDH mediates the degradation of the compatible osmolyte, proline, which is used to support the demand for nitrogen, carbon, and energy under carbohydrate limitation [7]. ASN1 and ASP3 are key enzymes of the biosynthesis of asparagine, which is utilized for intracellular nitrogen storage and nitrogen transport via vascular tissue especially when carbohydrate supply is limited [18]. It is conceivable that nutrient demand from the developing pod promotes a signal of decreased sugar level in leaf, leading to GmbZIP53A-mediated induction of amino acid catabolism-related genes which are necessary for nutrient translocation from senescing leaf to developing seed.

Starvation signals enhanced the expressions of ATGs and autophagy (Figure 3). Recent studies revealed that intracellular levels of sugar and BCAA play important roles in autophagy induction via AMP-activated protein kinase (AMPK)/Snf1-related protein kinase (SnRK) and target of rapamycin (TOR), respectively [2]. It has been shown that decrease of intracellular BCAA level accelerates autophagy by inactivation of TOR. BCATs appeared to function in the degradation of BCAA to provide an alternative carbon source under stress conditions in plants, animal, and

bacteria [25]. Barley BCATs and *Arabidopsis* BCAT2 are induced by drought stress and sucrose starvation, respectively [7, 20]. It was reported that AtbZIP1 and AtbZIP53 bind to specific sequence motif elements, ACGT-box and ACTCAT-box, in the promoter regions of ProDH, ASN1, and BCAT [7]. According to soybean genome sequence data base, the 5′-promoter regions within 300 bp from starting ATG codon of *GmBCAT* and *GmATG8i* genes possesses 3 boxes similar to consensus motives of the ACGT-box and ACTCAT-box. It is possible that GmbZIP53A plays pivotal roles in the expression of both *GmBCAT* and *GmATG8i* via binding to ACGT-box and ACTCAT-box. A significant induction of *GmBCAT* in soybean seedling under severe starvation possibly resulted in an increased degradation of BCAA by GmBCAT. It can be assumed that decreased BCAA pool of soybean seedling under nutrient starvation induces autophagy via TOR signal.

Increasing evidence suggests that the network of various nutrient signals, such as sugars and amino acids, regulate plant development, senescence, and primary metabolisms. Therefore, identification and molecular dissection of the signaling molecules involved in sugar signaling are important for research of autophagy and amino acid catabolism in higher plants.

Abbreviations

ASN1: Asparagine synthase 1
ASP3: Aspartate aminotransferase 3
ATG: Autophagy-related gene
BCAA: Branched chain amino acid
BCAT: Branched chain amino acid transaminase
PMSF: Phenylmethylsulfonyl fluoride
ProDH: Proline dehydrogenase
RT-PCR: Reverse transcription polymerase chain reaction
SDS: Sodium dodecylsulfate
TBS: Tris-buffered saline.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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