Leaf Senescence of Soybean at Reproductive Stage is Associated with Induction of Autophagy-related Genes, \textit{GmATG8c}, \textit{GmATG8i} and \textit{GmATG4}

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Abstract: Autophagy appears to function in bulk protein degradation and N remobilization in senescence. Depodding of soybean suppresses N remobilization from leaves, leading to “green stem syndrome”. Expression of autophagy-related genes (ATGs) and N contents of the leaves and stem was analyzed in soybean plants from none (control), half (50% depodding) and all (100% depodding) of flowers and pods were removed at the reproductive 5 stage. Total N content and SPAD in leaves of the plant after 100% depodding were retained at a constant level and those after 50% depodding gradually decreased from 4 to 5 wk after depodding, while those in the control plants rapidly decreased during this period. Expression of \textit{GmATG8c} and \textit{GmATG8i} in leaves peaked at 4 wk in control while the gene expression increased gradually after 50% and 100% depodding. The transient up-regulation of ATGs and the decline of leaf N content and SPAD occurred simultaneously. These results indicated that ATGs are involved in leaf senescence and N remobilization from leaf to pod.

Key words: ATG, Autophagy, Depodding, Green stem syndrome, Nitrogen, Soybean, SPAD.

Leaf senescence is a highly regulated developmental process that ends with the programmed cell death of leaf cells (Swidzinski et al., 2002). The senescence signal induces as set of senescence-associated genes that are involved in the degradation of cellular components. The resultant nutrients are translocated to other parts of the plant (Noodén et al., 1997). However, the progress of leaf senescence in maize at the grain-filling stage is affected by the source-sink balance between leaf and grain (Tollenaar, 1977). The source-sink balance at the grain-filling stage is an important factor in the regulation of leaf senescence at the whole-plant level (Tollenaar and Daynard, 1982; Miao et al., 2009).

Recent molecular and genetic studies with \textit{Arabidopsis} have shown that autophagy is involved in development, senescence, and cell differentiation, and that environmental stress is related to starvation, oxidative stress and pathogen attack (Bassham et al., 2006). Autophagy (self-eating) is an intracellular bulk degradation that is composed of nutrient-signal sensing and cytoplasm-to-vacuole transport.

The signal is made by generating autophagosomes, which recycle amino acids and energy for maintaining viability under the starvation stress. Plants with mutation of autophagy-related genes (\textit{ATG}) appeared to show early senescence phenotype (Doelling et al., 2002; Hanaoka et al., 2002) and/or higher susceptibility to various environmental stresses, such as oxidative stress (Hanaoka et al., 2002; Xiong et al., 2005). Many researchers have tried to identify genes encoding enzymes or proteins that are specifically induced by the remobilization of nitrogen, carbon and minerals during leaf senescence (Feller and Fisher, 1994). In legume crops like soybean, nitrogen supplied from nodule and fertilizer is the most critical factor for maintaining the growth rate of photosynthetic organs and generating flower buds at the vegetative growth stage (Matsumani et al., 2009; Nakamura et al., 2010). In turn, the efficiency of nitrogen translocation systems from vegetative to reproductive organs affects the yield at the
pod filling stage (Noodén et al., 1997).

In senescent leaf cells, the central vacuole is the largest lytic compartment and contains most of the cellular hydrolytic activity (e.g., 80–100% of acid protease and 50–100% of acid nuclease activities; De, 2000). In many cases, autophagic mechanisms appear to be responsible for de novo formation of vacuolar compartments in plants (Robinson and Hinz, 1997).

Nitrogen in grain is derived from nutrients that are taken up during the grain-filling stage and the nutrients are remobilized from vegetative organs in maize (Pan et al., 1986). The onset of grain filling may be a critical phase for N supply within the plant because of the decrease in uptake of the nutrients. The maize plant progresses to maturity (Christensen et al., 1981), mainly due to the reduced transport of carbohydrates to the roots. Senescence and photosynthetic rate of rice leaf at reproductive stage are affected by the supply of N condition from soil (Kumagai et al., 2009). Decreased N uptake during the grain-filling period may enhance nutrient remobilization from leaves and the stem, eventually leading to leaf senescence. However, regulation of biochemical changes occurring in the remobilization process is still unclear.

Therefore, to investigate the various events related to the primary assimilation or remobilization of nutrients in the progress of senescence. We developed an integrated model of the source-sink relationship and autophagy process at different stages of reproductive development.

Materials and Methods

1. Plant material and growth condition

Three to five seeds of soybean (Glycine max [L.] Merr. cv. Fukuyutaka) were sown in the pots (16 cm diameter × 20 cm height) filled with soil in the Kaizuka field, Kyushu University on 19 July 2007, and the seedlings were thinned to one plant per pot upon germination in order to get uniform plant stand. Chemical compound fertilizer, namekase (N:P2O5:K2O = 3:10:10) (5 g pot⁻¹) and lime (5 g pot⁻¹) were well mixed with the soil before sowing.

2. Treatments and samples collection

At the reproductive 5 stage (R5), beans begun to develop at one of the four uppermost nodes with completely unrolled leaf. Depodding treatments were given at this stage by removing half of the visible flower buds, open flowers and young pods (50% depodding), or all of the pods and flowers (100% depodding). Newly initiated flowers and pods were removed from depodded plants at 3–5 d interval. Samples for analysis were taken from the middle leaflets of the 2nd to 4th leaves below the uppermost leaf, which were not unrolled, and 6 times from the R5 stage until the leaf color changed to yellow or brown. Stems, seeds and shells were also sampled from the four uppermost nodes.

For RNA analysis, each sample (about 1 g) was dipped in liquid nitrogen for a while and then all were stored at −80°C until analysis.

At the final harvest, 10 plants in each treatment were examined for the following items: 1) yield and yield components such as yield per plant, 2) seed weight, 3) numbers of seeds, pods, branches, and total nodes per plant, 4) stem dry weight per plant, and 5) number of nodes per main stem and length of main stem.

3. Analysis of leaf SPAD value and total nitrogen content

Leaf chlorophyll levels were estimated on four uppermost fully expanded leaves on the main stem. The SPAD value was taken with a chlorophyllimeter (SPAD-502, Konica Minolta, Tokyo, Japan) at 1-wk intervals from R5. The value was recorded as an average of individual leaves (n = 6) at 0900–1200 hr.

To measure total nitrogen content (N content) of leaves and stem, we sampled the side leaflets of four uppermost leaves and stems at 1-wk intervals between R5 and R7 stages from control, 50% depodded and 100% depodded plants. The N contents of the samples were analyzed by the micro-Kjeldahl method (Jackson, 1973), using 100 μg N mL⁻¹ (NH₄)₂SO₄ as N standard solution. Absorbance at 625 nm was determined with a spectrophotometer (H-1800, Hitachi, Tokyo, Japan) to determine the N concentration.

4. Measurement of yield parameters and seed weight during seed development

To measure the dry weight (dw) and water content, we sampled leaves, stems, seeds and leaves at R5 to R7 stages from the control, 50% depodded and 100% depodded plants as in the analysis of N content. The water content of all samples was determined as the difference in the fresh weight and dry weight at a given time (fresh weight basis) with 3 replications for each treatment. The data were evaluated by analysis of variance (ANOVA) using Statview software (Statistical Analysis System, SAS Institute Inc., 1992–1998).

5. RNA extraction and Semi-quantitative RT-PCR analysis

BLAST search was conducted using APG genes (also known as ATG) of Arabidopsis thaliana in the Soybean Gene Index (SGI) at DFCI (http://compbio.dfci.harvard.edu/tgi/) and phytozome v.5.0 in JGI (http://www.phytozome.net/) identified soybean orthologs. SGI codes, accession numbers and specific primer sequences of the soybean genes and actin were shown in Table 1.

As previously described RNA preparation by the SDS/phenol/LiCl method, cDNA synthesis and PCR were carried out, (Imamura et al., 2008; Nang et al., 2009) using Rev TraAce reverse transcriptase (Invitrogen) and GoTaQ kit (Promega) in accordance with to the manufacturer’s manuals and the thermal cycler (PG-816 ASTEC, Fukuoka,
Table 1. Primers used for RT-PCR analysis.

| Gene name | AccessionNo./TC, SGI | Primer sequences |
|-----------|----------------------|-----------------|
| GmATG8c   | TC218668, Glyma15g11510 | F-AGGACCAGTGCCAAAAGCCCTGAACCTTTCTGAGGACGACGAC R-ATGAGGTCTGTCATTCTATTAGAGAGGATAGCTCTTAAATGAC |
| GmATG8f   | TC210141, Glyma05g04540 | F-TCTGGATCCCTCTCTCGACGAGGAAGAATGACA R-TGAATGTCGACATGCGATACAATCTGTGAAAG |
| GmATG8i   | AB453310, Glyma02g01180 | F-GCCGAAATTTGTTAGCTGTCGACAAACACTTAC R-GATAGTCGACACCTGCAAGATGTTGGA |
| GmATG4    | TC2298706, Glyma18g48380 | F-AGGCAAACAGTGCTCAGGATACACATAC R-ATGAGCGGATACACCACACTCTTG |
| GmATG7    | AB486013, Glyma12gg01250 | F-GCTGGATCCCTCTTCACGAGAGAATGACA R-TGATGTCGACACTTCACAAATTTAGAG |
| GmATG9    | AM085508, Glyma19g3630 | F-CATTGTGCACCACATGTAACATCTCTG R-CCTGTGCAGCAGCAAGAATCTTAA |
| GmATG18a  | TC229926, Glyma10g29320 | F-ATGTCTCCTAAACCTCGGTTCCGATGAG R-AGAGCTGGCGGTTGCAAGACCCCTGCCC |
| ACTIN     | V00450, Glyma08g15480 | F-GATGCTGATCCACATGTCGAGTTATATAT R-AGCCTTCGGAATTCACCATGTTG |

1TC, tentative consensus sequence; 2SGI, soybean gene index.

Results

1. Leaf SPAD value
The SPAD value in each treatment was sustained at 40–45 at 1–3 wk after depodding treatment (WAT) and then declined at various rates, depending on the treatments (Fig. 1). In the control, it rapidly dropped from 3 to 5WAT and finally reached the marginal level at 5WAT. In the 50% depodded, the value declined at relatively high rate from 4 to 5WAT, then gradually declined from 5 to 7WAT and reached 25, which was half of the initial level. In contrast, the value in 100% depodding groups declined more slowly than that of control and 50% depodding groups. The value was 35 at 8WAT. The change in the appearance of senescing leaves in each treatment (Fig. 4) was similar to that in SPAD (Fig. 1).

2. Total nitrogen content of leaves and stem in the seed-developing stage
After R5, N content of leaves changed differently depending on the treatments (Fig. 2A). In control, the N content declined from 32 mg g⁻¹ dw at 0WAT to 18 mg g⁻¹ dw at 4WAT and then reached 5 mg g⁻¹ dw at 6WAT (end of pod filling stage). N content in the 50% depodded group, dropped more slowly than in the control from 0 to 5WAT, then gradually declined from 5 to 7WAT and reached 25, which was half of the initial level. In contrast, the value in 100% depodded groups declined more slowly than that of control and 50% depodding groups. The value was 35 at 8WAT. The change in the appearance of senescing leaves in each treatment (Fig. 4) was similar to that in SPAD (Fig. 1).

Fig. 1. Effect of depodding treatment on SPAD values of soybean leaf at pod filling stage. All (100% depodding, closed circle), half (50% depodding, closed square) or none (control, open circle) of flower buds and pods were removed from soybean plants at the early stage of R5, and the depodding was repeated at 3–5-d intervals. SPAD values of leaves at the top of the main stem were measured at 1-wk intervals as described in Materials and Methods. Average of SPAD values (n=6) are indicated.

Fig. 2. Total nitrogen content of leaves and stem in the seed-developing stage. After R5, N content of leaves changed differently depending on the treatments (Fig. 2A). In control, the N content declined from 32 mg g⁻¹ dw at 0WAT to 18 mg g⁻¹ dw at 4WAT and then reached 5 mg g⁻¹ dw at 6WAT (end of pod filling stage). N content in the 50% depodded group, dropped more slowly than in the control from 0 to 5WAT, then gradually declined from 5 to 7WAT and reached 25, which was half of the initial level. In contrast, the value in 100% depodded groups declined more slowly than that of control and 50% depodding groups. The value was 35 at 8WAT. The change in the appearance of senescing leaves in each treatment (Fig. 4) was similar to that in SPAD (Fig. 1).

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| GmATG8i   | AB453310, Glyma02g01180 | F-GCCGAAATTTGTTAGCTGTCGACAAACACTTAC R-GATAGTCGACACCTGCAAGATGTTGGA |
| GmATG4    | TC2298706, Glyma18g48380 | F-AGGCAAACAGTGCTCAGGATACACATAC R-ATGAGCGGATACACCACACTCTTG |
| GmATG7    | AB486013, Glyma12gg01250 | F-GCTGGATCCCTCTTCACGAGAGAATGACA R-TGATGTCGACACTTCACAAATTTAGAG |
| GmATG9    | AM085508, Glyma19g3630 | F-CATTGTGCACCACATGTAACATCTCTG R-CCTGTGCAGCAGCAAGAATCTTAA |
| GmATG18a  | TC229926, Glyma10g29320 | F-ATGTCTCCTAAACCTCGGTTCCGATGAG R-AGAGCTGGCGGTTGCAAGACCCCTGCCC |
| ACTIN     | V00450, Glyma08g15480 | F-GATGCTGATCCACATGTCGAGTTATATAT R-AGCCTTCGGAATTCACCATGTTG |
mg g\(^{-1}\) dw at 7WAT. N content in the 50% depodded group rapidly dropped to 13 mg g\(^{-1}\) dw at 1WAT, but was maintained at the same level from 1 to 6WAT. On the other hand, N content in the 100% depodded group rapidly fell to 15 mg g\(^{-1}\) dw at 2WAT but dramatically increased to about 40 mg g\(^{-1}\) dw from 2 to 6WAT.

3. Physiological change after depodding and yield components of soybean plants

As previously reported by Noodén et al., (1980) soybean plants with 100% depodding reflowered and developed 130−200 pods per plant by 5WAT (data not shown). Plants after 100% depodding developed thicker stems (Fig. 3), shorter and thicker petiole and darker green leaves than the control. They retained leaves at a lower node those in when compared to the control and 50% depodded plants. The characteristic of leaf senescence was seen in the control at 4WAT, and leaf abscission was observed at 5WAT. Leaf abscission (Fig. 4) and seed maturation (Fig. 4, inserted) were both completed at 6WAT. In the 50% depodded group, leaf senescence, leaf abscission and pod maturation were delayed by 1−2 wk compared with the control, indicating “green stem syndrome”. In the 100% depodded group (Fig. 4), leaf senescence was delayed by 2−3 wk but leaf abscission was not observed even at 8WAT. There was no significant difference in the characteristics of vegetative growth, such as the length of main stem and the numbers of nodes in the main stem among the control, the 50% and 100% depodded group (Table 2). However, an increase in stem dw plant\(^{-1}\) (7.04 to 8.25 g) and seed weight (243.5 to 278.5 mg) and a decrease in seed number plant\(^{-1}\) (103.4 to 80.52) were observed in 50% depodding treatment compared with the control. Finally, the difference of seed yield plant\(^{-1}\) between control (25.13 g) and 50% depodding (22.34 g) was about 11%.

4. Effect of source-sink manipulation on expression of ATGs

In the control, GmATG8c and GmATG8i were expressed at a constant level from 0 to 3WAT, when leaves on the top of the stem were dark green (Fig. 4A). Then it was transiently up-regulated at 4WAT when the color of leaves started to change from green to yellow (Fig. 5A). Expression of GmATG8c and GmATG8i was not detected in senescent leaves at 5−6WAT when leaf senescence and abscission was completed. In 50% depodding, expression of GmATG8c and GmATG8i was at a constant level from 0 to 4WAT, but were slightly up-regulated at 5 and 6WAT (Figs.

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Table 2. The growth parameters, seed yield, and yield components of soybean growth under depodding treatments.

| Treatment          | Length of main stem (cm) | Nodes in main stem (plant\(^{-1}\)) | Number of pods (plant\(^{-1}\)) | Total no. of nodes (plant\(^{-1}\)) | Stem dry weight (g plant\(^{-1}\)) | Number of seeds (plant\(^{-1}\)) | Seed size (mg) | Seed yield (g plant\(^{-1}\)) |
|--------------------|--------------------------|------------------------------------|-------------------------------|-----------------------------------|------------------------------------|-------------------------------|---------------|---------------------------|
| Control            | 42.24 a                  | 13.56 a                            | 66.00 a                       | 50.33 a                           | 7.04 c                             | 25.13 a                      | 243.5 b       | 103.40 a                  |
| 50% depodding      | 41.65 a                  | 13.41 a                            | 52.33 b                       | 48.63 a                           | 8.29 b                             | 22.34 a                      | 278.5 a       | 80.5 b                    |
| 100% depodding     | 41.58 a                  | 13.11 a                            | 38.00 b                       | 38.00 a                           | 14.82 a                            | –                            | –             | –                         |

In a column for each treatments, values followed by the different letters are significantly different at P < 0.05, value is the mean of 10 plants.
Fig. 4. Photographs of senescing soybean plants in the control (A), 50% depodded (B) and 100% depodded (C) at 1-wk intervals after depodding are shown. Appearances of seed maturation in the control (A) and 50% depodded (B) plants are inserted in the upper right of each panel.

Fig. 5. Effects of depodding on expression of GmATG8c, GmATG8f, GmATG8i, GmATG4, GmATG7 and GmATG18a in soybean leaves. After depodding at the early stage of R5, total RNA was extracted from leaves of the control (A), 50% depodded (B) and 100% depodded (C) at 1-wk intervals, and then subjected to semi-quantitative RT-PCR. Synthesis of cDNA and RT-PCR with sets of gene specific primers (Table 1) for GmATG8c, GmATG8f, GmATG8i and actin were carried out as described in Materials and Methods. Appearances of senescing leaves at 1-wk interval after depodding are shown below each panel.

4B, 5B), although weaker and 1 wk later than that in the control plant. In the 100% depodded group, expression of GmATG8c and GmATG8i was relatively higher than those in the control and 50% depodded group from 0 to 3WAT, though it gradually increased uniformly throughout the seed filling stage (0–6WAT) (Figs. 4C, 5C). On the other hand, expression of GmATG8f was not detectable in any treatment (Fig. 5C).

In the control (Fig. 5A), GmATG4 was expressed at a low level during 0–3WAT and transiently up-regulated at 4WAT when the leaf started senescing. GmATG9 in the control was expressed with constant level at 0–3WAT and slightly up-regulated at 4WAT. Expression of GmATG4 and GmATG9 was not detected in completely senesced leaf at 5
The expression levels of GmATG4 and GmATG9 in the control were enhanced transiently at 4WAT of R5, which is similar to those of GmATG8c and GmATG8i. In 50% (Fig. 5B) and 100% depodding (Fig. 5C), expression of GmATG4 and GmATG9 was low or marginal at 0WAT and gradually increased from 2 to 5WAT. However, significant induction was not detected.

In contrast to GmATG8c, i.e., GmATG4 and GmATG9, expressions of GmATG7 and GmATG18a were detectable but not significantly changed in the control, 50% and 100% depodded plants during 0–6WAT (Fig. 5).

### Discussion

1. **Effects of source-sink manipulation on N content and senescence of leaves**

In the present study, the N content of leaf and stem in the control declined gradually from 33 and 28 mg g⁻¹ dw, respectively at 0WAT to 5 and 2 mg g⁻¹ dw, respectively at 6WAT (Fig. 2). Although SPAD values started to fall at the same time in the control and 50% depodded plants, the reduction was much slower in 50% depodded plants than in the control (Figs. 1, 5). N content was also relatively higher in the 50% depodded plants than in the control. Moreover, N content of stem significantly increased in the 100% depodded plants at 5–6WAT (Fig. 2B). This suggests that the stem in the 100% depodded group served as a sink organ for N, and it was translocated from leaf that did not senesce. This observation is consistent with our previous study that the final seed yield in 50% depodded group was 22.34 g plant⁻¹ and that in the control was 25.13 g plant⁻¹, respectively at 6WAT (Fig. 2). Although SPAD values started to fall at the same time in the control and 50% depodded plants, the reduction was much slower in 50% depodded plants than in the control (Figs. 1, 5). N content was also relatively higher in the 50% depodded plants than in the control. Moreover, N content of stem significantly increased in the 100% depodded plants at 5–6WAT (Fig. 2B). This suggests that the stem in the 100% depodded group served as a sink organ for N, and it was translocated from leaf that did not senesce. This observation is consistent with the findings reported by Crafts-Brandner and Egli. (1987).

As shown by SPAD values (Fig. 1) and appearance (Fig. 4), the leaves in the 50% and 100% depodded group maintained their green color and chlorophyll contents for 1 and 3 wk, respectively. After completion of leaf senescence and abscission in the control (Fig. 1), after depodding started to senesce. Egli et al. (1976) reported that depodding treatment elongated the period of N assimilation in remaining seed, hence increased the seed weight (weight seed weight in the control and 50% depodded groups was started to senesce. Egli et al. (1976) reported that depodding and abscission in the control (Fig. 1), after depodding per seed). This is consistent with our observation that the leaf senescence was accompanied with pod maturation. In general, senescence is regulated temporarily and spatially by a set of expression and/or repression of specific genes in relation to breakdown of organelles and degradation of cellular compounds (Smith et al., 1992). Previously, Hydrolytic enzyme activities (DNase, RNase and glucosidase) have been reported during corolla senescence in Ipomoea. These activities were dependent on both RNA and protein synthesis (Matile and Winkenbach, 1971). In the 100% depodding experiment, a significant amount of N was translocated to stem at 0 wk (Fig. 2B), whereas neither leaf senescence nor induction of ATGs were observed. Control plants were estimated to have accumulated about 2000 mg N in the seeds (25.1 g dw) (Table 2), i.e., about 80 mg N g⁻¹ dw in seed (Toda et al., 2003). In contrast, the 100% depodded plant accumulated 500 mg N in the stem (14.8 g dw), i.e., about 40 mg N g⁻¹ dw in stem (Table 2).

Therefore, it can be assumed based on the difference in N contents between pods in the control and stem in the 100% depodded plants. That N-sink performance of stem is not sufficient for inducing autophagy in leaves compared with that of pod. Our previous study indicated that expression of GmATG8c and GmATG8i in leaves was induced at 4WAT in leaf but decreases in seed from R5 to R6 along with seed maturation (Nang et al., 2008a). At the late pod-filling stage, enhanced-nutrient-up-take capacity and synthesis of storage protein in growing seed may stimulate a signal to the leaf, leading to enhancement of autophagy and senescence.

This study showed that a change of source-sink balance by depodding treatment at R5 had significant effects on ATG expression in leaves and caused “green stem syndrome”. This was indicated by the senescence delay (Fig. 4) and suppression of N content reduction (Fig. 2A) and SPAD (Fig. 1). Expression of GmATG8c, GmATG8i, GmATG4 and GmATG9 in leaves was induced at 4WAT in the control when leaf color started to turn yellow (Fig. 4A). Our previous study revealed that expression of GmATG8c, GmATG8i and GmATG4 significantly increased in soybean
hypocotyls when autophagy is induced under starvation stress (Nang et al., 2009). It has been also found that the Arabidopsis ATG8s mRNAs level in detached leaves mildly increased during the senescence (Doelling et al., 2002). ATG4 mRNA was induced in Arabidopsis suspension cultured cell when subjected to sucrose starvation (Rose et al., 2006). Toyooka et al. (2001) revealed that autophagic programmed cell death is accompanied with degradation of storage compounds in cotyledons of kidney bean during germination; it indicated that autophagy is important for translocating nutrients from cotyledon (source) to growing hypocotyls (sink). In the 50% depodded plants, leaf senescence was delayed and induction of ATG was also delayed by 1 wk. In the 100% depodded plants, expression of soybean ATG homologs gradually increased during 0–6WAT. Although flowers and pods were continuously removed from all nodes in the 100% depodded plants at R5, they were formed again before plants in the control reached the seed filling stage. Nutrients (amino acids, lipids and proteins) stored in leaves are assumed to be degraded and utilized for formation of new flower and pod. Thus, expression of ATGs is considered to be expressed continuously at a significant level even in 100% depodded plants. Light-shading treatment on leaves induces senescence associated genes, including ATG8 homolog in Arabidopsis and soybean (Otegui et al., 2005). The shading treatment also causes degradation of chloroplasts via autophagy in Arabidopsis (Wada et al., 2009), suggesting that deficiency of photosynthetic production also triggers autophagic process in plants. In addition to N remobilization, carbon remobilization from leaf to pod is essential for development of legume seeds where starch and lipid are synthesized as storage compound. Dam et al. (2009) reported that induction of β-amylase in leaf plays an essential role in carbon remobilization to developing legume seed. Recent studies revealed that leaf senescence is associated with autophagy of chloroplast (Wada et al., 2009) and induction of β-amylase (Doyl et al., 2007). Thus, the possibility should not be excluded that depodding treatment caused temporary carbon storage in the leaf and stem, which suppresses autophagy in the leaf leading to “green stem syndrome”. Our findings proved that both starvation stress and source-sink manipulation have significant effects on expression of GmATG8c, GmATG8i and GmATG4 (Nang et al., 2008b). This implies that the levels of sucrose and/or amino acids in the cytoplasm and the apoplastic space are monitored by specific nutrient sensors.

To our knowledge, this is the first report to focus on the expression profiles of autophagy-related genes in leaves using soybean plant at reproductive stage. This study shows that soybean homologs of ATG8s, ATG4 and ATG9 are significantly induced in senescent leaf and the expression is affected by the source-sink balance. Our findings indicate that autophagy plays important roles in translocation of nitrogen compounds from senescing leaf to developing seed. This study also suggested that the change in source-sink balance by depodding treatment causes “green stem syndrome” through suppression of autophagy and senescence in response to delayed translocation of nutrients from the leaf.

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