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Expression of sweet potato asparaginyl endopeptidase caused altered phenotypic characteristics in transgenic *Arabidopsis*

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ABSTRACT. We have previously isolated an asparaginyl endopeptidase, *SPAE*, from senescent leaves of sweet potato (*Ipomoea batatas* cv. Tainong 57). Gene expression of *SPAE* was activated and enhanced in natural and induced senescent leaves (Chen et al., 2004). In this report the full-length *SPAE* cDNA was constructed in the T-DNA portion of recombinant pBI121 vector under the control of *CaMV 35S* promoter and transferred to *Arabidopsis* with *Agrobacterium*-mediated floral dip transformation. Three transgenic *Arabidopsis* plants were isolated and confirmed by kanamycin-resistance and genomic PCR amplification of *SPAE*. Protein gel blot also demonstrated sweet potato *SPAE* expression in these transgenic plants. Phenotypic analysis showed that transgenic plants exhibited earlier floral transition from vegetative growth and leaf senescence than control. Transgenic plants also contained fewer siliques and a higher percentage of incompletely-developed siliques per plant than control. Based on these results we conclude that sweet potato asparaginyl endopeptidase, *SPAE*, may function in association with the senescence process, and its expression enhances or promotes senescence in transgenic *Arabidopsis* plants. The altered phenotypic characteristics in transgenic plants with *SPAE* gene expression were also discussed.

Keywords: Asparaginyl endopeptidase; Silique; *SPAE*; Sweet potato; Transgenic *Arabidopsis*.

INTRODUCTION

Leaf senescence has been considered as a type of programmed cell death and is the final stage of leaf development. Senescence is not simply a degenerative process, but also a recycling one, in which nutrients are translocated from the senescent cells to young leaves, developing seeds, or storage tissues (Buchanan-Wollaston, 1997; Quirino et al., 2000). Leaf cells undergo highly coordinated changes in structure, metabolism, and gene expression in a defined order during senescence. The earliest and most significant change in cell structure is the breakdown of the chloroplast (Makino and Osmond, 1991). Metabolically, the carbon assimilation (photosynthesis) is replaced by a catabolism of macromolecules and organelles which leads to the final cell death. During leaf senescence, breakdown of leaf proteins by proteases provides a large pool of cellular nitrogen for recycling (Makino and Osmond, 1991). In plants, three major degradation pathways have been described: (a) the ubiquitin-dependent pathway, (b) the chloroplast degradation pathway, and (c) the vacuolar degradation pathways (Vierstra, 1996). Among these pathways, vacuolar degradation is assumed to be involved in bulk protein degradation by virtue of the resident proteinases in the vacuole. Two types of vacuoles have been described in plants: (a) the storage vacuole and (b) the lytic central vacuole (Marty, 1999). Protein storage vacuoles are often found in seed tissues and accumulate proteins that are re-mobilized and used as the main nutrient resource for germination (Senyuk et al., 1998; Schlereth et al., 2001).

Most cells in vegetative tissues have a large central vacuole, containing a wide range of proteases in an acidic environment. Substrate proteins must be transported and sequestered into this vacuole for degradation. The role and function of vacuolar processing enzymes (VPEs) in association with vacuolar protein degradation and the nutrient recycling pathway in senescent leaves are generally not clear. Recently a novel group of plant VPEs was found in the developing seeds of the castor bean

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(Hara-Nishimura et al., 1991) and soybean (Scott et al., 1992; Hara-Nishimura et al., 1995), from mature seeds of the jack bean (Abe et al., 1993), and from germinating seeds of vetch (Becker et al., 1995). In castor bean and soybean seeds, VPEs were detected in the protein bodies and likely associated with the conversion of proproteins to their corresponding mature forms in vacuoles (Hara-Nishimura et al., 1991; Shimada et al., 1994; Shimada et al., 2003). VPEs also play a role in bulk degradation and mobilization of storage proteins during seed germination and seedling growth. An asparaginyl-specific cysteine endopeptidase, called "legumain-like proteinase" (LLP), was isolated from cotyledons of kidney bean (*Phaseolus vulgaris*) seedlings. It was the first proteinase ever known to extensively degrade native phaseolin in vitro, the major storage globulin of this grain legume (Senyuk et al., 1998). In vetch (*Vicia sativa*) seeds, the legumain-like VsPB2 and proteinase B together with additional papain-like cysteine proteinases were responsible for the bulk breakdown and mobilization of storage globulins during seed germination (Schlereth et al., 2000). In *Arabidopsis*, the seed protein profiles were compared between the wild type and a seed-type vacuolar processing enzyme *βVPE* mutant using a two dimensional gel/mass spectrometric analysis. A significant increase in accumulation of several legumin-type globulin propolypeptides was found in *βVPE* mutant seeds (Gruis et al., 2002). In sweet potato, an aspartic type protease was reported to degrade trypsin inhibitors, the major storage proteins (Hou et al., 2002).

The mechanism of VPE associated with bulk storage protein degradation in seed has been studied in *Vigna mungo*. A vacuolar cysteine proteinase, designated SH-EP, is synthesized in cotyledons of germinated *Vigna mungo* seeds and is responsible for degradation of seed proteins accumulated in protein bodies (protein storage vacuoles). SH-EP belongs to the papain proteinase family and has N-terminal and a C-terminal prosegments (Okamoto and Minamikawa, 1999; Okamoto et al., 1999). Okamoto and Minamikawa (1995) isolated a processing enzyme, designating it VmPE-1. It is a member of the VPEs and is involved in the post-translational processing of SH-EP. In addition, the cleavage sites of the in vitro processed intermediates and the mature form of SH-EP were identical to those of SH-EP purified from germinated cotyledons of *V. mungo*. Therefore, it is proposed that the VPE (VmPE-1)-mediated processing mainly functions in the activation of proSH-EP during seed germination (Okamoto et al., 1999). The activated SH-EP plays a major role in the degradation of seed storage proteins accumulated in cotyledonary vacuoles of *Vigna mungo* seedlings (Mitsuhashi et al., 1986).

We previously isolated a full-length cDNA of asparaginyl endopeptidase *SPAE* from sweet potato senescent leaves which exhibited high amino acid sequence homologies to plant VPEs, including kidney bean (*Phaseolus vulgaris*), spring vetch (*Vicia sativa*), and jack bean (*Canavalia ensiformis*) (Chen et al., 2004). Gene expression of *SPAE* was significantly enhanced in both natural and induced senescent leaves (Chen et al., 2004). In this report the full-length cDNA of *SPAE* was constructed in the T-DNA portion of recombinant pBI121 vector under the control of *CaMV 35S* promoter and transferred into *Arabidopsis* plants by *Agrobacterium*-mediated floral dip transformation. Expression of sweet potao *SPAE* in transgenic *Arabidopsis* plants and its association with altered phenotypic characteristics were investigated and discussed.

MATERIALS AND METHODS

Plant materials and sweet potato *SPAE*

The storage roots of sweet potato (*Ipomoea batatas* cv. Tainong 57) were grown in the greenhouse. Plantlets regenerated from the storage roots were used for experiments. *Arabidopsis thaliana* ecotype Columbia was the plant material for transgenic studies. The full-length cDNA of the sweet potato senescence-associated gene *SPAE* (GenBank accession no. AF260827), which encodes an asparaginyl endopeptidase (also known as a vacuolar processing enzyme), was constructed in the T-DNA portion of the pBI121 vector (Clontech) and used for the *Agrobacterium*-mediated floral dip transformation of *Arabidopsis* (Clough and Bent, 1998). Polyclonal antibody previously produced against sweet potato *SPAE* was used for protein gel blot hybridization (Chen et al., 2004).

Agrobacterium-mediated floral dip transformation of *Arabidopsis*

The full-length *SPAE* cDNA in recombinant pGEM-T easy vector was amplified with the following 5′ and 3′ primers containing an introduced *Sma*I (CCCGGG) restriction enzyme cutting site. The primer pair for *SPAE* (5′ primer: ATCGCCCGGG ATGATTCGCTCCGTCGTCGC and 3′ primer: ATCGCCCGGGTTATGCACTGAA TCCTCCTC) was used to amplify the full-length *SPAE* cDNA. After amplification the PCR products were first cloned into the pGEM-T easy vector (Promega), then released with restriction enzyme SmaI and subcloned into the T-DNA portion of pBI121 vector, which was digested with the same restriction enzyme (Figure 1). Both recombinant pGEM-T easy and pBI121 vectors were transformed into and replicated in *E. coli* DH5α cells. The correctness of these constructs in recombinant pGEM-T easy and pBI121 vectors was also confirmed by DNA sequencing using an ABI PRIZM 337 DNA sequencer. After confirmation, the correct recombinant pBI121 vector was transferred into *Agrobacterium tumefaciens* LBA4404 competent cells (Clonetech), with electroporation following the protocol supplied by the manufacturer (BIO-RAD). Transformed *Agrobacterium tumefaciens* cells were confirmed by PCR amplification using gene-specific primers as described earlier and utilized for *Arabidopsis* transformation with the floral dip method (Clough and Bent, 1998). After transformation, the *Arabidopsis* plant continued to grow

until seed set. These seeds were assigned as T0 seeds and collected for screening of transgenic T0 *Arabidopsis* seedlings.

Identification and characterization of transgenic *Arsbidopsis* plants

Transgenic T0 seedlings were identified and isolated after seed germination on half-strength MS salt medium with 1% sucrose, 500 μg/mL cefotaxine, and 50 μg/mL kanamycin under a regime of 22°C day/18°C night, 16 h light/8 h dark. The seedlings with a green phenotype were identified as putative transgenic T0 plants. These plants were then transferred to soil and grown in the growth chamber until flowering and T1 seed set. T1 seeds were collected and germinated on half-strength MS salt medium with 1% sucrose, 500 μg/mL cefotaxine, and 50 μg/mL kanamycin under the same light/dark regime. The T1 seedlings with a green phenotype were identified as putative transgenic plants and used for characterization as described below.

Genomic PCR amplification of *SPAE*

Putative transgenic T1 seedlings with a green phenotype grown on kanamycin-containing medium for 2 to 3 weeks after germination were transferred to soil and grown in a growth chamber under regime of 22°C day/18°C night, 16 h light/8 h dark. Leaf tissues of these putative transgenic T1 plants were collected for genomic PCR. About 0.5 g of putative transgenic T1 *Arabidopsis* and control plant samples were collected individually for genomic DNA isolation with the CTAB method (Chen et al., 2004). The same amount of purified genomic DNAs isolated from control and putative transgenic plants were used for PCR amplification with the primer pair (5′ primer: ATGATTCGCTCCGTCGTCGC and 3′ primer: TTATGCACTGAA TCCTCCTC) to confirm the presence of full-length *SPAE* cDNA in putative transgenic *Arabidopsis* plants (Jonson et al., 2000).

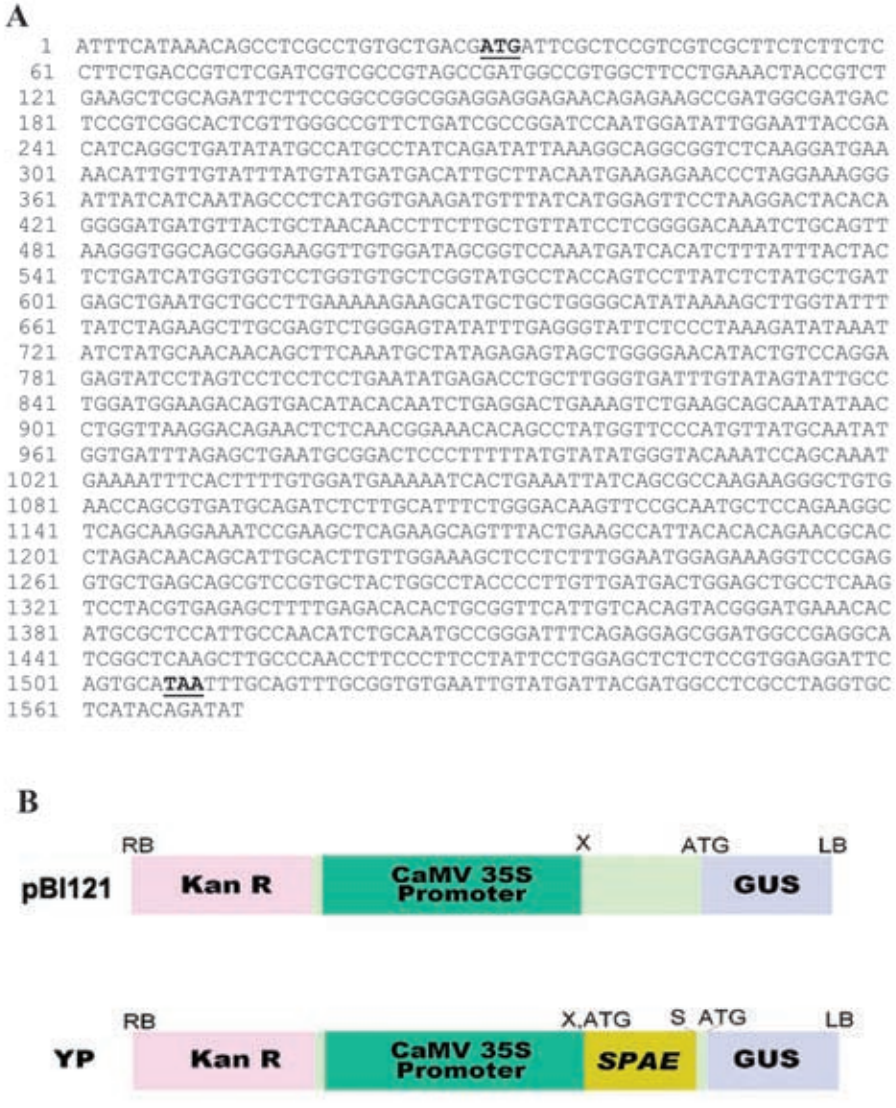

Figure 1. Construction of a full-length cDNA of sweet potato *SPAE* in T-DNA of recombinant pBI121 vector. A, The full-length cDNA sequence of sweet potato *SPAE*; B, Construction of the full-length cDNA (from ATG initiation codon to TAA stop codon) of sweet potato *SPAE* within the T-DNA portion of pBI121 vector. The upper is the unconstructed T-DNA portion of pBI121 (pBI121). The lower is the recombinant T-DNA portion of recombinant pBI121 (YP) which contains sweet potato *SPAE* full-length cDNA under the control of *CaMV 35S* promoter. X: *Xma*I; S: *Sma* I; ATG: start codon; Kan R: Kanamycin-resistance gene as a selectable marker; GUS: β-Glucuronidase; RB: right border of T-DNA; LB: left border of T-DNA.

Protein gel blot hybridization

Putative transgenic T1 seedlings with a green phenotype grown on kanamycin-containing medium for 2 to 3 weeks after germination were transferred to soil and grown in a growth chamber under a regime of 22°C day/18°C night, 16 h light/8 h dark. Leaf tissues of these putative transgenic T1 plants were collected for protein gel blot hybridization. About 0.3 g samples of transgenic T1 *Arabidopsis* and control plants were collected individually for total protein isolation, and ca. 100 μg total protein of each sample was used for protein gel blot hybridization with the polyclonal antibodiy against *SPAE* protein (Chen et al., 2004).

Analysis of morphological characteristics

Several phenotypic characters were observed and qualitatively or quantitatively compared between control and transgenic T1 plants. These characters included the time of transition from vegetative to reproductive growth, senescence of leaf, the silique and seed development, the number of seeds per silique, and the number of siliques per plant. Seedlings of transgenic T1 and control plants were grown under the same environmental conditions (22°C day/18°C night, 16 h light/8 h dark) in a growth chamber. The transition from vegetative phase to reproductive phase was compared and recorded once the inflorescence and flower had set. After flowering, the silique and seed development were also examined. *Arabidopsis* siliques were arbitrarily classified into four types (1 to 4) according to their morphologies after maturation. The morphology of different silique types, the number of seeds per silique of different types, and the percentage of incomplete silique development were compared and recorded among control and transgenic T1 plants.

RESULTS

Sweet potato *SPAE* gene is identified and expressed in transgenic *Arabidopsis* plants

In order to study the possible function of sweet ptato *SPAE*, its full-length cDNA was constructed in the T-DNA portion of pBI121 vector and transferred into *Arabidopsis* plants with the *Agrobacterium*-mediated floral dip transformation method. Transgenic *Arabidopsis* plants were isolated and identified using kanamycin resistance and genomic PCR amplification of *SPAE* full-length cDNA. In this experiment ca. 1 out of 5,000 to 10,000 seeds screened was identified as putative transgenic. Therefore, the transformation frequency was about 0.1% to 0.2%. Three putative transgenic T0 seedlings with green phenotype were isolated and designated YP1, YP2, and YP3. These putative transgenic plants were transferred to soil and grew until flowering and T1 seed had set. These T1 seeds were germinated on kanamycin-containing MS medium, and seedlings with green and white phenotypes were observed due to the meiotic segregation of the insert gene. Kanamycin-resistant T1 seedlings from YP1, YP2 and YP3 were identified as transgenic and utilized for further characterization.

Figure 2 shows the PCR products from genomic DNAs isolated from transgenic T1 seedlings and nontransformant control. The PCR products amplified with primers for *SPAE* full-length cDNA were detected and had molecular masses between 1.5 kb and 2.0 kb for all transgenic T1 seedlings of YP1, YP2 and YP3, but not for nontransformant control. These data demonstrate the presence of sweet potato *SPAE* gene in transgenic T1 seedlings. Protein gel blot hybridization with polyclonal antibody raised against *SPAE* (Chen et al., 2004) showed that a band with a molecular mass between 34 kDa and 40 kDa was detected from samples of sweet potato S1 senescent leaves and transgenic T1 *Arabidopsis* seedlings, but not from samples of sweet potato S0 mature young leaves and nontransformant *Arabidopsis* control (Figure 3). An extra band with a molecular mass between 40 kDa and 55 kDa was also detected from samples of transgenic T1 *Arabidopsis* plants, but not from samples of sweet potato S0/S1 mature young leaves and nontransformant *Arabidopsis* control (Figure 3). The reason is not clear. However, one of the possible explanation is that the extra band with higher molecular mass than the predicted mature form of *SPAE* protein may be due to the incomplete or lower processing efficiency of sweet potato *SPAE* precursor in transgenic T1 *Arabidopsis* plants than in sweet potato S1 senescent leaves. These data demonstrate that sweet potato *SPAE* can be expressed and processed into a mature form of *SPAE* protein in transgenic T1 *Arabidopsis* plants.

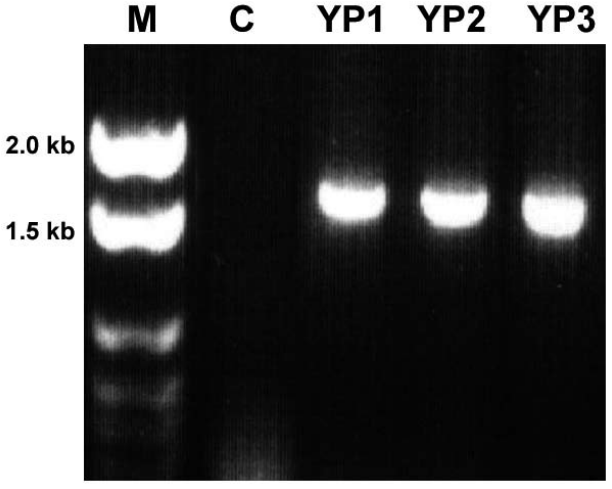

Figure 2. Genomic PCR analysis of putative transgenic T1 *Arabidopsis* plants. Total genomic DNA from nontransformant control and three isolated transgenic T1 *Arabidopsis* plants (YP1, YP2 and YP3) were analyzed for the presence of sweet potato *SPAE* gene. C and YP1/YP2/YP3 denote nontransformant control and transgenic *Arabidopsis* plants, respectively. M represents the molecular weight marker.

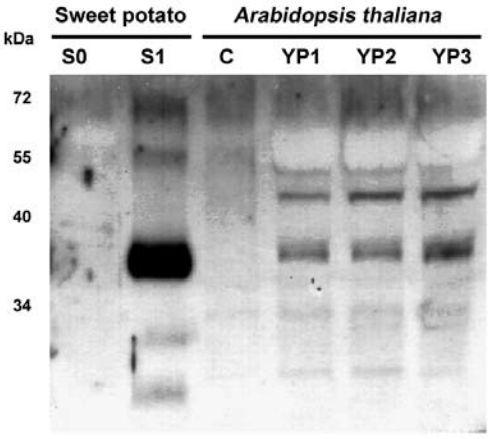

Figure 3. Protein gel blot hybridization of transgenic T1 *Arabidopsis* plants with rabbit polyclonal antibody against sweet potato *SPAE* protein. S0 and S1 represent sweet potato mature young and senescing leaves with 100% and 25% chlorophyll contents, respectively. C and YP1/YP2/YP3 denote nontransformant control and transgenic *Arabidopsis* plants, respectively.

Transgenic T1 *Arabidopsis* plants exhibited earlier flowering and leaf yellowing

In order to characterize the effects of sweet potato *SPAE* gene expression on the phenotypic properties of *Arabidopsis* transgenic plants, the growth patterns were compared and recorded among nontransformant control and transgenic T1 *Arabidopsis* plants. Transgenic T1 *Arabidopsis* plants expressing *SPAE* exhibited earlier floral transition from vegetative growth than nontransformant control ca. 30 to 35 days after seed germination (Figure 4A). Early flowering is generally considered a phenomenon of senescence. During seed and silique development, the lower leaves of *Arabidopsis* plants began to senesce, and a higher degree of leaf yellowing was observed for transgenic T1 *Arabidopsis* plants than for nontransformant control plants around 7 weeks after seed germination (Figure 4B). These data support the conclusion that sweet potato *SPAE* is a senescence-associated gene and its expression likely enhances or promotes senescence in transgenic *Arabidopsis* plant.

Transgenic T1 *Arabidopsis* plants contained higher percentage of incompletely-developed siliques and fewer siliques per plant

The phenotypic effects of sweet potat *SPAE* gene expression on the silique and seed development of transgenic T1 *Arabidopsis* plants were also investigated and recorded. First of all, four types of siliques were classified according to their morphologies and seed numbers per silique. Type 1 silique exhibited a fully-developed morphology (Figure 5A and 5B) with ca. 55 seeds per silique (Figure 6A). Type 2 silique displayed a slightly shorter and thinner morphology (Figure 5A and 5C) with ca. 32 seeds per silique (Figure 6A). Type 3 silique had an incompletely-developed morphology with a size drastically reduced to about one-half of the type 1 silique (Figure 5A and 5D) and ca. 20 seeds per silique (Figure 6A). Type 4 silique also showed a highly underdeveloped morphology with size reduced to about one-third of the type 1 silique (Figure 5A and 5E) and ca. 6 seeds per silique (Figure 6A). Types 2, 3 and 4 were grouped together as the incompletely-developed siliques, and the percentage was compared among transgenic T1 *Arabidopsis* plants and nontransformant control. Transgenic YP1 (25.43%), YP2 (23.98%), and YP3 (31.40%) plants contained much higher incompletely developed silique percentages than control (2.19%) (Figure 6B). In addition, transgenic YP1 (57), YP2 (78) and YP3 (57) had significantly fewer siliques per plant than control (99) (Figure 7). The percentages of silique numbers per plant of T1 transgenic *Arabidopsis* were about 60 to 80% that of control. These data demonstrate that *SPAE* gene expression may influence seed and silique development and result in reduced seed number per silique, smaller silique numbers per plant, and a higher percentage of incompletely developed siliques per plant in transgenic *Arabidopsis* plants.

DISCUSSION

In this report, three transgenic *Arabidopsis* plants were isolated and identified with the floret dip transformation method (Clough and Bent, 1998). Genomic PCR and protein gel blot analysis confirmed that these *Arabidopsis* plants (YP1, YP2 and YP3) were transgenic and contained the foreign sweet potato *SPAE* gene in their genomes (Figure 2), which can be expressed as mature protein products (Figure 3). Two bands were detected in transgenic T1 *Arabidopsis* plants. The lower band, with molecular mass between 34 kDa and 40 kDa, has a band position similar to that of the positive control from sweet potato S1 senescent leaves. The putative mature form of *SPAE* after removal of N-terminal and C-terminal prosegments contains 325 amino acids and has a predicted molecular mass ca. 36 kDa (Chen et al., 2004). Therefore, the lower band likely represents the mature form of *SPAE*. The upper band with molecular mass between 40 kDa and 55 kDa was close to the predicted molecular mass of the putative intermediate form of *SPAE*, which contained 442 amino acids after removal of N-terminal prosegment and had a predicted molecular mass ca. 49 kDa. Therefore, the upper band likely represents the intermediate form of *SPAE*. Similar results have also been observed and reported for various plant vacuolar processing enzymes, including *Vigna mungo* VmPE-1 (Okamoto et al., 1999), *Arabidopsis βVPE* (Gruis et al., 2002), *Arabidopsis γVPE* (Kuroyanagi et al., 2002; Rojo et al., 2003). These data also support the conclusion that transgenic *Arabidopsis* plants may contain similar processing mechanisms for correcting sweet potato *SPAE* processing and thus can produce mature sweet potato *SPAE* protein products.

Transgenic *Arabidopsis* plants exhibited an earlier floral transition from vegetative growth and leaf senescence (Figure 4). Early transition from a vegetative phase to the reproductive phase has been reported as a type of senescence. The reasons and mechanisms that allow sweet potato *SPAE* gene expression to promote earlier floral transition and enhance senescence in transgenic *Arabidopsis* plants are not clear. However, Raper et al. (1988) and Rideout et al. (1992) hypothesized that floral transition is stimulated by an imbalance in the relative availability of carbohydrate and nitrogen in the shoot apical meristem. Therefore, whether *SPAE* expression can interfere with the endogenous metabolic balance, which in turn leads to accelerated senescence, awaits further investigation.

Expression of sweet potato *SPAE* in transgenic *Arabidopsis* plants caused reduced seed number per silique, an elevated number of incompletely-developed silique, and smaller silique numbers per plant (Figures 5, 6 and 7). The reasons for the altered phenotypic characteristics in transgenic *Arabidopsis* by sweet potato *SPAE* expression are not clear. However, sweet potato *SPAE* is in close association with the plant vacuolar processing enzymes of seeds from phylogenic analysis (Chen et al., 2004). These enzymes have been implicated in the degradation and mobilisation of the storage proteins called globulins during seed germination and seedling growth in *Phaseolus vulgaris* (Senyuk et al., 1998), *Vigna mungo* (Okamoto et al., 1999), *Vicia sativa* (Schlereth et al., 2000; Schlereth et al., 2001), and *Arabidopsis thaliana* (Gruis et al., 2002). In *Vigna mungo*, *VmPE-1* has been demonstrated to increase in the cotyledons of germinating seeds and was involved in the post-translational processing of a vacuolar cysteine endopeptidase, designated SH-EP, which degraded seed storage proteins (Okamoto and Minamikawa, 1999). Whether it is inappropriate pre-degradation of globulin-type storage protein during seed development and maturation by constitutively expressed sweet potato *SPAE* in transgenic *Arabidopsis* that results in partial repression of seed and silique development and in turn lead to a higher incompletely-developed silique percentage (Figures 5 and 6) and lower silique numbers

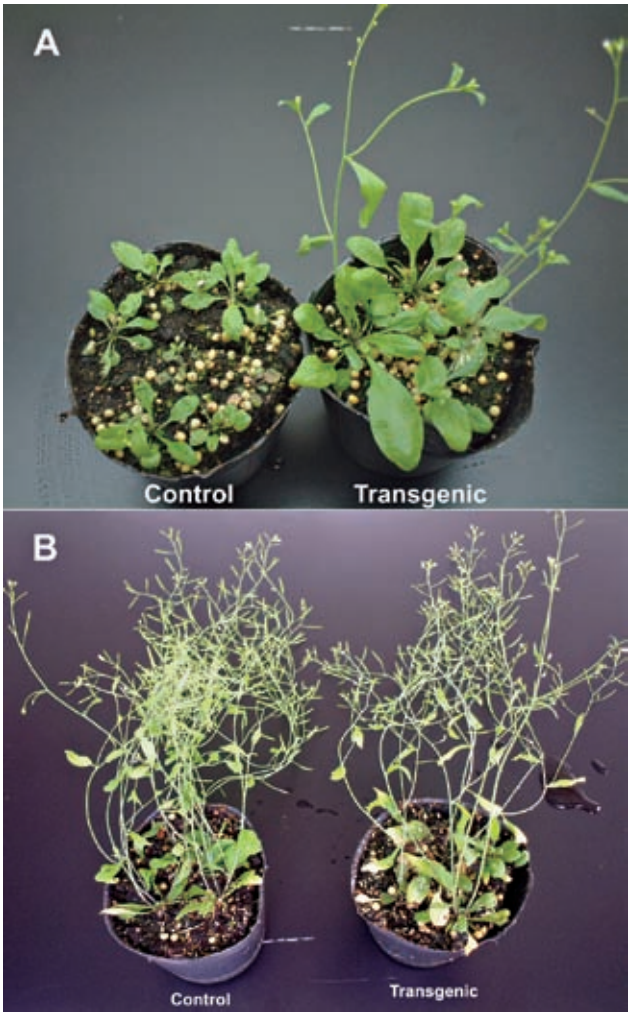

Figure 4. Comparison of growth patterns between nontransformant control and transgenic T1 *Arabidopsis* plants, YP3. A, Floral transition from vegetative growth was observed at ca. 30 to 35 days after seed germination; B, Leaf senescence during seed and silique development was recorded at ca. 6 to 7 weeks after seed germination.

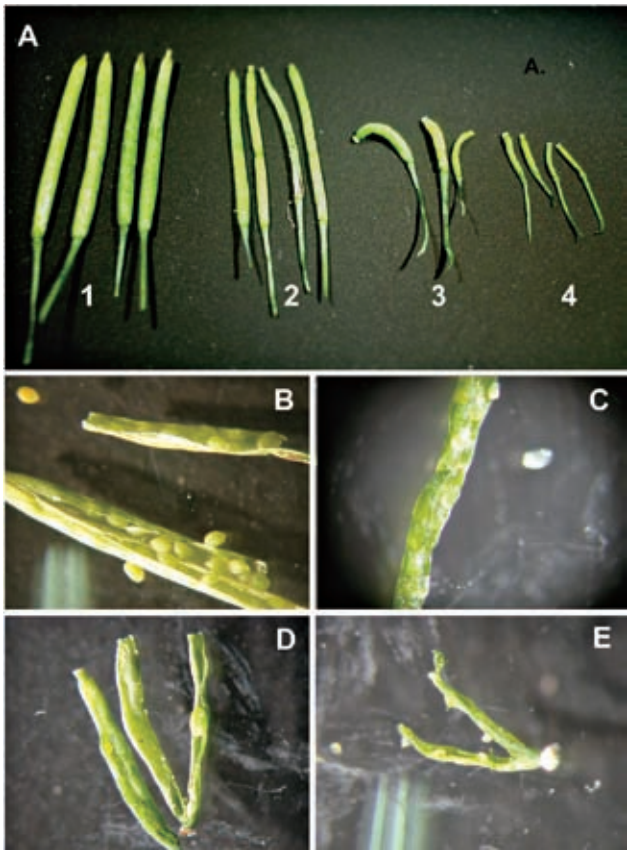

Figure 5. Classification of siliques according to their morphology. A, There are four types of siliques (types 1, 2, 3 and 4) classified; B, Dissection of type 1 silique; C, Dissection of type 2 silique; D, Dissection of type 3 silique; E, Dissection of type 4 silique.

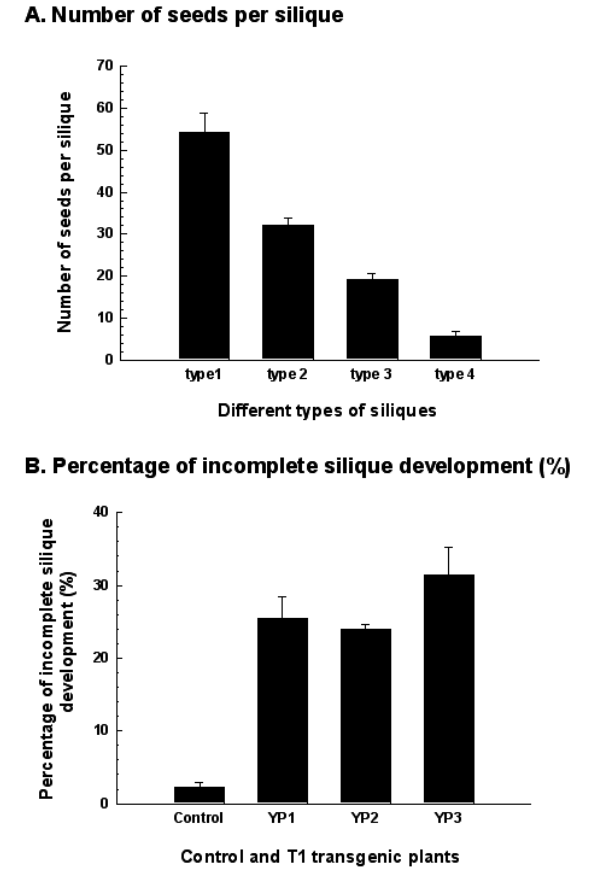

Figure 6. Comparison of seed number per silique among different silique types and incompletely-developed silique percentage between control and transgenic T1 plants. A, Comparison of seed number per silique among different silique types; B, Comparison of incompletely-developed silique percentage among control and transgenic T1 plants. C and YP1/YP2/YP3 denote nontransformant control and transgenic *Arabidopsis* plants, respectively. The data are from the average of five plants per treatment and are shown as mean ± SE.

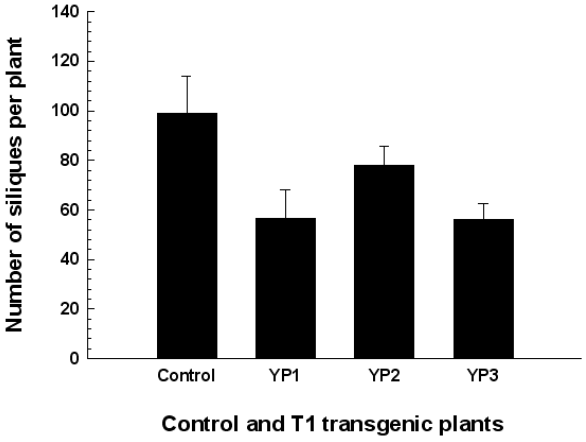

Figure 7. Comparison of silique number per plant among control and transgenic T1 plants. C and YP1/YP2/YP3 denote nontransformant control and transgenic *Arabidopsis* plants, respectively. The data are from the average of five plants per treatment and are shown as mean ± SE.

per plant (Figure 7) awaits further investigation. Based on these results we conclude that sweet potato asparaginyl endopeptidase, *SPAE*, may function in association with the senescence process, and its expression enhances or promotes senescence in transgenic *Arabidopsis* plants.

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甘藷天冬醯胺肽鏈內切酵素的表現造成轉殖阿拉伯芥植株性狀改變

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我們先前從甘藷老化葉片分離出一種天冬醯胺肽鏈內切酵素 asparaginyl endopeptidase，基因表現研究顯示其在天然及誘導的老化葉片中會被活化及增加表現量 (Chen et al., 2004)。本研究報告將全長 *SPAE* cDNA 構築於 pBI121 載體內的 T-DNA 部分，並且受 *CaMV 35S* 啟動子的調控。利用農桿菌感染阿拉伯芥花序的方法進行植株轉殖，轉殖植株利用其抗抗生素 kanamycin 的特性進行篩選並且以 genomic DNA 擴增的方法來確認，西方默點雜交結果證實 *SPAE* 於轉殖植株中有表現。表型分析結果顯示轉殖植株比對照組表現出較早開花及葉片老化，而且平均每株轉殖植株比對照組含較少的長莢果數目及較高的發育不完全果莢百分比。根據這些結果結論甘藷天冬醯氨肽鏈內切酵素 asparaginyl endopeptidase 其功能與老化過程有關，且其基因表現可以增加或促進轉殖植株老化。

關鍵詞：天冬醯胺肽鏈內切酵素；長莢果；*SPAE*；甘藷；轉殖阿拉伯芥植株。