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Hormonal Regulation of Expression of a Gene encoding Pod Storage Protein in Common Bean Plants

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Abstract

Pod storage protein (PSP) accumulates in both developing pods and wounded leaves of common bean (*Phaseolus vulgaris*). Since jasmonic acid (JA) is known to be involved in wound responses, we examined hormonal regulation of *PSP* expression. Methyl jasmonate (MeJA)-induced expression of *PSP* was blocked by both salicylic acid (SA) and ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). The *PSP* promoter fused to β-glucuronidase (GUS) coding region was introduced into *Arabidoposis thaliana* using *Agrobacterium* infection. No MeJA-inducible GUS expression was found in the transformants. The chimeric gene was then introduced into stems of common bean using particle bombardment. Exogenously applied MeJA enhanced GUS activity, and this was prevented by both SA and ACC. Deletion analysis of the promoter indicated that the region between positions-747 and-555 included *cis*-regulatory elements for JA induction and ethylene suppression, and that *cis*-elements for SA-suppression were located in the region downstream of position -86.

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Key words: jasmonic acid, *Phaseolus vulgaris*, pod storage protein, plant wounding responses, promoter.

Abbreviations

ACC, 1-aminocyclopropane-1-carboxylic acid; GUS, β-glucuronidase; JA, jasmonic acid; Luc, luciferase; MeJA, methyl jasmonate; PSP, pod storage protein; SA, salicylic acid; VSP, vegetative storage protein.

Introduction

Damage to plants by herbivorous insects induces many defense genes needed to protect against insects and pathogens invading from the wound (Kessler and Baldwin, 2002). Wounding induces jasmonic acid (JA) biosynthesis (Creelman *et al.*, 1992), which activates transcription of a set of genes. JA produces a systemic response because it is released from the wound into the entire plant via volatile methyl jasmonate (MeJA; Farmer and Ryan, 1990). Wounding also induces formation of ethylene, which upregulates defense-related genes including chitinase and β-1,3-glucanase (Beerhues and Kombrink, 1994). In addition, ethylene is known to regulate transcription of some JA-inducible genes synergistically or antagonistically. In *Arabidopsis* plants, expression of a defensin gene, *PDF1.2*, is induced by both JA and ethylene (Penninckx *et al.*, 1998). Expression of a gene for the *Arabidopsis thaliana* vegetative storage protein (VSP), *AtVSP*, was induced by JA (Berger *et al.*, 1995), and its induction was suppressed by ethylene (Rojo *et al.*, 1999). Salicylic acid (SA) is known to induce expression of pathogenesis-related proteins (Raskin, 1992) and to inhibit wound-induced JA production and JA-elicited gene expression (Engeberth *et al.*, 2001, Manners *et al.*, 1998). Genetic analysis of *Arabidopsis* mutants showed that SA suppresses JA-induced genes by cross-talk be-

tween SA and JA signaling pathways (Glazebrook, 1999; Raymond and Farmer, 1998).

We found that pod removal enhanced accumulation of a 28-kD protein in the pods that formed subsequently (Zhong *et al.*, 1997). We previously postulated that excess nutrients - due to the removal of the pod as a sink - were later transported to the new pods, and that pod removal induced the synthesis of this protein as a temporary reserve of such nutrients. This protein was designated pod storage protein (PSP), and its corresponding cDNA was cloned and sequenced. RNA blotting using the cDNA as a probe showed that the increase in PSP in the pods of depodded plants was regulated at the level of mRNA abundance. The deduced amino acid sequence of the PSP cDNA exhibited 65-71% identity with those of VSPs from soybean (Mason *et al.*, 1988; Staswick, 1988). It is known that expression of the *VSP* genes is stimulated by wounding and JA (Staswick, 1994), and we showed that PSP accumulation in leaves was also induced by wounding and MeJA, with the accumulation being regulated by its mRNA level (Zhong *et al.*, 1999).

In the present study, we investigated PSP accumulation in wounded leaves, and examined the effects of JA, ethylene and SA on expression of *PSP* in leaves. We also cloned genomic clones for *PSP* and determined its sequence. The promoter region fused to β-glucuronidase (GUS) reporter gene was introduced into *Arabidopsis thaliana* plants, and the GUS expression in transgenic plants examined. We also performed deletion analysis of the *PSP* promoter fused to the GUS reporter gene in common bean seedlings.

Materials and Methods

Plant materials

Dry seeds of common bean (*Phaseolus vulgaris* L. cv Goldstar) were purchased from Sakata Seed (Yokohama, Japan). Common bean plants were grown in a phytotron (Koitotron, Koito, Yokohama, Japan) under 13 h of light (190 μE s^{-1} m^{-2}) and 11 h of darkness daily at 27℃. For extraction of protein and RNA, 2-cm-diameter leaf disks were cut from the leaf of the plants, frozen immediately in liquid nitrogen, and then stored at -20℃ or -80℃ until use. *Arabidopsis thaliana* (L.) Heynh. ecotype Colombia was used as the host for transformation. Germination was induced by a 48-h incubation in darkness at 4℃ followed by white light at 21℃.

Extraction of proteins

All manipulations were performed at 0-4℃. Leaf disks were powdered with liquid nitrogen, and each leaf disk was homogenized with 1 ml of 50 mM sodium phosphate (pH 7.2) and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 20000 × *g* for 10 min, and the resultant supernatant was used as a crude extract. Protein contents were measured by a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

SDS-PAGE and protein blotting

SDS-PAGE was performed as described in Laemmli (1970), and the immunoblot method used followed Zhong *et al.* (1997).

RNA extraction and RNA blot analysis

Preparation of total RNA from plant tissues and RNA blot analysis were performed as described in Zhong *et al.* (1997).

Construction of genomic library and screening of genomic clones for the PSP gene

The DNA from leaves of bean seedlings was isolated as described in Yamauchi *et al.* (1989). The DNA digested with *Mbo*I was size-fractionated through a 10-40% sucrose gradient. The DNA fragments, 10-20 kb in length, were ligated to the EMBL3-*Bam*HI-digested arm (Stratagene, La Jolla, CA, USA) and packed in vitro. Approximately 5 × 10^5 recombinant bacteriophages were obtained and amplified. This amplified library was screened by ^{32}P-labeled cDNA for PSP and subclones were constructed in pBluescriptII (Stratagene). The sequences of the clones were determined by an automated DNA sequencer (ABI Model 310, PE Applied Biosystems, Foster City, CA, USA).

Determination of transcription start sites by primer extension analysis

Total RNA was prepared from wounded leaves of common bean plants. Primer extension analysis was performed with the total RNA and the primer corresponding to the sequence from positions +108 to +84 as described in Sakurai *et al.* (1988) except for the reverse transcriptase reaction with ^{32}P-dCTP.

Construction of a T-DNA plasmid and transformation of Arabidopsis thaliana

A fragment of about 0.8kb cut with *Hin*dIII from λgPSP1 was subcloned into pBluescriptII. From this plasmid, a fragment of about 0.8 kb was amplified by PCR using BcaBEST Sequencing Primer M13-20 (Takara Bio, Otsu, Japan) and primer PSP01 (ACTTCAGATCTCACTCACTGCTTCT). After the resulting product was digested with restriction enzymes *Hin*dIII and *Bgl*II, the fragment was ligated to vector pBI101 (Jefferson *et al.*, 1987)

digested with *Hin*dIII and *Bam*HI. The resulting plasmid (designated pBIPSP1) was introduced into *Agrobacterium tumefaciens* (strain EHA101) by electroporation. Transformation of *Arabidopsis thaliana* was performed by the floral dip method (Clough and Bent, 1998), and transgenic plants were selected on the basis of their kanamycin resistance. GUS activity in plants was detected with X-Gluc as described in Yamauchi *et al.* (1996).

Construction of plasmids for a transient assay of the PSP promoter

PSP promoter (approximately 0.8 kb) was amplified by PCR as described above. The amplified fragment, digested with restriction enzymes *Hin*dIII and *Bgl*II, was ligated to vector pBI221 (Jefferson *et al.*, 1987) digested with *Hin*dIII and *Bam*HI, and the resulting plasmid was designated pPSP-748. Deletion mutants were constructed using PCR with primer PSP01 and a primer for the creation of the *Hin*dIII site.

Particle bombardment

We introduced reporter plasmids into common bean seedlings using a biolistic device (IDERA III, Tanaka, Sapporo, Japan). To normalize the GUS activity, pDO432 (Ow *et al.*, 1986), in which the CaMV35S promoter is fused to the luciferase (Luc) gene, was used as an internal control. All plasmids were mixed at a molar ratio of 3:1 (reporter:internal control). Reporters and effectors were introduced into 2-week-old seedlings by particle bombardment as described in Yamauchi (1997). After the bombardment, the seedlings were incubated in shooting buffer [50 mM sodium succinate (pH 5.5) and 50 mM $CaCl_2$] at room temperature with shaking for 24 h. Enzyme activity was measured as described in Sutoh and Yamauchi (2003).

Wounding and application of chemicals

Wounding and the application of chemicals were performed on plants with a sixth leaf as described in Zhong *et al.* (1999). The third leaf was wounded by cutting at 20 positions with a razor blade. Half-diluted Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 0.05% (w/v) Tween 20 was used as a chemical solvent. The third, fourth and fifth leaves in the seedling were immersed in the solution in a plastic bag for 5 seconds. For analysis of the response against MeJA of transgenic *Arabidopsis*, 2-week-old seedlings were transferred to MS medium containing 3% sucrose and 0.8% agar in the presence or absence of 20 μM MeJA, and then incubated for 24 h.

Results and Discussion

Wound-inducible synthesis of PSP

For analysis of wound-inducible expression of *PSP*, leaves of common bean plants were incised with a razor blade. Wounding of the fourth leaf increased the amount of PSP in that leaf after 4 h, and this continued to increase until 48 h (**Fig. 1**). In contrast, the PSP mRNA level peaked at 24 h. This

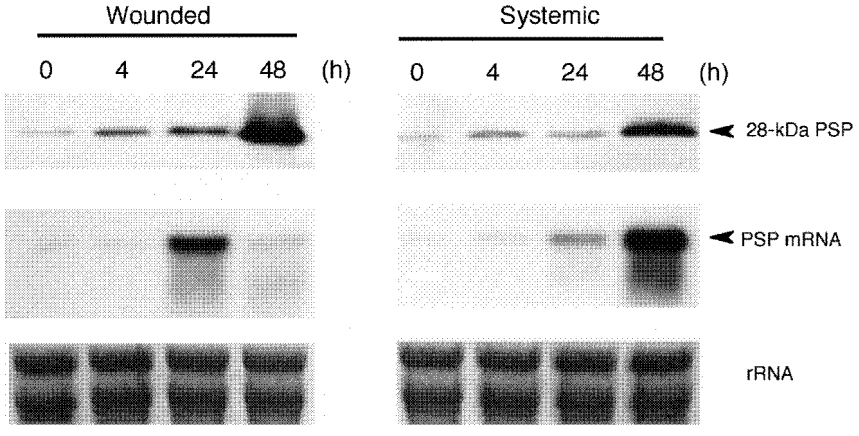

Fig. 1 Wound-inducible expression of *PSP* in common bean leaves. Top panels, immunological detection of PSP; middle panels, RNA blot analysis of PSP mRNA; bottom panels, rRNA stained with methylene blue. Numbers indicate hours after wounding. Each sample was prepared from three different plants. "Wounded" indicates the wounded fourth leaf, and "Systemic" indicates the sixth leaf from each wounded plant. Extracts (30 μg protein) were separated by 12.5% SDS-PAGE followed by immunoblotting using the antiserum against PSP. Total RNA (10 μg) was glyoxylated and separated by electrophoresis on a 1.4% agarose gel, blotted onto a nylon filter, and hybridized with the ^{32}P-labeled cDNA for PSP. Arrowheads in the top and middle panels indicate 28-kDa PSP and PSP mRNA, respectively.

increase preceded that in the amount of protein. The systemic effect of wounding the fourth leaf on temporal changes in the amount of PSP and its mRNA level was also examined by monitoring changes in the sixth leaf. The amount of PSP increased slightly after 4 h and vigorously from 24 h to 48 h. The PSP mRNA level increased 24 h after wounding, and this increase continued up to 48 h. Increases in the amount of PSP and its mRNA level in the systemic leaf followed changes in the wounded leaf.

Hormonal regulation of expression of PSP

The wounding of plants is known to induce JA biosynthesis, which in turn induces the expression of wound-responsive genes. Application of MeJA (10 μM) to leaves of common bean plants enhanced the accumulation of PSP and its mRNA (**Fig. 2**). We examined the effects of SA and ethylene on this MeJA-induced expression of *PSP*. Application of SA (1 mM) suppressed the accumulation of PSP and its mRNA induced by MeJA. Application of 1-aminocyclopropane-1-carboxylic acid (ACC, 1 mM), the immediate precursor of ethylene, onto leaves prevented the MeJA-induced increases in PSP and its mRNA.

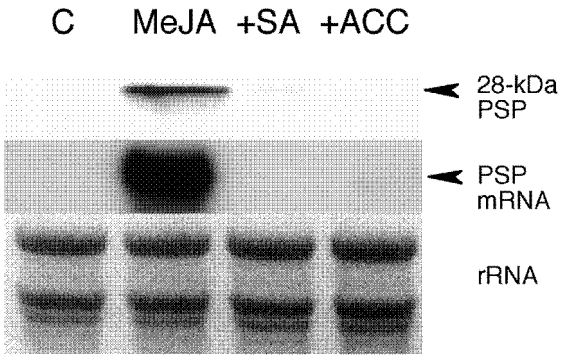

Fig. 2 The effect of application of SA and ACC on expression of PSP induced by MeJA. Top panel, immunological detection of PSP; middle panel, RNA blot analysis of PSP mRNA; bottom panel, rRNA stained with methylene blue. Lane C, leaves treated with 1/2MS medium and 0.05% Tween 20; lane MeJA, leaves treated with 10 μM MeJA solution; lane +SA, leaves treated with a solution containing 10 μM MeJA and 1 mM SA; lane +ACC, leaves treated with a solution containing 10 μM MeJA and 1 mM ACC. Leaves were collected 24 h after treatment. Immunoblotting and RNA blotting were performed as described in the legend of Fig. 1. Arrowheads in the top and middle panels indicate 28-kDa PSP and PSP mRNA, respectively.

Gene organization

To determine the sequence of *PSP*, two clones were obtained from the genomic library of common bean, and their restriction maps were compared to that of the genomic clone obtained by PCR (accession no. AB000585). The maps of the two clones indicated that one clone, designated λgPSP1, contained the 5'-upstream region and the first exon, and that the other clone, λgPSP2, included the second and third exons and the 3'-downstream region (**Fig. 3**). Primer extension analysis was performed to determine the transcription start sites of *PSP*: the two sites detected were 102 bp and 83 bp upstream from the initiation codon (data not shown), the first of which was desiganted +1 (**Fig. 4**). Because the two sequences TAAAAAT and GATAAT are located at 34 bp and 30 bp upstream from each of the transcription start sites, respectively, they likely correspond to TATA boxes. Sequencing indicated that *PSP* consists of three exons 479 bp, 123 bp and 323 bp in length, and two introns 293 bp and 273 bp in length.

Comparison of the promoter regions between PSP-related genes

There is little information on *cis*-acting elements and *trans*-acting factors involved in the JA response. A short inverted repeat (CGTCA/TGACG) was found to be involved in the JA response of a lipoxygenase 1 gene from barley (Rouster *et al.*, 1997). Another short inverted repeat (AGCAC/GTGCT) was shown to be JA-responsive in *AtVSP1* (Guerineau *et al.*, 2003). However, these inverted repeats were not found in the *PSP* promoter. The JA-responsive element was determined as a GCC motif in the promoter region of the strictosidine synthase gene (*Str* of *Catharanthus roseus*) which interacts with the transcription factor ORCA2 (Menke *et al.*, 1999). Overexpression of ORCA2 activates the transcription of *Str* without JA. A JA-responsive element in the *Arabidopsis PDF1.2* promoter was identified as a GCC box (Brown *et al.*, 2003) however, the GCC motif was not found in the promoter region of *PSP*.

Figure 5 shows a comparison of the promoter regions between*PSP* and *VSP* genes from soybean (*VspA, VspB*; Rhee and Staswick, 1992a, b) and *Arabidopsis* (Utsugi *et al.*, 1998). Three putative regulatory elements - W-box, G-box, and I-box - were found in these regions. A W-box is known to be an elicitor response element, which interacts with the transcription factor WRKY (Rushton *et al.*, 1996) however, it is unclear whether elicitors induce expression of *VSP* genes. A G-box is known to interact with bZIP transcription factors and to

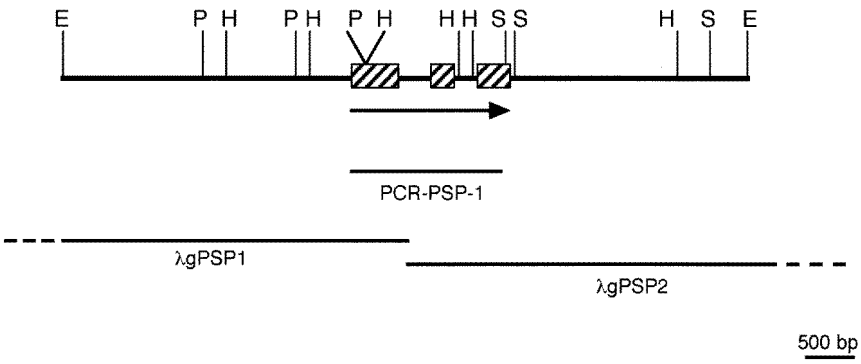

Fig. 3 Structure of *PSP* showing the restriction map of the *Eco*RI-digested fragment. The shaded boxes indicate the regions of introns. The arrow under the map denotes the direction of transcription. A genomic clone, named PCR-PSP-1, and two λ clones, λgPSP1 and λgPSP2, are aligned under the map. Restriction enzyme sites are labeled as follows: E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Spe*I.

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-747 AAGCTTTTAAGTGATGTTATTTATTAGAGATTCATTATTACCGTACACCACCAATTTTTCAAAACTAGCA

-677 AATAATAACTGATTTTTGATAATAAATAAATTATTTAATACAAATAATTGCAGAATACTTATACTTATTA

-607 TTTTTAAAGGGTCCTAATATTTTTAACGAAAATTATATTTGAAAAGTAAAACATATTCACGTCACAATAA
                                                    G-box
-537 AATATTTAAAATTAAAAAATAAACTAAATACAACTAAAATATTAACTTTTGAATTGTTAAGTGTTGGAGA

-467 TGTCACATGAACTAAAGAGTAGAACATTTATATATAAGTGAATATAAACTTTATTTTATAAGTTCGGTTT

-397 TATAAGGTTGAGTTAAACTTATTAAAGTCAATTTTTTATAATATGTTATCATAATCATTCTGAAGTTTAT
                           W-box
-327 CCTAACGATTATTTATTGGGTTTATCAGGTCACCTGCTATCGAATTGTTACTGAATCACCTATAATATAT

-257 ACTCTCAGACATAAGTTGGTAATATCGACGTGAGCAAGTGGTGGAGATTACACATATAATATGAACAACA
                           G-box
-187 TATATTATTGAACTTATCCGGTCATTCGCTATCGAATTGTTACCGAATCACCGGTGTGAGAAACAAGTTG
                I-box
-117 ATAATATCTAGGTGAGAAAGTGATGGAGATTACACATATGATGCACGTACAAGATTGAAAAAGTAAACTA
                                                G-box
 -47 AATTATTGGAGTGTAAAAATTAGGTTACAGATGGCAGATAATTTATGGTGGAGGTACAGAGGCAGCGTGT
                TATA box                TATA box
+24 ATTTAAGGATGCAAAGCATGGAGGAAGAAGATGCAGAAGGTAGTTGAGATAAGAAGAAGAAGAAGCAGTG

+94 AGTGAGTTATGAAGTGCCTCGTGTTCTTTGTTGCTGCAGTTTTGGTGGCATCTCAATGCCATGGCGCTTC
          M  K  C  L  V  F  F  V  S  A  V  L  V  A  S  Q  C  H  G  A  S
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Fig. 4 Nucleotide sequence of the promoter region of *PSP*. Nucleotides are numbered from the transcription start sites (shown by open circles). Filled circles indicate ends of the promoter regions for deletion constructs. Putative transcriptional regulatory elements are underlined.

function as a coupling element with another *cis*-element involved in ABA induction (Hobo *et al.*, 1999) and light responses (Schindler *et al.*, 1992). The region between positions -585 and -535 of the *VspB* promoter confers MeJA responsiveness (Mason *et al.*, 1993). Because this region of *VspB* promoter contains a G-box and a C-rich sequence, it is possible that both sequences cooperatively regulate the MeJA response. However, the sequence corresponding to the C-rich sequence was not found in the *PSP* promoter. An I-box is known to be involved in light regulation and the circadian-clock regulation of a gene for an *Arabidopsis* chlorophyll a/b binding protein (Rose *et al.*, 1999). Carbohydrate is synthesized and accumulates in leaves during the daytime, to be transported from leaves to sink organs during the nighttime, and a starch-biosynthesis gene is also regulated by the circadian clock (Mérida *et al.*, 1999). Therefore, if the circadian clock regulates expression of *PSP*, that acts as a temporary storage protein, this regulation may involve the I-box in the promoter.

Analysis of PSP promoter in transgenic Arabidopsis plants

To investigate the spatial and hormonal regulation of the *PSP* promoter, we introduced GUS reporter gene fusions into *Arabidopsis thaliana* and per-

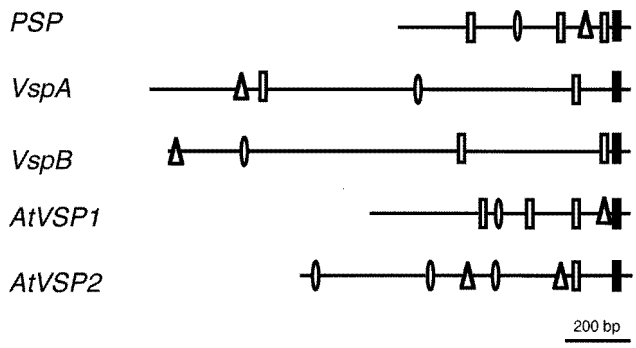

Fig. 5 Comparison of the promoter regions among *PSP* and *VSP* genes. The schematic representation was derived from the promoter region of the following sources: *PSP* from common bean, *VspA* and *VspB* from soybean (Rhee and Staswick, 1992a, b), and *AtVSP1* and *AtVSP2* from *Arabidopsis thaliana* (Utsugi et al., 1998). Symbols indicate the following structures: ellipse, W-box; triangle, I-box; open box, G-box; filled box, TATA box.

formed histochemical analysis of the transgenic plants. We obtained six independent transformants, designated L1-L6, of which transformants L1-L5 contained a single copy of the chimeric GUS gene as identified on the basis of their kanamycin resistance. In the seedlings before flowering, GUS activity was detected in lateral root primordia of all the transformants (**Fig. 6A**). This activity was also detected in trichomes of young leaves in four of the lines (L1, L2, L4, and L5; **Fig. 6B**): in flowering plants, GUS activity was also detected in the stigmata of these transformants (**Fig. 6C**). GUS activity appeared in immature siliques of transformants L5 and L6, but disappeared in mature siliques (**Fig. 6D, E**). The expression of *AtVSP* genes was induced in seedlings by exogenously applied MeJA (Benedetti *et al.* 1995), however, the application of MeJA to the seedlings of these transformants did not change GUS activity (data not shown). Furthermore, wounding did not affect the pattern of GUS activity in the seedlings and did not induce GUS activity in the leaves (data not shown). Therefore, we presume that the responses against wounding and JA were absent in these transgenic *Arabidopsis* plants.

Previous immunoblot and RNA blot analyses have indicated that *PSP* is expressed in young leaves, flowers, and pods of common bean plants (Zhong *et al.*, 1999). In addition, the removal of pods induce PSP accumulation in roots. The seedlings of these transformants were grown on a medium including 3% sucrose, which may have affected the GUS activity in the roots during these previous experiments. Thus, the pattern of expression of *PSP* in common bean and *Arabidopsis thaliana* was similar, except that GUS activity was detected in the roots of all the transgenic *Arabidopsis* plants. However, exogenously applied MeJA did not increase GUS activity in the seedlings of the transformants.

Analysis of PSP promoter with transient expression in common bean seedlings

To examine whether the promoter region from -747 to +100 contained *cis*-acting elements involved in the MeJA response, we constructed *PSP* promoter:GUS fusions and introduced it into stems of common bean seedlings using particle bombardment (**Fig. 7**). Although biolistics physically damages the organ, MeJA still enhanced the activity 1.65-fold relative to that of the control. Exogenously applied SA and ACC decreased the activities by 41% and 58%, respectively, as compared with incubation with MeJA alone. The region from -747 to +100, including the *cis*-acting elements is involved in activation by MeJA and suppression by both SA and ethylene.

The promoter of *AtVSP1* has been analyzed by both transgenic *Arabidopsis* and a transient assay with tobacco protoplasts (Guerineau *et al.*, 2003). Lower induction factors were detected in transient assays than in the *Arabidopsis* transformants. This discrepancy is probably attributable to an activation of *AtVSP1*-GUS expression in protoplasts, possibly following the release of JA by plant cells during protoplast isolation. The reason for the low induction shown in **Fig. 7** may be similar. JA released from the cells damaged by particle bombardment increased the GUS activity of pPSP-747. However, JA enhanced gene expression, and hence deletion analyses were performed to define the region containing regulatory sequences.

Deletion analysis of PSP promoter

Deletion of the region between -747 and -555 increased GUS activity as compared to -747 (**Fig. 7**). In addition, this deletion removed both the enhancement by MeJA and suppression by ACC. Deletion to position -400 decreased GUS activity but not the response against MeJA, and both ACC and SA suppressed GUS activity. Deletion to position -237 increased GUS activity without MeJA. Moreover, MeJA suppressed GUS activity from pPSP-237, and its suppression was lost by exogenously applied ACC. Deletion to position -86 also produced high GUS activity without MeJA and suppression by MeJA. In addition, ACC suppressed GUS activity of pPSP-86 in the presence of

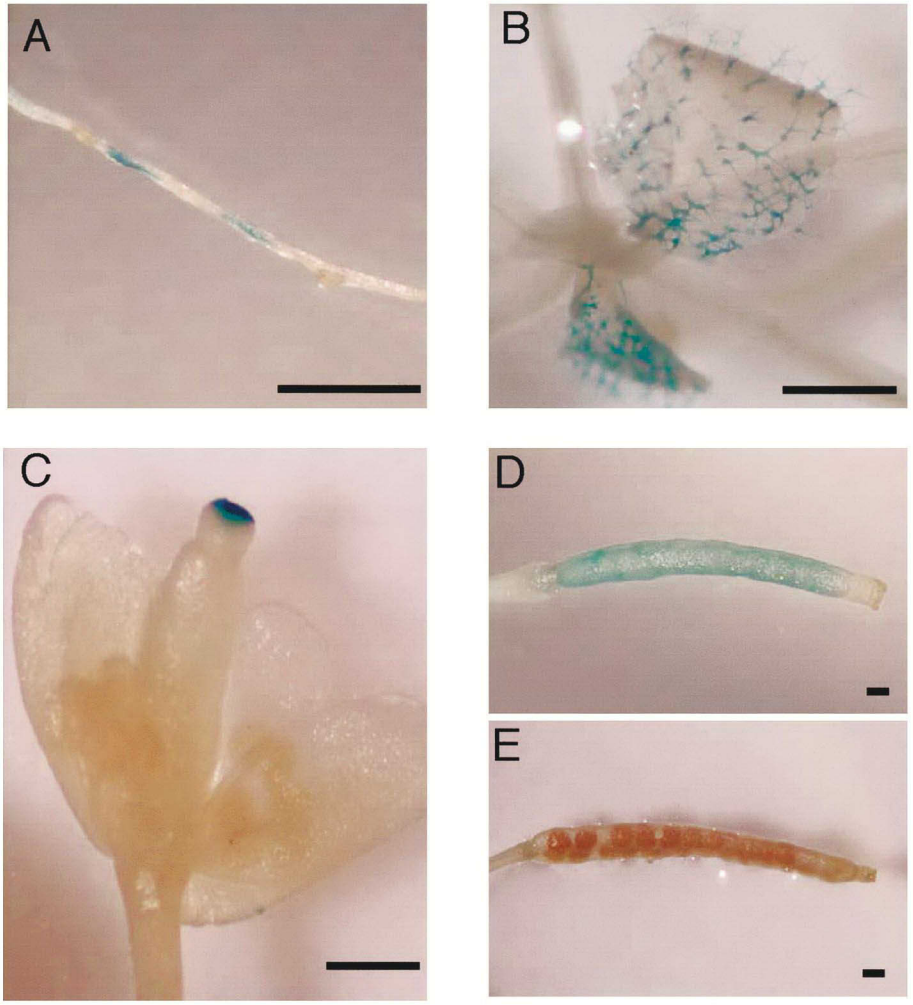

Fig. 6 GUS expression derived from the promoter region of *PSP* in transgenic *Arabidopsis* plants. GUS activity was detected with X-Gluc as a substrate : (A) root of seedling of transformant L6 ; (B) young leaves of seedlings of transformant L1 ; (C) flower of transformant L1 ; (D) immature silique of transformant L5 ; (E) mature silique of transformant L5. Scale bars, 0.5 mm.

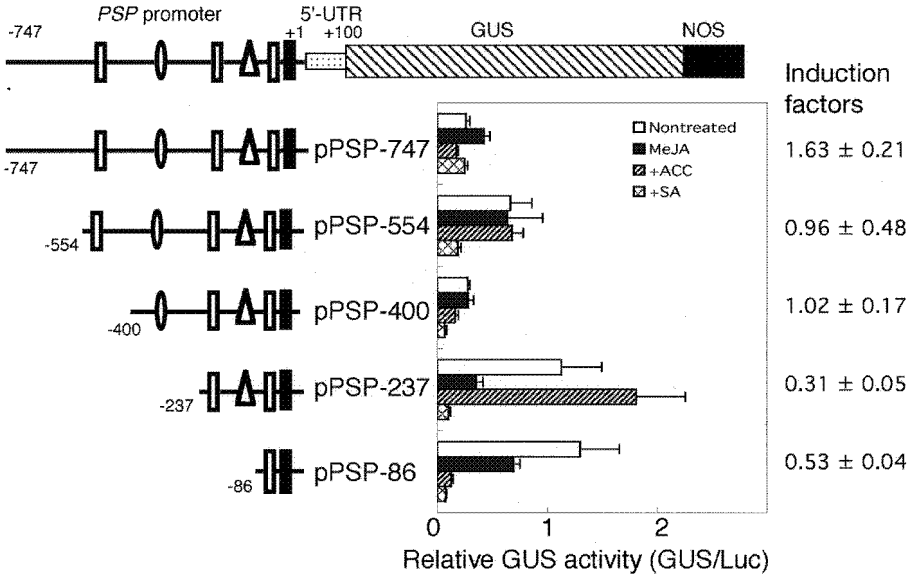

Fig. 7 Deletion analysis of the promoter region of *PSP* using the transient assay. A schematic of the forefather construct, pPSP-747, is shown at the top, which indicates the of *PSP* promoter (*PSP* promoter), the 5'-untranslated region (5'-UTR) from +1 to +100, GUS coding region (GUS), and terminator of nopaline synthase gene (NOS). The symbols in *PSP* promoter region are as in **Fig. 5**. The numbers to the left of the construct diagrams indicate the 5' end of each deletion, results of transient expression experiments with different constructs are shown in the middle, and MeJA induction factors are indicated on the right. For estimation of transformation efficiency, luciferase (Luc) reporter construct pDO432 was cobombarded with each deletion construct. After bombardment, the stems were incubated in shooting buffer for 24 h without plant hormones (Nontreated), with 10 mM MeJA (MeJA), 10 μM MeJA and 1 mM ACC (+ACC), or 10 μM MeJA and 1 mM SA (+SA). Relative GUS activity was calculated by dividing the GUS to Luc ratio from each construct by that obtained from pBI221. Induction factor was calculated by dividing GUS activity incubated with MeJA by that without hormone. Bars indicate means and standard errors of the GUS/Luc activities (n=4).

MeJA, and exogenously applied SA lowered GUS activities in all of the constructs. These results suggest that the *cis*-elements for MeJA enhancement of expression of PSP are located between -747 and -555, which includes the *cis*-element for ethylene suppression, and that the region downstream of position -86 contains the *cis*-element for the suppression by SA. Our results suggest that the transcription factors involved in JA and ethylene signaling differ from those involved in SA signaling, and that ethylene affects signal transduction from JA to transcription of *PSP*.

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