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Wound-response regulation of the sweet potato *sporamin* gene promoter region

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Abstract

Sporamin, a tuberous storage protein of sweet potato, was systemically expressed in leaves and stems by wound stimulation. In an effort to demonstrate the regulatory mechanism of wound response on the *sporamin* gene, a 1.25 kb *sporamin* promoter was isolated for studying the wound-induced signal transduction. Two wound response-like elements, a G box-like element and a GCC core-like sequence were found in this promoter. A construct containing the *sporamin* promoter fused to a *β-glucuronidase* (*GUS*) gene was transferred into tobacco plants by *Agrobacterium*-mediated transformation. The wound-induced high level of GUS activity was observed in stems and leaves of transgenic tobacco, but not in roots. This expression pattern was similar to that of the *sporamin* gene in sweet potatoes. Exogenous application of methyl jasmonate (MeJA) activated the *sporamin* promoter in leaves and stems of sweet potato and transgenic tobacco plants. A competitive inhibitor of ethylene (2,5-norbornadiene; NBD) down-regulated the effect of MeJA on *sporamin* gene expression. In contrast, salicylic acid (SA), an inhibitor of the octadecanoid pathway, strongly suppressed the *sporamin* promoter function that was stimulated by wound and MeJA treatments. In conclusion, wound-response expression of the *sporamin* gene in aerial parts of plants is regulated by the octadecanoid signal pathway.

Abbreviations: GUS, β-glucuronidase; MeJA, methyl jasmonate; NBD, 2,5-norbornadiene; SA, salicylic acid; ABA, abscisic acid

Introduction

Sporamin is the most abundant protein in the tuberous roots of sweet potato and is thought to be a storage protein that functions as a nutritional resource for tuberous root germination (Maeshima *et al*., 1985; Hattori *et al*., 1989). On the other hand, sporamin was found to strongly inhibit trypsin activity (Yeh *et al*., 1997a), and insect-defense capabilities were confirmed in insect bioassays with transgenic tobacco (Yeh *et al*., 1997b) and cauliflower (Ding *et al*., 1998). These results suggested that sporamin functions not only as a storage protein for nutrient supply but also as a factor against herbivore attacks. Thus far, at least ten sweet potato *sporamin* genes have been isolated and characterized (Hattori *et al*., 1989; Chen *et al*., 1997). These genes belong to a large multigene family that is divided into subfamilies A and B. There is over 90% nucleotide homology among intra-subfamily genes, and about 80% nucleotide homology among inter-subfamily genes (Hattori *et al*., 1989).

In field-grown plants, sporamin was shown to be strongly associated with tuberous roots, with only very low amounts produced in stems, and almost none in leaves (Maeshima *et al*., 1985; Chen *et al*., 1997). However, large amounts of sporamin were accumulated in leaf-petiole cuttings under high concentrations of sucrose, glucose or fructose cultured conditions

(Hattori *et al*., 1990; Ohto *et al*., 1992). A 0.96 kb *sporamin* promoter of the *gSPO-A1* gene coding for a subfamily A-sporamin has been characterized. Sucrose-induced β-glucuronidase (GUS) activity in transgenic tobacco containing *gSPO-A1* promoter/*GUS* fusion gene presented a distinct spatial pattern, where GUS activity was predominantly observed in stems. Furthermore, strong activity was detected in the internal phloem of the vascular system, nodes and, especially, at the base of the axillary buds. In addition, weak activity was detected in pith parenchyma cells (Hattori *et al*., 1990; Ohta *et al*., 1991).

Effects of osmotic stress on the activity of the *sporamin* promoter has been thoroughly investigated (Ohta *et al*., 1991; Ohto *et al*., 1992). Recently, it was reported that wounding could induce systemic expressions of this gene in leaves (Yeh *et al*., 1997a). In this study, we confirmed the wound-response expression of the *sporamin* gene and studied the signal transduction in sweet potato plants. In order to further investigate wound modulation on the *sporamin* promoter, a 1.25 kb 5′-flanking DNA fragment of the *sporamin* gene was isolated with an improved genome walking method. The promoter sequence was then fused with a *GUS* reporter gene, and the construct was delivered into tobacco plants by *Agrobacterium tumefaciens*-mediated transformation. We investigated the wound activation process of the *sporamin* promoter in a variety of transgenic tobacco plant vegetative tissues, including leaves, stems and roots, in which little or no *sporamin* promoter activity was detected under normal conditions. We also examined the role of wound-signaling compounds, such as methyl jasmonate (MeJA), salicylic acid (SA), abscisic acid (ABA) and ethylene in regulation of the wound-response *sporamin* promoter. Finally, the characteristics of the wound signal transduction pathway for *sporamin* genes are discussed.

Materials and methods

Plant materials and growing conditions

Sweet potato (*Ipomoea batatas* cv. Tainong 57) plants were grown in pots at 28 °C in 16 h light (2000 lux)/8 h dark (16 L/8 D). Tobacco (*Nicotiana tabacum* cv. W38) plants were transformed with *Agrobacterium tumefaciens* LBA4404 by using the leaf-disk method (Hörsch *et al*., 1985). Primary transformants (T_0) harboring the 1.25 kb sweet potato *sporamin* promoter/*GUS* fusion gene were selected on MS agar medium (Murashige and Skoog, 1962) containing 200 μg/ml kanamycin. Then they were transferred to pots and grown in 16 L/8 D at 28 °C. Two independently transformed lines, Tspoa11 and Tspoa37, were selected as representatives to study *sporamin* promoter-driven expressions. Seeds from the two lines were germinated on MS selection medium, and grown for 2 weeks at 28 °C in 16 L/8 D. The young seedlings (about 3 cm in height) were used in various treatments to study wound-response activation.

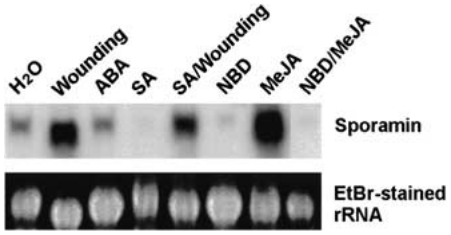

Figure 1. Effects of various wound signal compounds on s*poramin* gene expression in sweet potato leaves. Leaves of sweet potato were wounded after leaf-petiole cuttings were cultured in H_2O for 3 days. Exogenous ABA (0.1 mM), SA (0.2 mM), NBD (2.5 ml/l; v/v) and MeJA (0.05 mM) were applied to cultured solutions, respectively. SA/wounding, wounding treatment for 12 h after SA pretreatment for 30 min. NBD/MeJA, leaf-petiole cuttings were pretreated with NBD for 30 min before MeJA treatment for 12 h. RNA was extracted from the leaves after various treatments. Total RNAs (20 μg) were separated on a 1% agarose gel and hybridized with a *sporamin* cDNA probe.

DNA cloning and sequence

A 21-mer oligonucleotide, GSP2, 5′-GAACTTGGG-AGAAAAGCTAAG-3′ (Figure 2), was synthesized based on the *sporamin* genomic clone *gSPOR5-31* (Wang *et al*., 1995). The *Pspoa* fragment, a 1.25 kb of 5′-untranslated region of the *sporamin* gene, was amplified with GSP2 primer from sweet potato genomic DNA using an improved PCR-based genome walking method (Siebert *et al*., 1995). The *Pspoa* DNA fragments were ligated into a pUC19 vector at the *Sma*I site and transformed to *Escherichia coli* XL1 blue. The nucleotide sequence was determined by the dideoxynucleotide termination method by means of ^{35}S-dATP. The promoter sequence was analyzed with the Wisconsin GCG Software Package version 9.0 (Devereux *et al*., 1984). Then, the *Pspoa* DNA fragment was ligated into pBI101 binary vector (Clontech) which contained the *GUS* gene as a reporter. This construct was named pBI101/*Pspoa*.

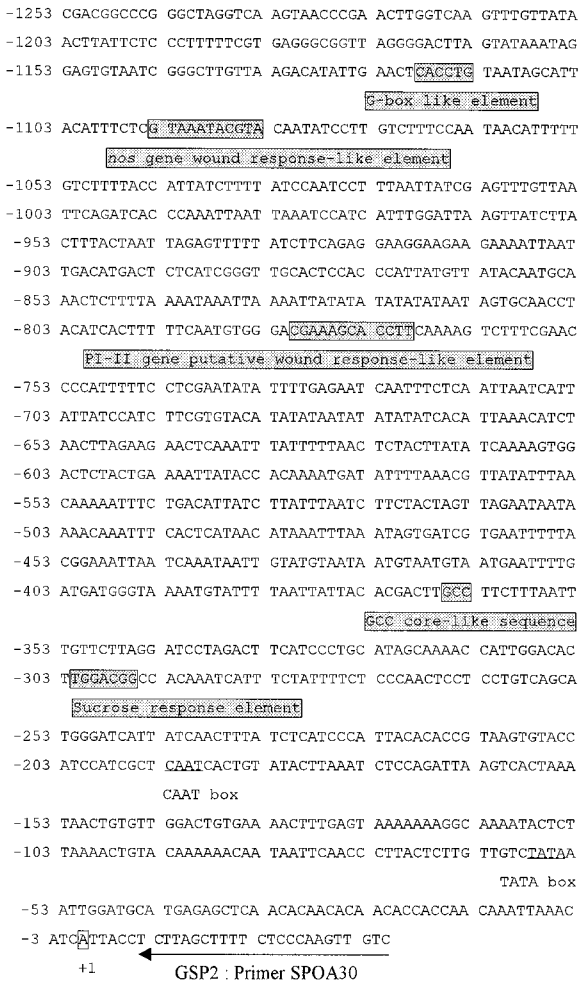

Figure 2. Nucleotide sequence of *sporamin* promoter region, *Pspoa*. The transcriptional start site is labeled +1. The putative TATA and CAAT box were underlined. The sucrose response element, wounding response-like elements, GCC core-like sequence and G box-like element are boxed.

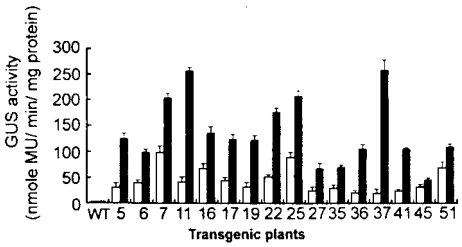

Figure 3. GUS activity of individual transgenic tobacco plants in response to wounding treatment. One middle leaf of mature T_0 tobacco plants, harboring the pBI101/*Pspoa* construct, was injured. Leaves of the injured plant were harvested to determine GUS activity. The number for each transgenic line is indicated. Values are means ± SD of three independently prepared samples. Open bar, GUS activity before wounding. Solid bar, GUS activity after wounding.

Northern blot analysis

The total RNA was extracted by the rapid guanidinum hydrochloride/sarcosinate method that was described by Yeh *et al*. (1991). The ^{32}P-labeled *sporamin* cDNA was used as a hybridization probe.

Wounding treatment

The wounding on plants was carried out as follows. A leaf in the middle section of a mature tobacco (about 30 cm high) or a sweet potato plant (about 15 cm high) was wounded with scissors. Then, 1.5 cm strips of leaf tissue from wounded and unwounded plants were sampled with a razor at the indicated time points, and about 1 cm long sections of stems and roots of mature tobacco were cut for GUS activity assays. For transgenic T_1 generation seedlings, a 1 cm long wound was randomly inflicted on a leaf with forceps.

Application of methyl jasmonate and abscisic acid

Leaf-petiole cuttings of sweet potato were prepared as described by Ohto *et al*. (1992). These cuttings (about 15 cm high) were pre-cultured in H_2O for 3 days and then moved to 50 μM MeJA and 100 μM ABA for 12 h, respectively. On the other hand, prior to fluorometric GUS activity assays of transgenic tobacco, solutions of MeJA (50 μM and 100 μM) or ABA (50 μM and 100 μM) were sprayed onto leaves of mature tobacco (about 30 cm high). Then samples were harvested after being chemically treated for 12 h. Sodium phosphate buffer, pH 7.0, was used as a control solution. For histochemical GUS activity staining on tobacco T_1 generation, seedlings (about 3 cm in height) were treated by immersing the whole plants in solutions for 12 h. To identify the role of ethylene on the MeJA-response pathway in sweet potato leaves, leaf-petiole cuttings were equilibrated with 2,5-norbornadiene (NBD, 2.5 ml/l) for 30 min prior to MeJA treatment for 12 h.

Salicylic acid treatments

Leaf-petiole cuttings of sweet potato were cultured with 200 μM SA for 12 h, and then the *sporamin* gene expression was detected by northern blot analysis. The mature T_0 transgenic tobacco plants (about 30 cm in height) and T_1 seedlings (3 cm) harboring the pBI101/*Pspoa* plasmid were treated with SA solutions (200 μM or 1 mM) by spraying and immersing, respectively. Then the GUS activity was determined.

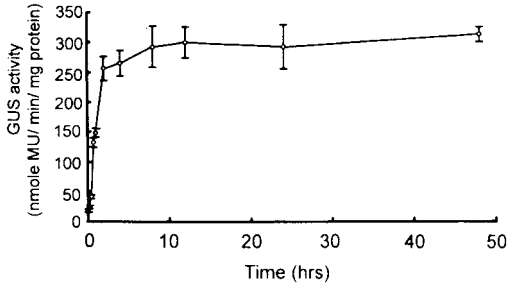

Figure 4. Time course of wound activation of the *sporamin* promoter/*GUS* fusion gene in transgenic tobacco plants (Tspoa37). One middle leaf of mature transgenic tobacco plants harboring the pBI101/*Pspoa* construct was wounded. GUS activity in wounded leaves was measured at indicated time point. Values are means ± SD ($n = 3$).

In order to analyze SA effects on the wound response pathway, leaf-petiole cuttings of sweet potato, mature transgenic T_0 tobacco plants and T_1 tobacco seedlings were treated with SA for 30 min before the leaves were wounded. Then, leaf samples were harvested 12 h after wounding. To determine the role of SA in the signal pathway of MeJA regulations on *sporamin* gene expressions, T_1 seedlings were treated with 200 μM SA before the seedlings were immersed in 50 μM MeJA.

Fluorometric GUS activity assays

Plant tissues were ground into a fine powder using liquid nitrogen with a mortar and pestle, and suspended in GUS extraction buffer (50 mm sodium phosphate buffer pH 7.0, 10 mm Na_2EDTA, 10 mm 2-mercaptoethanol, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100). The supernatant was collected after centrifugation at 12,000 × g for 10 min at 4 °C. Fluorometric assays were performed using 4-methylumbelliferyl-β-D-glucuronide substrate (Jefferson, 1987). The total protein content of the extracts was determined by using the Bradford (1976) method.

Histochemical GUS activity staining

Leaf tissues excised from transgenic mature tobacco plants (T_0) and whole T_1 seedlings were stained for observation of GUS activity by the method described by Jefferson *et al*. (1987).

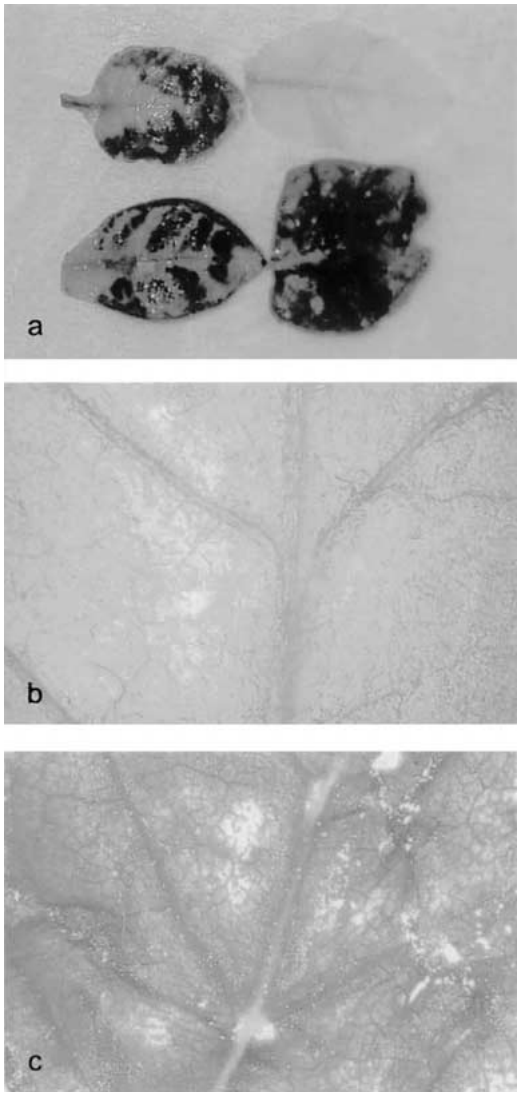

Figure 5. Histochemical GUS staining in wounded and unwounded leaf tissues of transgenic tobacco plants (Tspoa37). a. Leaf harvested under normal conditions (upper right). Unwounded leaves (upper and lower left) and wounded leaf (lower right) harvested from a wound-treated transformant. b. Leaf tissue harvested before wounding. c. Leaf tissue harvested after wounding for 12 h.

Results

Expression patterns of sporamin gene in sweet potato

Induction of the *sporamin* gene expression was observed in sweet potato leaves 12 h after wounding (Figure 1). Then we examined whether the *sporamin* gene expression in non-tuberous plant parts was activated by wound-related transduction signals, such as ABA, SA and MeJA. The data showed that the ex-

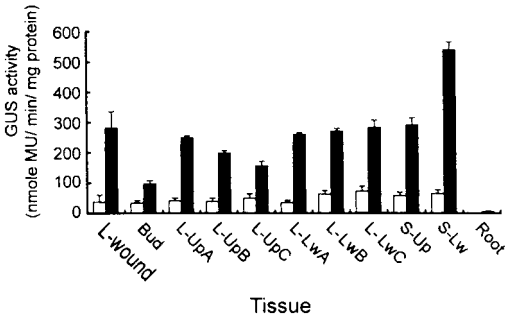

Figure 6. Tissue-specific activity of the *sporamin* promoter in transgenic tobacco plants (Tspoa37). A middle leaf of a mature transgenic tobacco plant (about 30 cm high) was wounded. Tissues were harvested from different parts of plants to determine GUS activity 12 h after wounding. Values are means ± SD ($n = 3$). Open bar, unwounded plant; solid bar, wounded plant; L, leaf; S, stem; Up, upper; Lw, lower. A, B and C indicates positions from tip to base on stems.

pression of the *sporamin* gene was hardly induced by ABA treatment (Figure 1). Treatment with 200 μm SA prior to wounding clearly suppressed gene expression induced by wound treatment (Figure 1). On the other hand, treatment with MeJA (50 μm) enhanced the expression of *sporamin* genes, but the effect of MeJA was inhibited by 0.25% v/v of NBD (Figure 1).

Cloning and characterization of the sporamin promoter

In order to analyze *sporamin* promoter function in response to wound stress, the 1.25 kb 5′ non-coding region of *sporamin* DNA (Figure 2), *Pspoa*, was isolated from the sweet potato genome. The transcriptional start site had been identified and designated as +1 on the *Pspoa* sequence (Figure 2). A sucrose response element 'TGGACGG' was identified on *gSPO-A1* promoter by Hattori *et al.* (1990), and it was also present at −296 to −302 of *Pspoa* (Figure 2). An element, CGAAAGCACCTT (−770 to −781), had 83% identity with the wound-inducible sequence CGTAAGTACCTT on the potato proteinase inhibitor II *(PI-II)* promoter (Palm *et al.*, 1990). Another element, GTAAATACGTA (−1084 to −1094), had 82% identity with the wound-response Z element GCACATACGTA on the nopaline synthase (*nos*) promoter (An *et al.*, 1990). A G box-like element, CACCTG (−1114 to −1119), was similar to a conserved MeJA-responsive domain, CACGTG (Williams *et al.*, 1992; Mason *et al.*, 1993). The GCC core sequence, a jasmonic acid (JA)-responsive element (Menke *et al.*, 1999), was present at −366 on this promoter (Figure 2). Then, the *Pspoa* fragment was ligated to the *GUS* gene. This construct was transformed into tobacco for analyzing the regulation of the *sporamin* promoter.

Tissue-specific expression of sporamin promoter in response to wounding

To determine whether the sweet potato *sporamin* promoter could confer a wound-inducible expression pattern in tobacco, GUS activity was measured in leaves of the tobacco T_0 plants from 16 transformed lines harboring the pBI101/*Pspoa* construct. A high level of GUS activity was observed in Tspoa11 and Tspoa37 transgenic lines (Figure 3). GUS activity was induced within 15 min after wound treatment (Figure 4). Then, the activity continuously increased and reached the maximum level at 12 h (the activity was 12.5-fold higher than that detected at 15 min after injury), and the activity remained at this level until 48 h after wounds were inflicted (Figure 4). In addition, the histochemical staining data showed significant GUS activity in wounded and unwounded leaves that were incubated for 12 h after wounding (Figure 5), and GUS activity increased in systemic leaves (Figure 5a). The highest level of GUS activity in wounded tobacco was observed in the lower stems (Figure 6, S-Lw). In general, GUS activity was 4- to 7-fold higher in leaves of wounded plants than before wound treatment (Figure 6). However, GUS activity was never detected in roots (Figure 6). At 12 h after the leaves were wounded, high GUS activity in the aerial parts of wounded T_1 seedlings of Tspoa37 were detected by histochemical staining (Figure 7b). Thus, the wounding signal activates the *sporamin* promoter only in the stems and leaves, and the response to wound infliction is systemically restricted to aerial plant parts. GUS activity was present in the meristem of both wounded and unwounded plants (Figure 7).

Effects of MeJA and ABA on sporamin promoter in transgenic tobacco

GUS activity of transgenic tobacco increased 37-fold and 20-fold after spraying with 50 μM MeJA and 100 μM MeJA, respectively (Figure 8). A level of 50 μM MeJA induced a great response from the *sporamin* promoter in aerial parts of the plant. The histochemical staining assay revealed that GUS activity was very high in aerial parts of 50 μM MeJA-treated Tspoa37 T_1 seedlings, but non-existent in root regions (Figure 7c). The result of histochemical staining on

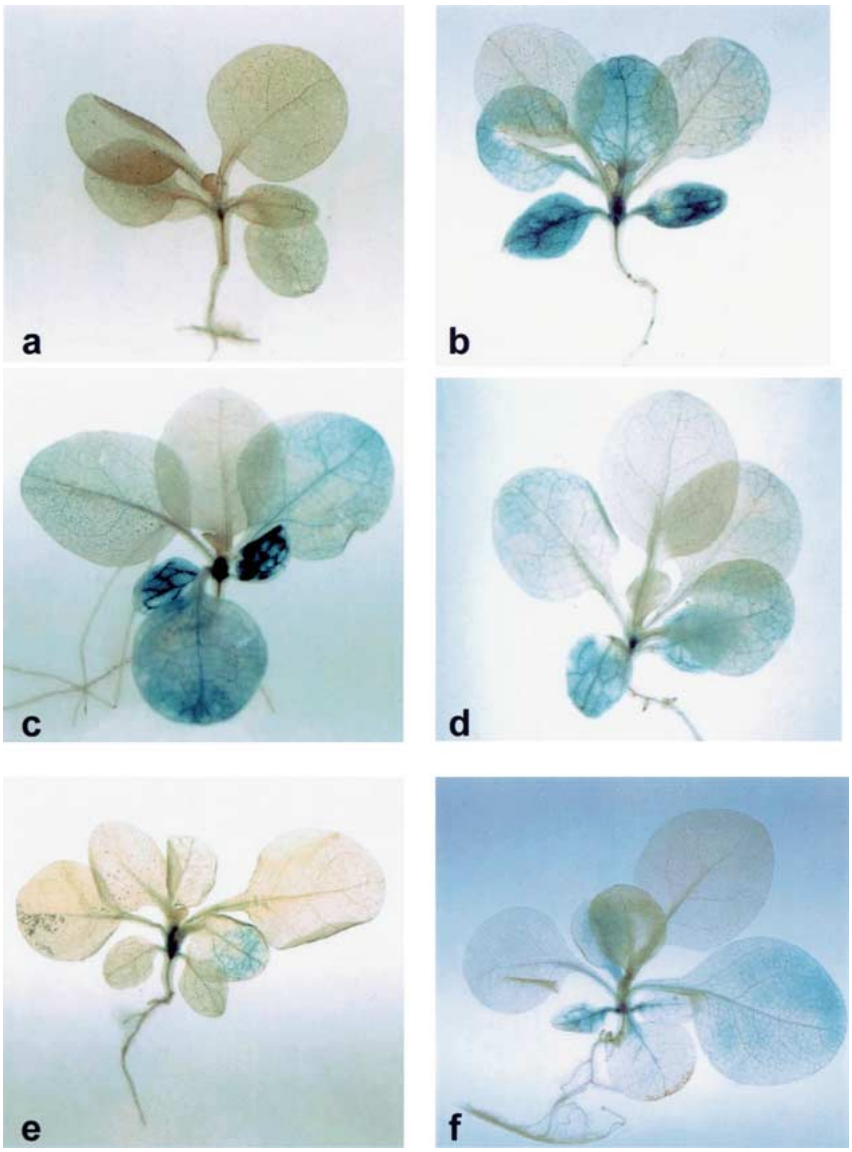

Figure 7. Characterization of *GUS* reporter gene expression in T_1 seedlings of transgenic tobacco Tspoa37. Seedlings (about 3cm in height) were individually treated with 0.15 M sodium phosphate buffer (control) (a), wounding activation (12 h) (b), 50 μM MeJA (c), 200 μM SA treatment for 30 min before wound treatments for 12 h (d), 200 μM SA for 30 min before 50 μM MeJA treatment for 12 h (e), and 100 μM ABA (f).

T_1 seedlings and fluorometric GUS activity assay on mature leaves of T_0 plants showed that ABA slightly increased GUS activity levels in transgenic tobacco (Figure 7f and Figure 8).

Effects of salicylic acid on wound-response expression of the sporamin promoter in transgenic tobacco

The inhibitory effect of SA on *sporamin* gene expression was examined in mature Tspoa37 transgenic T_0 plants and their T_1 seedlings. GUS activities in the mature leaves of wounded transgenic tobacco were repressed by SA (Figure 8). Supplying SA to seedlings prior to wounding or MeJA treatments decreased GUS

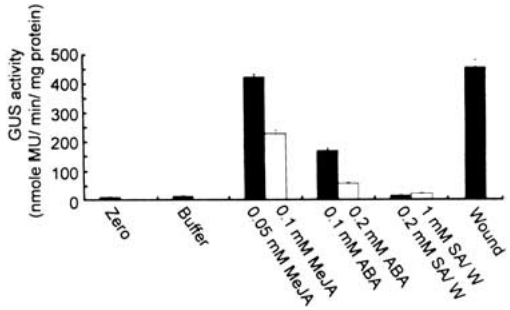

Figure 8. Effects of wound-related signal compounds on *sporamin* promoter activity in transgenic tobacco plants (Tspoa37). Mature transgenic tobacco plants were sprayed with MeJA, ABA, SA or sodium phosphate buffer, respectively. Then, leaves were harvested to determine GUS activity. Values are means ± SD ($n = 3$). Zero, unsprayed control; Buffer, 0.15 M sodium phosphate buffer; MeJA, methyl jasmonate; ABA, abscisic acid; SA/W, wounding treatment for 12 h after SA treatment for 30 min.

activity significantly (Figure 7d and e). Thus, SA effectively inhibited wound- and MeJA-stimulated expression on the *sporamin* promoter.

Discussion

Sporamin genes of sweet potato were modulated to express in aerial tissues by wounding

Previous studies have demonstrated that *sporamin* genes were strongly expressed in tuberous roots and were inducible in leaves in response to some osmotic compounds like PEG, sucrose and polygalactose (Hattori *et al*., 1990; Takeda *et al*., 1995). Recent studies have shown that wounding can induce systemic expression of *sporamin* genes in leaves and stems (Imanishi *et al*., 1997; Yeh *et al*., 1997a). The 1.25 kb *sporamin* promoter sequence contained two elements that were similar to wound-response elements on *nos* and *PI-II* gene promoters, respectively (An *et al*., 1990; Palm *et al*., 1990). In addition, a G box-like element and a GCC-like core sequence were also present on the *sporamin* promoter (Figure 2). These elements were possibly involved in plant defense responses (Williams *et al*., 1992; Menke *et al*., 1999). Transferring the *sporamin* promoter/*GUS* gene fusion construct into tobacco plants enabled us to determine the regulatory pattern of the *sporamin* promoter. Results showed that wounding induced GUS activity in all aerial plant parts, but not in roots (Figure 7b). These results confirmed our previous observations that *sporamin* expression was modulated by wounding in aerial tissues, while it is always present at high levels in tuberous roots growing under non-stressful conditions (Chen *et al*., 1997; Yeh *et al*., 1997a).

Wound activation of the sporamin gene utilized the octadecanoid signal transduction pathway

After wounding, JA rapidly accumulate in leaves and stems via the octadecanoid pathway (Creelman *et al*., 1992; Creelman and Mullet, 1995; Conconi *et al*., 1996). This accumulation modulated expressions of several defense genes, such as those for proteinase inhibitors (Creelman *et al*., 1992; Farmer and Ryan, 1992; Doares *et al*., 1995). JA is one of the endogenous and wound-induced signals involved in regulating the expression of defense genes. Wounds and MeJA significantly activated the *sporamin* gene in leaves and stems of sweet potato and transgenic tobacco (Figures 1, 7b, 7c and 8), but the effect was down-regulated by SA, an inhibitor of the octadecanoid signal transduction pathway (Figures 1, 7d, e and 8). Wound activation of the *sporamin* gene in leaves and stems of the sweet potato is quite similar to the wound activation of the proteinase inhibitor II gene (*pin* 2) in tomato, in that the *pin 2* gene was up-regulated by MeJA and ABA and blocked by SA in the signal transduction pathway (Doares *et al*., 1995; Peña-Cortés *et al*., 1995). In this study, ABA induced *sporamin* promoter activity in transgenic tobacco, but the level of expression was significantly lower than that stimulated by MeJA (Figures 7 and 8). Compared to other primary signals, such as systemin and JA, ABA usually does not strongly induce systemic wound-response genes (Birkenmeier and Ryan, 1998). The exact role of ABA is still controversial. Some wound-inducible genes, such as *ipomoielin* in sweet potato and *AtLox2* gene in *Arabidopsis thaliana*, are also MeJA-inducible but not responsive to ABA (Bell and Mullet, 1993; Imanishi *et al*., 1997). These observations indicated that MeJA-mediated wound-induced gene expression was not necessarily coupled with ABA.

Several research groups have demonstrated that neither wounding nor JA were able to induce expression of defense genes, such as the *pin* gene, in the presence of ethylene action inhibitors (Weiss and Bevan, 1991; O'Donnel *et al*., 1996). In some cases, ethylene could act synergistically with jasmonate to induce higher expression levels of defense genes, such as *PR-1b* and *osmotin* (*PR-5*), than jasmonate alone (Xu *et al*., 1994). Ethylene either functioned downstream of MeJA in the signaling cascade or increased

the potency of its effects. According to northern blot analysis, a competitive inhibitor of ethylene, NBD, blocks the MeJA effect on *sporamin* gene expression (Figure 1) and therefore suggests that ethylene plays a role in the regulation of MeJA enhancing *sporamin* gene expression.

In summary, the *sporamin* promoter is a wound-response promoter that is expressed exclusively in tuberous roots under non-stressful growing conditions. Wounding induces *sporamin* expression in the aerial parts of plants. Some of the plant's signaling compounds, such as MeJA and ethylene, also effectively activate the *sporamin* promoter; however, SA plays a role as down-regulator. Therefore, we propose that *sporamin* gene expression is modulated via the octadecanoid pathway. *Sporamin* promoter functions in transgenic tobacco might be useful to understand the expression patterns of the *sporamin* gene in response to herbivore attacks and other environmental stresses on sweet potato.

Acknowledgements

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References

An, G., Costa, M.A. and Ha, S.B. 1990. Nopaline synthase promoter is wound inducible and auxin inducible. Plant Cell 2: 225–233.

Bell, E. and Mullet, J.E. 1993. Characterization of the *Arabidopsis* lipoxygenase gene responsive to methyl-jasmonate and wounding. Plant Physiol. 103: 1133–1137.

Birkenmeier, G.F. and Ryan, C.A. 1998. Wound signaling in tomato plants. Plant Physiol. 117: 687–693.

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Chen, J.C., Chen, Y.M. and Yeh, K.W. 1997. Isolation and characterization of new sporamin gene members from sweet potato (*Ipomoea batatas).* Taiwania 42 (1): 34–42.

Conconi, A., Miquel, M. and Ryan, C.A. 1996. Changes in the intracellular lipid composition and free fatty acids of tomato leaves in response to wounding. Plant Physiol. 111: 717–803.

Creelman, R.A. and Mullet, J.E. 1995. Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc. Natl. Acad. Sci. USA 92: 4114–4119.

Creelman, R.A., Tierney, M.L. and Mullet, J.E. 1992. Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc. Natl. Acad. Sci. USA 89: 4938–4941.

Devereux, J., Haeberli, P. and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. 12: 195–387.

Ding, L.C., Hu, C.Y., Yeh, K.W. and Wang, P.J. 1998. Development of insect-resistant transgenic cauliflower plants expressing the trypsin inhibitor gene isolated from local sweet potato. Plant Cell Rep. 17: 854–860.

Doares, S.H., Narvaez-Vasquez, J., Conconi, A. and Ryan, C.A. 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol. 108: 1741–1746.

Farmer, E.E. and Ryan, C.A. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4: 129–134.

Hattori, T., Nakagawa, S. and Nakamura, K. 1990. High-level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown *in vitro* on sucrose medium. Plant Mol. Biol. 14: 595–604.

Hattori, T., Yoshida, N. and Nakamura, K. 1989. Structural relationship among the members of multigene family coding for the sweet potato tuberous roots storage proteins. Plant Mol. Biol. 13: 563–572.

Hörsch, R.B., Fry, J. E., Hoffmann, N.L., Eichholtz, D., Rogers, S.G. and Fraley, R.T. 1985. A simple and general method for transferring genes into plants. Science 227: 1229–1231.

Imanishi, S., Kito-Nakamura, K., Matsuoka, K., Morikami, A. and Nakamura, K. 1997. A major jasmonate-inducible protein of sweet potato, ipomoelin, is an ABA-independent wound-inducible protein. Plant Cell Physiol. 38: 643–652.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. 1987. GUS fusion: β-glucuronidase as a sensitive and versatile gene fusion marker. EMBO J. 6: 3901–3908.

Maeshima, M., Sasaki, T. and Asahi, T. 1985. Characterization of major proteins in sweet potato tuberous roots. Phytochemistry 24: 1899–1902.

Mason, H. S., DeWald, D. B. and Mullet, J. E. 1993. Identification of a methyl jasmonate-responsive domain in the soybean *vspB* promoter. Plant Cell 5: 241–251.

Menke, F.L.H., Champion, A., Kijne, J.W. and Memelink J. 1999. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene *Str* interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. EMBO J. 18: 4455–4463.

Murashige, T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497

O'Donnel, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O. and Bowles, D. J. 1996. Ethylene as a signal mediating the wound response of tomato plants. Science 274: 1914–1917.

Ohta, S., Hattori, T., Morikami, A. and Nakamura, K. 1991. High-level expression of a sweet potato sporamin gene promoter: β-glucuronidase (GUS) fusion gene in the stems of transgenic tobacco plants is conferred by multiple cell type-specific regulatory elements. Mol. Gen. Genet. 225: 369–378.

Ohto, M., Nakamura-Kito, K. and Nakamura, K. 1992. Induction of expression of genes coding for sporamin and β-amylase by polygalacturonic acid in leaf-petiole cuttings of sweet potato. Plant Physiol. 99: 422–427.

Palm, C.J., Costa, M.A., An, G. and Ryan, C.A. 1990. Wound-inducible nuclear protein binds DNA fragments that regulate a proteinase inhibitor II gene from potato. Proc. Natl. Acad. Sci. USA 87: 603–607.

Peña-Cortés, H., Fisahn, J. and Willmitzer, L. 1995. Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proc. Natl. Acad. Sci. USA 92: 4106–4113.

Siebert, P.D., Chenchik, A., Kellogg, D.E., Lukyanov, K.A. and Lukyanov, S.A. 1995. An improved PCR method for walking in uncloned genomic DNA. Nucl. Acids Res. 23: 1087–1088.

Takeda, S., Kowyama, Y., Takeuchi, Y., Matsuoka, K., Nishimura, M. and Nakamura, K. 1995. Spatial patterns of sucrose-inducible and polygalacturonic acid-inducible expression of genes that encode sporamin and ß-amylase in sweet potato. Plant Cell Physiol. 36: 321–333.

Wang, S.J., Lin, C.T., Ho, K.C., Chen, Y.M. and Yeh, K.W. 1995. Nucleotide sequence of a sporamin gene in sweet potato. Plant Physiol. 108: 829– 830.

Weiss, C. and Bevan, M. 1991. Ethylene and wound signal modulate local and systemic transcription of *win2* genes in transgenic potato plants. Plant Physiol. 96: 943–951.

Williams, M.E., Foster, R. and Chua, N.H. 1992. Sequences flanking the hexameric G-box core CACGTG affect the specificity of protein binding. Plant Cell 4: 485–496.

Xu, Y., Chang, P.F.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M. and Bressan, R.A. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell 4: 1077–1085.

Yeh, K.W., Chen, J.C., Lin, M.I., Chen Y.M. and Lin, C.Y. 1997a. Functional activity of sporamin from sweet potato (*Ipomoea batatas*): a tuberous storage protein with trypsin inhibitor activity. Plant Mol. Biol. 33: 565–570.

Yeh, K.W., Juan, R.H. and Su, J.C. 1991. A rapid and efficient method for RNA isolation from plants with high carbohydrate content. Focus 13: 102– 103.

Yeh, K.W., Lin, M.I., Tunan, S.J., Chen, Y.M., Lin, C.Y. and Kao, S.S. 1997b. Sweet potato (*Ipomoea batatas*) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against *Spodoptera litura*. Plant Cell Rep. 16: 692–696.