Plant Molecular Biology **51:** 831–838, 2003.

A novel oxidative stress-inducible peroxidase promoter from sweetpotato: molecular cloning and characterization in transgenic tobacco plants and cultured cells

Kee-Yeun Kim[1,3], Suk-Yoon Kwon[1], Haeng-Soon Lee[2], Yunkang Hur[3], Jae-Wook Bang[3] and Sang-Soo Kwak[1,*]

[1]*Laboratory of Environmental Biotechnology,* [2]*Laboratory of Plant Cell Biotechnology, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Oun-dong 52, Yusong-gu, Daejeon, 305-333 Korea; (*author for correspondence; e-mail sskwak@kribb.re.kr);* [3]*Department of Biology, Chungnam National University, Yusong, Daejeon, 305-763 Korea*

Received 30 November 2001; accepted in revised form 13 September 2002

Key words: cultured cells, inducible promoter, *Ipomoea batatas*, oxidative stress, peroxidase, promoter-deletion analysis

Abstract

A strong oxidative stress-inducible peroxidase (POD) promoter was cloned from sweetpotato (*Ipomoea batatas*) and characterized in transgenic tobacco plants and cultured cells in terms of environmental stress. A POD genomic clone (referred to as *SWPA2*) consisted of 1824 bp of sequence upstream of the translation start site, two introns (743 bp and 97 bp), and a 1073 bp coding region. *SWPA2* had previously been found to encode an anionic POD which was highly expressed in response to oxidative stress. The *SWPA2* promoter contained several *cis*-element sequences implicated in oxidative stress such as GCN-4, AP-1, HSTF, SP-1 reported in animal cells and a plant specific G-box. Employing a transient expression assay in tobacco protoplasts, with five different 5′-deletion mutants of the *SWPA2* promoter fused to the β-glucuronidase (GUS) reporter gene, the 1314 bp mutant deletion mutant showed about 30 times higher GUS expression than the CaMV 35S promoter. The expression of GUS activity in transgenic tobacco plants under the control of the −1314 *SWPA2* promoter was strongly induced in response to environmental stresses including hydrogen peroxide, wounding and UV treatment. Furthermore, GUS activity in suspension cultures of transgenic cells derived from transgenic tobacco leaves containing the −1314 bp *SWPA2* promoter-GUS fusion was strongly expressed after 15 days of subculture compared to other deletion mutants. We anticipate that the −1314 bp *SWPA2* promoter will be biotechnologically useful for the development of transgenic plants with enhanced tolerance to environmental stress and particularly transgenic cell lines engineered to produce key pharmaceutical proteins.

Abbreviations: GUS, β-glucuronidase; MS, Murashige-Skoog; PCR, polymerase chain reaction; POD, peroxidase; *SWPA2*, sweetpotato peroxidase anionic 2

Introduction

Plant cell suspension cultures are very important in the field of plant biotechnology for development of transgenic plants, for mass propagation and for the biosynthesis of key pharmaceutical proteins. Cell aggregates, so-called calli, formed from the wounded parts of explants might have a high potential to produce various antioxidant components to overcome oxidative stress derived by wounding. However, there are no detailed reports on the biochemical studies of cultured cells

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AF4532791 (*SWPA2* genomic clone).

in terms of antioxidants. Cultured plant cells might be a good source of material for studies of antioxidant mechanisms and mass production of antioxidants, because they are considered to be grown under high oxidative stress conditions.

In previous reports, we have investigated the levels of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD), and low molecular weight antioxidants such as ascorbate and glutathione in 100 cell lines derived from different plant species (Kim *et al.*, 1994; You *et al.*, 1996; Ahn *et al.*, 1999; Lee *et al.*, 2000). Among the antioxidant activities investigated, plant cultured cells were found to have much higher levels of antioxidant enzymes such as SOD and POD than differentiated plant tissues. Particularly, a sweetpotato (*Ipomoea batatas*) cell line produced a very high level of POD activity (Kwak *et al.*, 1995). Thus, we established an efficient production system of POD in suspension cultures of sweetpotato, showing that the A2 POD isoenzyme composes ca. 7.5% of total soluble cell protein. We isolated four POD cDNAs, three anionic ones, *swpa1*, *swpa2*, *swpa3*, and one neutral cDNA, *swpn1*, from suspension cultures of sweetpotato. Moreover, we have characterized their expressions in terms of oxidative stresses, all four genes were predominantly expressed in cultured cells of sweetpotato (Huh *et al.*, 1997; Kim *et al.*, 1999). Interestingly, *SWPA2* was particularly strongly expressed under these conditions. In contrast, it was not expressed in differentiated plant tissues. Furthermore, *SWPA2* was strongly induced by environmental stresses such as wounding, chilling and ozone in leaves of sweetpotato, suggesting that this gene was regulated by a stress-inducible promoter.

Production of pharmaceutical proteins in cultured plant cells has been actively studied due to its potential commercial utility. Only a small number of low-molecular-weight compounds derived from plants such as shikonin and taxol have been successfully produced in plant suspension cultures (Fujita *et al.*, 1984; Yukimune *et al.*, 1996; Hong, 2000). However, with recent advance in plant metabolic engineering, protein expression in plant cell suspension cultures has significant potential (Yun *et al.*, 1995; Shinmyo *et al.*, 1998, Herbers and Sonnewald, 1999; Doran, 2000; Giddings *et al.*, 2000). A powerful expression system with an appropriate promoter is key requisite for expression of foreign genes efficiently in cultured plant cells. So far, several stress-induced genes have been isolated from plants and studied by functional analysis of their 5′-promoter regions in transgenic plants, those activated by drought (Iuchi *et al.*, 1996a, b), wounding (Kawaoka *et al.*, 1994), and cold (Thomashow, 1998), ascorbate peroxidase (Mittler *et al.*, 1998), phenylalanine ammonia-lyase (Ohl *et al.*, 1990), chalcone synthase (Doerner *et al.*, 1990), proteinase inhibitors (Keil *et al.*, 1990), pathogenesis-related proteins (Ohshima *et al.*, 1990) and β-fructosidase (Stum and Chrispeels, 1990). In this study, we describe a novel oxidative stress-inducible POD promoter cloned from sweetpotato (*Ipomoea batatas*) and its subsequent characterization using an *in vitro* transient assay, transgenic tobacco plants and cultured cells. The results highlight the potential utility of the *SWPA2* promoter for the development of stress-tolerant transgenic plants and importantly for the generation of transgenic cell lines to synthesize key biological materials.

Materials and methods

Construction of genomic library and screening

Total genomic DNA was isolated from plants of sweetpotato, according to the method of Dellaporta (1983). The genomic DNA was digested with *Sau*3AI, ligated into the *Bam*HI site of λBlueSTAR *Bam*HI Arms vector, and packaged with PhageMaker *In Vitro* Lambda Packaging system kits (Novagen), according to the manufacturer's instructions. The library was screened with a radiolabeled full-length *swpa2* cDNA probe by standard plaque lift methods (Sambrook *et al.*, 1989). The probe was prepared from cDNA labeled with [α-^{32}P]dCTP by means of the Rediprime kit (Amersham). The library screening was carried out by the method of Sambrook *et al.* (1989).

Plasmid construction

Chimeric genes for the transient expression assay are constructed as follows: A *Sal*I/*Bam*HI fragment of *SWPA2* promoter synthesized by PCR was cloned into the same site of pBI101 (Clontech, Palo Alto, CA). A series of deletion of *SWPA2* promoter is generated by PCR amplifications (a DNA Thermal Cycler with *Ex Taq* DNA polymerase and sequence-specific primers in 50 μl reaction mixtures). Six primers, named 1(Δ354), 2(Δ602), 3(Δ968), 4(Δ1314), 5(Δ1824), and 6, based on the nucleotide sequences of *SWPA2*, were synthesized. The sequence of the upstream primer 1 (5′-ACGCGTCGACTTACTTTGTGATTCTA-3′), primer 2 (5′-ACGCGTCGACAATGGACGAATTATTAGT-3′),

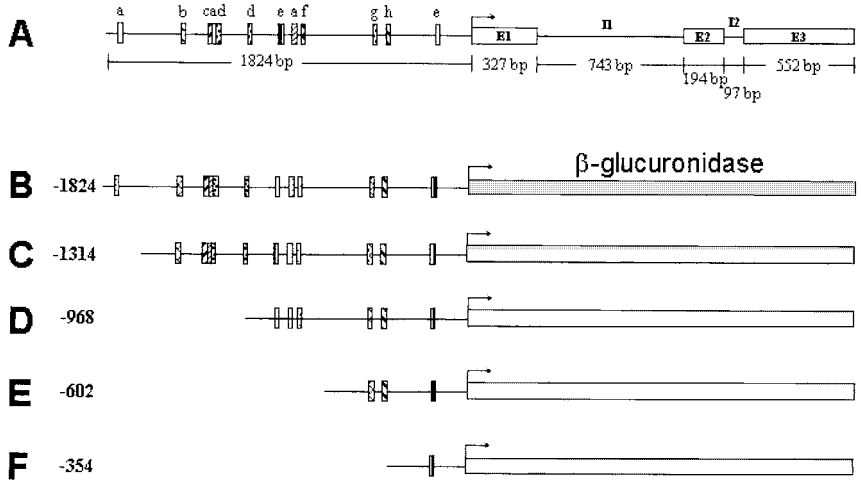

Figure 1. Functional architecture of sweetpotato (*Ipomoea batatas*). Possible *cis* elements involved in oxidative stress: a, GCN-4; b, Oct-1; c, HSE; d, AP-1; e, CATT box; f, TATA box; g, G-box; h, Sp-1. Introns 1 and 2 are denoted by I1 and I2, respectively. Exons 1, 2 and 3 are denoted by E1, E2 and E3, respectively. The GenBank accession number for the *SWPA2* genomic clone is AF453791.

primer 3 (5′-ACGCGTCGACGGTCGGAACGTT TTTT-3′), primer 4 (5′-ACGCGTCGACGCCATGAT CAGATCGATA-3′), or primer 5 (5′-ACGCGTCGAC CTTCAATATTTTGTCTGTATT-3′) contains a *Sal*I restriction enzyme site. The sequence of the downstream primer 6 (5′-CGGGATCCGGTCAAAGGAAA AT-3′) contains a *Bam*HI restriction enzyme site to create the new restriction enzyme site. Purified PCR products were digested with *Sal*I/*Bam*HI, and subcloned into pBI101, and were designated pBS354, pBS602, pBS968, pBS 1314, and pBS 1824.

Isolation of protoplasts from tobacco BY-2 cells

A suspension-cultured cell line of tobacco BY-2 (*Nicotiana tabacum* L. cv. Bright Yellow 2) was used for transient expression by PEG method (Nagata, 1987). The cells were subcultured by transferring 1.0 ml of a 7-day culture suspension into 100 ml of a fresh medium (pH 5.8) composed of MS salts supplemented with 200 mg/l KH_2PO_4, 1.0 mg/l thiamine, 100 mg/l myo-inositol, 0.2 mg/l 2,4-D, and 3% sucrose, and agitating the cells in a gyratory shaker (130 rpm) at 26 °C in the dark. To prepare protoplasts, 3-day old cultured cells were harvested and washed once with 0.4 M mannitol by centrifugation (100 × *g* for 3 min). They were then re-suspended in an enzyme solution (pH 5.5) containing 1% cellulase Onozuka RS (Yakult Honsha), 0.1% Pectolyase Y-23 (Seishin Pharmaceutical), 10 mM MES and 0.4 M mannitol, and incubated for 1 h at 30 °C with gently pipetting every 15 min. Protoplasts were collected by centrifugation (100 × *g* for 3 min) and washed three times with the CPW solution (pH 5.8). The protoplasts were re-suspended at a density of 2 × 10^6/ml in the CPW solution. The tubes were shaken slowly by hand, and 200 μl PEG solution (25% PEG_{6000} (Serva), 100 mM $Ca(NO_3)_2$, 450 mM mannitol pH 9.0 with KOH and sterilized by filtration) and DNA (20 μg) were added. The mixture was shaken carefully again, and incubated for 15 min at RT. DNA uptake was stopped by the addition of 5 ml 275 mM $Ca(NO_3)_2$. The protoplasts were collected by centrifugation (100 × *g* for 3 min), re-suspended in 1 ml of a protoplast culture medium except that it contained 0.4 M mannitol and 1% sucrose instead of 3% sucrose, and cultured at 26 °C for 20 h in the dark.

Fluorometric GUS activity and staining

Extraction of proteins and GUS activity in crude extracts was determined florometrically as described (Jefferson, 1987). Histochemical staining for GUS activity was performed essentially as described by Jefferson (1987) with X-gluc as a substrate. Protein content of sample extracts was determined according to the method of Bradford (1976) by using a BioRad Protein Assay Kit with BSA as a standard. Data are presented as the mean of GUS activity from at least three independent determinations.

Preparation of transgenic tobacco plants and cultured cells

Agrobacterium tumefaciens strain LBA4404 harboring each pBS1314, pBS 1824 or pBI121 was used to transform tobacco plants (*Nicotiana tabacum* cv. Xanthi) by the leaf disk method (Horsch *et al.*, 1985). Transformants were selected on MS (Murashige and Skoog, 1962) medium supplemented with 200 mg/l kanamycin and 300 mg/l claforan. Kanamycin-resistant shoots were directly formed on cut edges of leaf discs. Rooting of kanamycin-resistant plantlets was carried out in the selectable MS medium without growth regulators. After acclimation, the plants were transplanted to potting soil and maintained in a greenhouse. For callus induction, leaf segments from *in vitro* transgenic plants were incubated for 17 days on medium, containing MS salts (Duchefa Biochemical, Netherlands), 3% sucrose, 2 mg/l 1-naphthaleneacetic acid (NAA), 0.25 mg/l 6-benzyl-aminopurine (BA) and 1 mg/l thiamine-HCl.

Stress treatment

For stress treatment, the 3rd and 4th leaves from the top of plants grown in a greenhouse were used. For hydrogen peroxide (H_2O_2) treatment, leaf disks (7 mm in diameter) were floated on 0.4 M sorbitol solution containing 1 mM H_2O_2 in Petri dish under continuous light of 6000 lux at 25 °C for 48 h, and then the GUS activity was measured. For wounding stress, leaf disks were wounded by pressing with a needle puncher as described previously (Huh *et al.*, 1997), and GUS activity was measured after keeping them in growth chamber for 48 h. For UV treatment, leaf disks in a petri dish were exposed to UV-C light ($1\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) of clean bench for 4 h, and GUS activity was measured after keeping samples in a growth chamber for 24 h. All data is a mean ± SE of three replicates

Results and discussion

Isolation and molecular characterization of a genomic POD clone

A genomic library of sweetpotato was screened with an anionic POD cDNA, *SWPA2*, which encodes the predominant POD isoenzyme A2 in suspension cultures of sweetpotato. This enzyme comprises ca. 7.5% of total soluble protein in such cells (Kwak *et al.*, 1995; Kim *et al.*, 1999). One genomic clone (referred to as *SWPA2*) was obtained that strongly hybridized after a third screening. The *SWPA2* clone contains a 3.8 kb fragment harboring 1.8 kb upstream of the translation start, two introns of 734 bp and 96 bp and three exons of 327 bp, 194 bp and 552 bp (Figure 1A). All introns were AT-rich and had consensus GT and AG dyads at the 5′ and 3′ termini, respectively.

A promoter motif search of the *SWPA2* sequence was carried out to define putative *cis* elements using the software program TESS in our Computational Biology & Informatic Laboratory. Several motifs corresponding to known *cis*-regulatory signals of eukaryotic genes were found to be present in the sequence (Figure 1A). The TATA box was located at positions −765 to −759 upstream of the translation start site, whereas CAAT boxes were found at positions −898 to −895 and −116 to −111. Interestingly, sequence elements with homology to those found in other stress-induced plant genes such as GCN-4, HSE, AP-1 were identified in the *SWPA2* promoter region. Three GCN-4 motifs were discovered at positions −837 to −823, −1179 to −1167 and −1716 to −1704. This element is known to be involved in oxidative stress in animal cells (Hilson *et al.*, 1990; Konig and Richmond, 1993). Two regions at positions −1183 to −1179 and −1188 to −1184 contain a core sequence ATAAN which is highly homologous to the heat shock element (HSE) (Treuter *et al.*, 1993). The *SWPA2* promoter region also contains two sequence motifs, consensus sequences RSTGACTMANN, at positions −1012 to −1022 and −1162 to −1172, with substantial homology to AP-1, which is present in various reactive oxygen species-modulated plant gene promoters (Lee *et al.*, 1987; Kathryn, 1996; Muller *et al.*, 1997; Grant *et al.*, 2000). In RSTGACTMANN, R, S, M and N mean the mixed base, in which R, S, M and N represent A or G, C or G, A or C, and A or G/G or T, respectively. Other *cis*-acting elements, such as Sp-1, Oct-1 and the G-box are present, which have been implicated in stress responses. In addition, the repeat sequences (TTTTATT) were detected at seven positions. The sequence analysis of *SWPA2* promoter suggested that the *SWPA2* gene may be induced in response to oxidative stress.

Analysis of SWPA2 promoter deletion mutants

To investigate the promoter activity of the *SWPA2* gene, five deletion mutants of this region (Figure 1B–F) were studied in a transient-expression assay em-

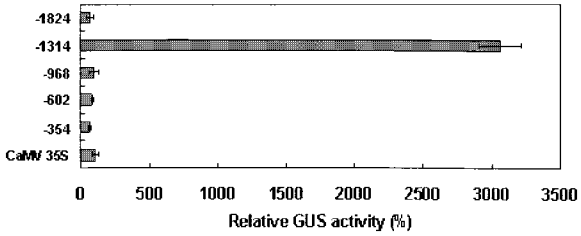

Figure 2. Functional analysis of *SWPA2* promoter deletion mutants in tobacco (BY-2) protoplasts. Deletion mutants defined as numbers of base pairs from the site of translation. GUS activity is expressed relative to that supported by CaMV 35S promoter, which was 5176 ± 96 pmol/min per mg protein. Data are mean ± SE of three replicates.

ploying tobacco (BY-2) protoplasts (Figure 2). The activity of *SWPA2* promoter mutants was examined by measuring the activity of the β-glucuronidase (GUS) reporter enzyme. The −1314 fragment supported the highest levels of GUS activity, which was about 30 times higher than that supported by the 35S promoter of CaMV. A deletion from −1314 to −968 slightly reduced GUS activity compared to the CaMV 35S promoter. Further deletion to −354 gradually reduced *SWPA2* expression to two-thirds those of the CaMV 35S promoter. The 1824 bp fragment showed a lower level of expression than the CaMV 35S promoter. Taken together, these results suggested that positive *cis* elements may be located in the regions between −1314 and −968, whereas negative *cis* elements may exist in the regions between −1824 and −1314. The *cis* elements such as GCN-4, AP1 and HSE in the region from −1164 to −1188 may therefore be important for gene expression. The characterization of exact positive and negative *cis* elements remains to be determined.

The SWPA2 promoter is highly responsive to oxidative stress

To determine *SWPA2* promoter activity in whole plants, in response to various oxidative stresses, the −1824 and −1314 *SWPA2* promoter deletion mutants were introduced into tobacco (*Nicotiana tabaccum* cv. Xanthi) by *Agrobacterium tumefaciens*-mediated transformation using strain LBA4404. Transgenic tobacco plants were selected and confirmed by PCR for the presence of the respective p*SWPA2*::GUS and CaMV 35S::GUS cassettes. Regenerated tobacco plants harboring the different constructs exhibited wild-type morphology and growth when compared with non-transgenic plants (data not shown).

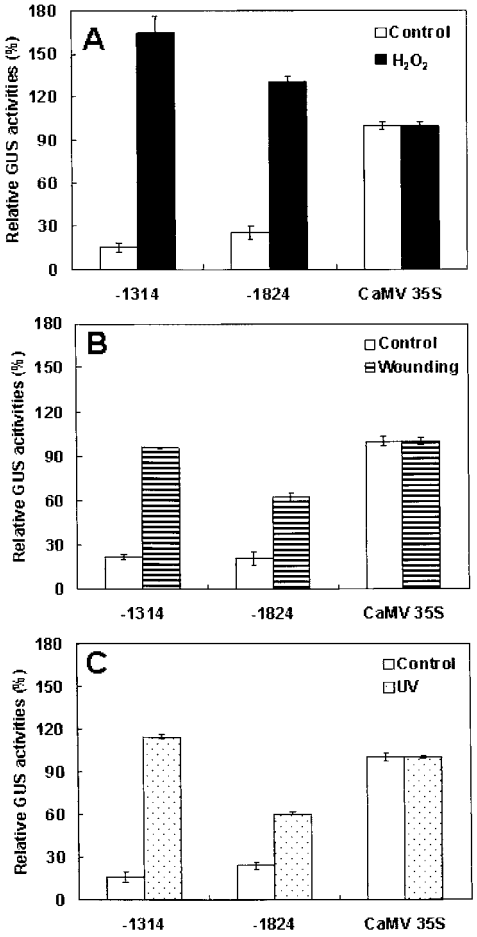

Figure 3. Activation of GUS activity mediated by *SWPA2* deletion mutants in transgenic tobacco plants cued by distinctive environmental stresses. A. GUS activity at 48 h after 1 mM hydrogen peroxide treatment. B. GUS activity at 48 h after mechanical wounding. C. GUS activity at 24 h after UV treatment. Data are mean ± SE of three independent replicates.

We determined GUS activity in the leaves of T_0 transgenic tobacco plants under various stresses such as hydrogen peroxide, wounding and UV (Figure 3). GUS activity in CaMV 35S::GUS transformants was not significantly modulated after either stress. However, GUS activity in the −1824 and −1314 transformants was induced after 48 h of H_2O_2 treatment. The average GUS activity in these transformants was ca. 1.53 and 1.25 times higher than in transgenic plants containing CaMV 35S::GUS (Figure 3A).

In the case of wounding treatment (Figure 3B), GUS activity in the −1824 and −1314 transformants, was detected at a low level (29% compared to pBI121) before wounding treatment. However, GUS activity in these transformants was increased by ca. 3.6 and

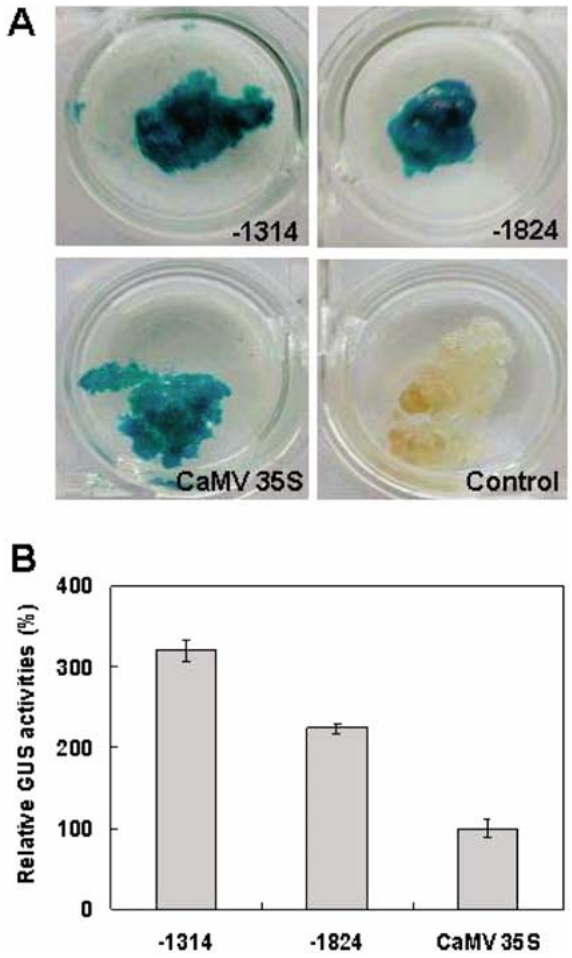

Figure 4. Expression of GUS protein in calluses derived from leaves of transgenic and non-transgenic tobacco plants. GUS staining (A) and GUS specific activity (B, pmol/min per mg protein) after 30 days of subculture. The calluses were cultured on MS medium supplemented with 2 mg/l NAA, 0.25 mg/l BA and 3% sucrose. GUS activity is expressed relative to that supported by the CaMV 35S promoter, which was 4707 ± 522 pmol/min per mg protein. Data are mean ± SE of three independent replicates.

2.5 times compared to that established by the CaMV 35S promoter after 72 h wounding treatment. The GUS activity in −1314 transformants after wounding was 96% of the level measured in CaMV 35S:GUS transformants.

UV-C treatment also enhanced GUS activity elevated by the −1824 and −1314 promoter deletion mutants (Figure 3C). GUS activity had increased ca. 5.6 and 2.5 times after 24 h of UV treatment. Interestingly, GUS activity in transformants established by the −1314 *SWPA2* promoter was 20% higher than that established by the CaMV 35S promoter, whereas the

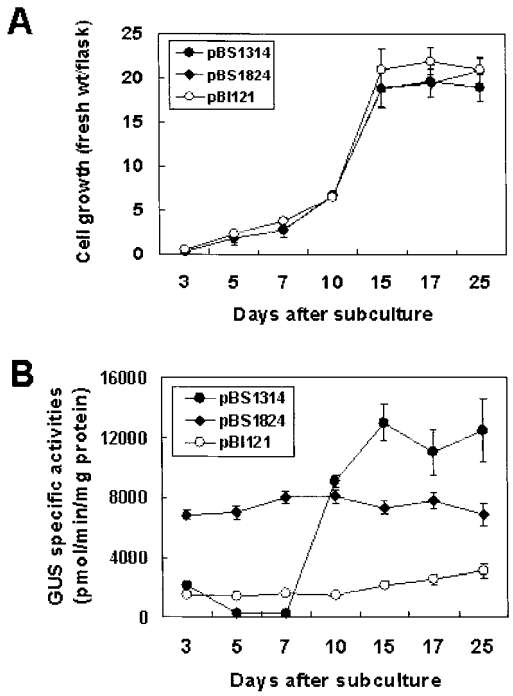

Figure 5. Expression of GUS activity following subculture of cells derived from leaves of transgenic and non-transgenic tobacco plants. Cell growth (A) and GUS specific activity (B, pmol/min per mg protein) in three cell lines. pBS1314 and pBS1824 transformants indicate the transgenic cells containing the 1314 bp and 1824 bp promoter/GUS fusion gene in pBI101.1 vector, respectively. Data are mean ± SE of three replicates.

−1824 *SWPA2* promoter showed lower GUS expression than that established by the CaMV 35S promoter.

The SWPA2 promoter directs high expression in transgenic tobacco cell lines

Transgenic calluses were successfully developed from the leaves of transgenic plants containing *SWPA2* promoter deletion mutants. Transgenic calluses containing −1314 and −1824 *SWPA2* promoters were strongly stained with X-gluc, whereas those containing the CaMV 35S promoter showed weak GUS staining, reflecting the GUS activity of each deletion promoter (Figure 4A). Transgenic calluses containing the −1314 and −1824 *SWPA2* promoters showed ca. 3.3 and 1.9 times higher GUS activity than those containing the CaMV 35S promoter after 30 days of subculture (Figure 4B).

After establishment of suspension cultures from these calluses, their respective GUS activities were investigated (Figure 5). The cell growth of −1314, −1824 and CaMV 35S cell lines was similar in pat-

tern, showing a typical sigmoidal growth curve, with maximum growth at 15 days after subculture (DAS) (Figure 5A). Hence, there is no significant difference in cell growth among these three cell lines. GUS activity established in CaMV 35S::GUS-containing cells showed a constitutive low level of GUS expression regardless of the cell growth phase. However, the GUS activity in the −1314::GUS cell line showed a minimum level at 5 and 7 DAS, but markedly increased GUS activity from 7 DAS, which reached a maximum level at 15 DAS and sustained a high level of GUS activity with further cultures (Figure 5B). In the case of the −1824::GUS cell line, a high level of constitute GUS was observed during every growth phase. As expected, the GUS activity established by the −1314 *SWPA2* promoter showed high levels at stationary growth phase (15 DAS), reflecting the accumulation of *SWPA2* transcripts during cell growth (Kim *et al.*, 1999). The GUS activities of −1314::GUS and −1824::GUS cell lines were ca. 6.2 and 1.8 times higher than that recorded in the CaMV 35S::GUS cell line. From the results of transgenic plants (Figure 3) it can be concluded that GUS activity will be significantly increased in response to oxidative stress. The GUS expression under stress conditions in transgenic cell lines is under study.

In a previous study, we discovered that *SWPA2* was not expressed in any tissues of differentiated plants, whereas this gene was strongly expressed in cultured cells, and induced by wounding, chilling and ozone in intact leaves (Kim *et al.*, 1999). High GUS activity in transgenic tobacco cell lines containing −1314 and −1824 *SWPA2* promoters well reflected the POD expression in cell cultures of sweet potato (Kim *et al.*, 1999).

There are some differences in GUS expression among experimental systems such as protoplasts, leaves and cultured cells and deletion mutants of the *SWPA2* promoter, suggesting the existence of complicated factors among experiments in terms of stress. The difference among experiment systems remains to be determined.

The −1314 *SWPA2* promoter can be considered as an oxidative stress-inducible promoter, since it has several *cis* elements involved in oxidative stress in animal cells (Figure 1), and it may regulate GUS expression in transgenic plants and cultured cells by oxidative stress (Figures 3 and 5). In conclusion, the results indicate that an oxidative stress-inducible *SWPA2* promoter, particularly the −1314 promoter, from sweetpotato, will be biotechnologically useful for the development of stress-tolerant plants and for the development of transgenic cell lines to produce useful biological materials. In addition, the *SWPA2* promoter may have high utility for studies investigating oxidative stress signal transduction. The transcription factors in relation to positive and negative *cis* elements are currently under investigation.

Acknowledgements

This work was supported by a research grant (CGM0300111) from the Crop Functional Genomics Center and a research grant (# PF002111-01) from the Plant Diversity Research Center, the Korean Ministry of Science and Technology. We are grateful to Prof. Gary Loake, University of Edinburgh, UK, for his valuable comments to the manuscript.

References

Ahn, Y.O., Choi. Y.H., Kwon, S.Y., Lee, H.S., Kim, S.W., Park, I.H. and Kwak, S.S. 1998. Free radical scavenging activity and ascorbate content in various plant cell lines. Kor. J. Plant Tiss. Cult. 25: 289–293.

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

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. Maize DNA minipreps. Maize Genet. Coop. Newsl. 57: 26–29.

Doerner, P.W., Stermer, B., Schmid, J., Dixon, R.A. and Lamb, C.J. 1990. Plant defense gene fusions in transgenic plants: Tools for identification of novel inducers. Bio/technology 8: 845–848.

Doran, P.M. 2000. Foreign protein production in plant tissue cultures. Curr. Opin. Biotechnol. 11: 199–204.

Fujita, Y., Takahashi, S. and Yamada, Y. 1984. Selection of cell lines with high productivity of shikonin derivatives through protoplasts of *Lithospermum crythrorhizon*. In: Third European Congress on Biotechnology, Verlag Chemie, Weinheim, pp. I-161–166.

Giddings, G., Allison, G., Brooks, D. and Carter, A. 2000. Transgenic plants as factories for biopharmaceuticals. Nature Biotechnol. 18: 1151–1155.

Grant, J.J., Yun, B.Y. and Loake, G.J. 2000. Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. Plant J. 24: 569–582.

Herbers, K. and Sonnewald, U. 1999. Production of new/modified proteins in transgenic plants. Curr. Opin. Plant Biol. 10: 163–168.

Hilson, P., de Froidmont, D., Lejour, C., Hirai, S.I., Jacquemin, J.M. and Yaniv, M. 1990. Fos and Jun oncogenes transactivate chimeric or native promoters containing AP1/GCN-4 binding sites in plant cells. Plant Cell 1: 651–658.

Hong, S.S. 2000. Mass production of paclitaxel by plant cell culture. In: Proceedings of International Symposium on New Strategy for

Drug Development in Post-Genome Era, . 19–20 October, Seoul Education Cultural Center, p. 77.

Horsch, R.B., Fry, J.E., Hoffmann, N.L., Eichholtz, D. and Rogers, S.G. 1985. A simple and general method for transferring genes into plants. Science 227: 119–123.

Huh, G.H., Lee, S.J., Bae, Y.S., Liu, J.R. and Kwak, S.S. 1997. Molecular cloning and characterization of cDNAs for anionic and neutral peroxidases from suspension cultured cells of sweet potato and their differential expression in response to stress. Mol. Gen. Genet. 255: 382–391.

Iuchi, S., Yamaguchi-Shinozaki, K., Urao, T. and Shinozaki, K. 1996a. Characterization of two cDNAs for novel drought-inducible genes in the highly drought-tolerant cowpea. J. Plant Res. 109: 415–424.

Iuchi, S., Yamaguchi-Shinozaki, K., Urao, T., Terao, T. and Shinozaki, K. 1996b. Novel drought-inducible genes in the highly drought-tolerant cowpea: cloning of cDNAs and analysis of the expression of the corresponding genes. Plant Cell Physiol. 37: 1073–1082.

Jefferson, R.A. 1987. Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol. Biol. Rep. 5: 387–405.

Kathryn, Z. 1996. Induction of the mammalian stress response gene GADD153 by oxidative stress: role of AP-1 element. Biochem. J. 314: 547–554.

Kawaoka, A., Kawamoto, T., Sekine, M., Yoshida, K., Takano, M. and Shinmyo, A. 1994. A *cis*-acting element and a *trans*-acting factor involved in the wound-induced expression of a horseradish peroxidase gene. Plant J. 6: 87–97.

Keil, M., Scnchez-Serrano, J., Schell, J. and Willmitzer, L. 1990. Localization of elements important for the wound-inducible expression of a chimeric potato proteinase inhibitor II-CAT gene in transgenic tobacco plants. Plant Cell 2: 61–70.

Kim, K.Y., Huh, G.H., Lee, H.S., Kwon, S.Y., Hur, Y. and Kwak, S.S. 1999. Molecular characterization of cDNAs for two anionic peroxidases from suspension cultures of sweet potato. Mol. Gen. Genet. 261: 941–947.

Kim, S.K., Kwak, S.S., Jung, K.H., Min, S.R., Park, I.H. and Liu, J.R. 1994. Selection of plant cell lines for high yields of peroxidase. J. Biochem. Mol. Biol. 27: 132–137.

Konig, P. and Richmond, T.J. 1993. The X-ray structure of the GCN-4-bZIP bound to ATF/CREB site DNA shows the complex depends on DNA flexibility. J. Mol. Biol. 233: 139–154.

Kwak, S.S., Kim, S.K., Lee, M.S., Jung, K.H., Park, I.H, and Liu, J.R. 1995. Acidic peroxidase from suspension-cultures of sweet potato. Phytochemistry 39: 981–984.

Lee, J.E., Ahn, Y.O., Kwon, S.Y., Lee, H.S., Kim, S.W., Park, I.H. and Kwak, S.S. 2000. Glutathione contents in various plant cell lines. Kor. J. Plant Tiss. Cult. 27: 57–61.

Lee, W., Mitchell, P. and Tjian, R. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 49: 741–752.

Muller, J.M., Rupec, R.A. and Baeuerle, P.A. 1997. Study of gene regulation by NF-κB and AP-1 in response to reactive oxygen intermediates. Methods 11: 301–312.

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

Mittler, R., Feng, X. and Cohen, M. 1998. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell 10: 461–473.

Nagata, T. 1987. Interaction of plant protoplast and liposome. Meth. Enzymol. 148: 34–39.

Ohl, S., Hedrick, S.A., Chory, J. and Lamb, C.J. 1990. Functional properties of a phenylalanine ammonia-lyase promoter from *Arabidopsis*. Plant Cell 2: 837–848.

Ohshima, M., Itoh, H., Matsuoka, M., Murakami, T. and Ohashi, Y. 1990. Analysis of stress-induced or salicylic acid-induced expression of the pathogenesis-related 1a protein gene in transgenic tobacco. Plant Cell 2: 95–106.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Plainview, NY.

Shinmyo, A., Shoji, T., Bando, E., Nagaya, S., Nakai, Y., Kato, K., Sekine, M. and Yoshida, K. 1998. Metabolic engineering of cultured tobacco cells. Biotechnol. Bioeng. 58: 229–332.

Stum, A. and Chrispeels, M. 1990. cDNA cloning of carrot extracellular fructosidase and expression in response to wounding and bacterial infection. Plant Cell 2: 1107–1119.

Thomashow, M.F. 1998. Role of cold-responsive genes in plant freezing tolerance. Plant Physiol. 118: 1–7.

Treuter, E., Nover, L., Ohme, K. and Scharf, K.D. 1993. Promoter specificity and deletion analysis of three heat stress transcription factors of tomato. Mol. Gen. Genet. 240: 113–125.

You, S.H., Kim, S.W., Kim, S.H., Liu, J.R. and Kwak, S.S. 1996. Selection and isozyme analysis of plant cell lines for high yields of superoxide dismutase. Kor. J. Plant Tiss. Cult. 23: 103–106.

Yukimune, Y., Tabata, H., Higashi, Y. and Hara, Y. 1996. Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. Nature Biotechnol. 14: 1129–1132.

Yun, D.J., Hashimoto, T. and Yamada, Y. 1992. Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition. Proc. Natl. Acad. Sci. USA 89: 11799–11803.