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# Differential expression of four sweet potato peroxidase genes in response to abscisic acid and ethephon

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**Abstract**

Expression of four peroxidase (POD) genes, three anionic PODs (swpa1, swpa2 and swpa3), and one neutral POD (swpn1) isolated from suspension cultures of sweet potato (*Ipomoea batatas*) were analyzed by measuring the accumulation of transcripts in suspension cultured cells and leaves of sweet potato in response to the stress-related plant hormones abscisic acid (ABA) and ethephon (an ethylene generating chemical). The four genes responded differently to ABA (0.1 mM) and ethephon (0.1 mM) in cultured cells and leaves. In suspension cultures, ABA reduced the expression levels of swpa1, swpa2, and swpn1, but did not affect the level of swpa3. Ethephon strongly increased expression levels of swpa3 and swpn1, and slightly increased the level of swpa1. The expression level of swpa2 was reduced. Expression levels in intact leaves, however, were significantly changed by this treatment. Expression of the swpa1 and swpa2 genes was induced 15 min after ABA treatment, followed by a decrease to a basal level after 3 h. A strong re-expression occurred after 12 h. Expression of the swpa3 and swpn1 genes occurred from 3 to 24 h after treatment. All four genes were differentially expressed 12 h after ethephon treatment. The swpa2 gene was strongly expressed immediately after ethephon treatment. The results indicate that each POD gene is differentially regulated by ABA and ethylene in whole plants and in cultured cells in vitro. 

*Keywords:* *Ipomoea batatas*; Convolvulaceae; Peroxidase; Abscisic acid; Ethephon; Gene expression

## 1. Introduction

Peroxidase (POD, EC 1.11.1.7) is an ubiquitous enzyme that reduces hydrogen peroxide in the presence of an electron donor. POD plays a significant role in many physiological processes, including lignification, and is widely used in industry (Krell, 1991; Siers, 1991). POD activity in plants increases in response to a variety of physical, chemical, and biological stresses (Endress et al., 1980; van Huystee, 1987; Bowles, 1990; Miller and Kelley, 1989). However, uncertainty remains as to which POD isoenzyme is involved in a specific reaction. Transient kinetic studies have recently demonstrated that only cationic PODs form a stable compound III in the course of IAA oxidation, indicating that each isoenzyme is involved in a unique reaction (Gazaryan et al., 1999). Most higher plants have a number of POD isoenzymes which are usually classified as anionic, neutral, and cationic, based on their isoelectric points (Intapruk et al., 1993). Each isoenzyme is thought to serve a different function during plant cell growth and development. However, their actual physiological roles in plant physiology are still unclear. Since many POD isoenzymes exist in plants, it is important to isolate the genes that encode each isoenzyme and to characterize the expression and proper-

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ties of the gene products in vivo. Cultured plant cells are a good material for POD production and for the study of antioxidative mechanisms because they can be grown under high oxidative stress conditions (Takeda et al., 1990; Yamada et al., 1987; Kim et al., 1994; Kwak et al., 1995).

Previous studies have established an efficient POD production system in suspension cultures of sweet potato (*Ipomoea batatas*) in which enhanced POD activity was attained by chemical elicitation using the stress-related plant hormones abscisic acid (ABA) or ethephon (an ethylene generating chemical) (Kim et al., 1994; Kwak et al., 1995; Kwak et al., 1996). Four POD cDNAs (anionic swpa1, swpa2, swpa3, and neutral swpn1) have also been isolated and characterized from suspension cultures of sweet potato in response to environmental stress (Huh et al., 1997; Kim et al., 1999). Herein, we describe expression of four POD genes in response to either ABA or ethephon in suspension cultures and leaves of sweet potato with a view to understand the physiological function of PODs in relation to chemical stress.

## 2. Results and discussion

### 2.1. Expression of POD genes in cultured cells

In untreated sweet potato control cells, the expression patterns of the four POD genes were different during cell growth from 12 days after subculture (DAS). This indicates that expression of these genes is differently regulated during cell growth (Fig. 1). The expression level of swpa1 was constant from 12 to 21 DAS, whereas the level of swpa2 progressively increased during the same period. The expression levels of swpa3 and swpn1 reached a maximum at 14 DAS, then decreased thereafter.

ABA treatment strongly reduced expression levels of all genes at 1 day after treatment (DAT). At 3 DAT, the expression levels of swpa1 and swpa2 were inhibited by ABA, compared to control cells, whereas the levels of swpa3 and swpn1 were not significantly affected. At 10 DAT, ABA reduced expression levels of swpa1, swpa2, and swpn1. However, the level of swpa3 was slightly increased. Ethephon strongly increased expression levels of swpa3 and swpn1, and slightly increased the level of swpa1. The swpa2 level was reduced.

ABA mediates many physiological and developmental processes in plants in response to various environmental stresses (Moore, 1989). In potato tissue culture, ABA induces an anionic POD associated with suberization (Espelie and Kolattukudy, 1985). Ethylene accelerates maturation, senescence, and abscission in plants (Birecka et al., 1979). These phenomena are often accompanied by an increase in POD activity. Ethylene increases POD activity in sweet potato, cucumber, pea, tomato, and tobacco (Abeles et al., 1988; Cassab et al., 1988; Gahagan et al., 1968), and also affects POD mRNA expression in suspension cultured cells of peanut (Breda et al., 1993). In etiolated pea seedlings, ethylene increases POD activity at 3 DAT (Cassab et al., 1988).

ABA and ethephon addition both enhance total POD activity by approximately 50% in suspension cultures of sweet potato without a significant effect on cell growth when added to the growth medium at the late logarithmic growth phase (Kwak et al., 1996). In this study, expression levels of the four studied POD genes in cultured cells were reduced by ABA treatment, while the levels of swpa3 and swpn1 were increased by ethephon treatment (Fig. 1). Based on native gel analysis, there are at least 10 POD isoenzymes in cultured cells of sweet potato (Kim et al., 1994). POD genes other than those studied herein are probably responsible for ABA induced enhancement of POD activity, since there was no correlation between the expression levels of the four studied POD genes and POD activity after ABA treatment. These results indicate that each POD gene is probably regulated by ABA and ethephon in different ways.

### 2.2. Expression of POD genes in leaves

The four studied POD genes were not expressed in healthy sweet potato leaves (Huh et al., 1997; Kim et al., 1999) (Fig. 2). Expression levels of swpa1 and

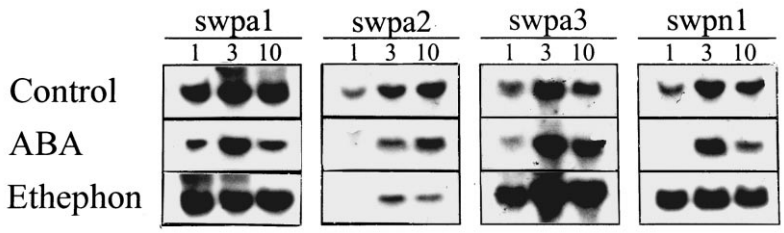


Fig. 1. Expression of sweet potato POD genes in suspension cultured cells of sweet potato in response to abscisic acid and ethephon. ABA or ethephon (final conc. 0.1 mM) was applied to suspension cultures 11 days after subculture. RNA was extracted at 1, 3, and 10 days after treatment (DAT). Equal amounts (40 μg) of each sample were loaded in each lane.

swpa2 were slightly induced immediately after ABA treatment (0 h after treatment, HAT). Expression levels were increased at 0.25 HAT, then decreased to a basal level at 3 and 6 HAT (Fig. 2A). The two genes were re-expressed at 12 HAT at the maximum level, then decreased thereafter. Expression levels of swpa3 and swpn1 were slightly induced from 3 to 24 HAT, but their levels were lower than the levels of swpa1 and swpa2. The four genes were differentially expressed 12 h after ethephon treatment (Fig. 2B). The swpa2 gene was strongly expressed immediately after ethephon treatment, followed by a decrease to the basal level at 6 h, then strongly re-expressed at 12 h.

The expression of swpa2 was strongly induced in leaves by both ABA and ethephon, but reduced in cultured cells. The ABA induced expression levels of swpa3 in leaves were very low compared to the other genes, whereas levels of swpa3 in cultured cells were not changed by ABA. The ethephon induced expression levels of swpn1 were very low in leaves and high in cultured cells. These results indicate that gene expression of POD isoenzymes is different between in vitro cultured cells and whole plants, and that each POD gene is regulated by ABA and ethylene in different ways. Promoter analysis of each POD gene is required to understand the gene expression mechanism of POD isoenzymes. Characterization of the swpa2 promoter is under investigation based on environmental stress and the nature of the *cis*-elements and *trans*-acting factors involved in regulation of gene expression.

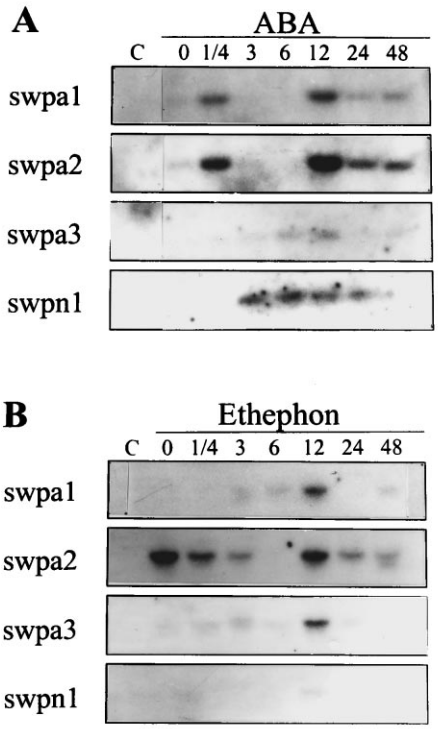


Fig. 2. Expression of sweet potato POD genes in leaves of sweet potato in response to abscisic acid (A) and ethephon (B). After ABA or ethephon (final conc. 0.1 mM) was applied to leaves, total RNA was isolated from leaves at 1/4, 3, 6, 12, 24, and 48 h after treatment (HAT). Equal amounts (40 μg) of each sample were loaded in each lane.

## 3. Experimental

### 3.1. Plant material and cell cultures

The SP-47 cell line selected by the small cell-aggregate method from cell suspension cultures of sweet potato was used (*I. batatas*) for a high POD yield (Kim et al., 1994). Cells subcultured at 10-day intervals (1 g fr. wt) were inoculated into 50 ml of LS (Linsmaier and Skoog, 1965) basal medium supplemented with 1 mg $l^{-1}$ 2,4-D and 30 $g^{-1}$ sucrose (LS1D medium) in a 300 ml conical flask and cultured for 21 days at 25°C in the dark (100 rpm). Sweet potato plants were grown in a greenhouse for two months after vegetative propagation.

### 3.2. Chemical treatment

After addition of either ABA (final conc. 0.1 mM) or ethephon (final conc. 0.1 mM) to the cultures at 11 DAS, cells were harvested 1, 3, and 10 DAT for preparation of total RNAs. The leaves were used for chemical treatment of intact leaves. An ABA or ethephon solution (final conc. 0.1 mM) was applied until the entire leaf was infiltrated. Total RNAs of the fourth leaf from the shoot tip were extracted 15 min, 3, 6, 12, 24, and 48 h after treatment. Authentic ABA and ethephon were purchased from Sigma and Wako Chemicals, respectively. Treatments were repeated three times.

### 3.3. POD genes and northern blot analysis

The POD cDNAs were isolated from suspension cultures of sweet potato (Huh et al., 1997; Kim et al., 1999). Total RNAs from suspension cells and leaves were extracted by the guanidium thiocyanate/CsCl method (Sambrook et al., 1989) and hybridized with cDNA specific probes using the 3′-untranslated sequence regions. Forty micrograms of total RNAs were fractionated on 0.8% agarose formaldehyde gel and transferred onto a nylon membrane by capillary transfer. Probes were labeled with [α-$^{32}$P]dCTP using a Rediprime Kit (Amersham). Hybridization was carried out at 65°C for 18 h in a solution of 6 × SSPE, 0.5% SDS, 10 × Denhardt's solution, and denatured salmon sperm DNA (100 μg/ml). The membranes were rinsed once with 2 × SSC containing 0.5% SDS at room tem-

perature, then washed twice with 0.1 × SSC containing 0.1% SDS at 65°C.

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