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Indole-3-butyric acid suppresses the activity of peroxidase while inducing adventitious roots in *Cinnamomum kanehirae*

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ABSTRACT. Indole-3-butyric acid (IBA) effectively promoted rooting in *Cinnamomum kanehirae*. The peroxidase (POX) activity significantly decreased in the IBA-treated tissues as compared with the controls. The lower amounts of lignin content in IBA-treated cuttings is correlated with the decline of POX activity. We suggest that the inhibition of POX activity may lead to the redifferentiation processes induced by IBA, which produces the new root primordia during the formation of adventitious roots. In this investigation, we also cloned POX cDNA from the young roots. A full-length cDNA of the POX gene designated was cloned by 5′ and 3′ RACE. According to the analysis of the promoter elements of *CKPX3*, we found some elements related to auxin response, lignification, pathogen invasion and stress response. Our results suggest that *CKPX3* may have auxin responsive elements within its promoter which are suppressed by auxin response factors that are regulated by exogenously applied IBA during the adventitious rooting.

Keywords: Adventitious root; *Cinnamomum kanehirae*; Indole-3-butyric acid; Lignification; Peroxidase.

Abbreviations: IAA, indole-3-acetic acid; **IBA,** indole-3-butyric acid; **NAA,** naphthaleneacetic acid; **PCR,** polymerase chain reaction; **POX,** peroxidase.

INTRODUCTION

Cinnamomum kanehirae Hayata is an endemic timber species used in the manufacture of high quality furniture in Taiwan. The exploitation of *C. kanehirae* has resulted in its population's rapid decline. In addition to its entomophilous characteristic and not easily to collect sufficient seeds, cutting seedlings are used as planting materials in silvicultural practices. Adventitious root formation, an obligatory phase of plant vegetative propagation, involves a complex process of redifferentiation, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primodia (Aeschbacher et al., 1994). Generally, adventitious root formation leads to three recognition phases: the induction, initiation and expression phases (Gaspar et al., 1992). These series phases are associated with changes in endogenous auxin concentration. In most cases, high levels of IAA are associated with the promotion of adventitious roots (Sagee et al., 1992).

Auxin is involved in root growth and developmental processes, such as root elongation, lateral root and adventitious root formation. Recent reports described how auxin and mechanical signals regulated the spacing of pericycle founder cells, then regulated the patterning of new lateral root primordia (Dubrovsky et al., 2008; Ditengou et al., 2008; Peret et al., 2009). Lanteri et al. (2008) found that nitric oxide triggered phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber.

Class III plant peroxidase (EC 1.11.1.7) (POX) are ubiquitous, heme-containing glycosylated isozymes. These enzymes catalyze the reduction of H_2O_2 from numerous electron donors such as phenolic compounds, lignin precursors, auxin or secondary metabolites (Hiraga et al., 2001). They are mainly localized in cell walls and vacuoles, implicated in a broad range of important physiological responses, including structural protein deposition, phenylpropanoid cross-linking of pectins, formation of lignin and suberin (Christensen et al., 1998; Quiroga et al., 2000), and auxin metabolism (Gazaryan et al., 1999). Many studies on adventitious root formation have suggested a fundamental role of POXs in controlling rooting. Changes in POX activity and POX isoform patterns have been proposed as biochemical markers of the successive rooting phases (Syros et al., 2004).

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The POX-dependent production of phenolic radicals often results in the generation of reactive oxygen species (ROS) and has been reported to catalyze H_2O_2 dependent and H_2O_2 independent mechanism for ROS generation, respectively. Reactive oxygen species (ROS) produced as intermediates in the reduction of O_2 to H_2O (superoxide radical, hydrogen peroxide, hydroxyl radical), are generally regarded as harmful products of oxygenic metabolism causing cell damage in plants. However, oxygen radical chemistry can also play a useful role if it takes place outside of the protoplast. The oxygen radical chemical mechanism of cell wall loosening involved in extension growth of plant organs has been studied mostly by analyzing auxin-induced growth responses. From the kinetics of auxin-induced coleoptile growth it can be predicted that the wall loosening reaction is initiated by the hormone (Schopfer et al., 2006).

Gaspar et al. (1992) have observed a direct relationship between the time of maximum POX activity and that of the formation of the meristematic structures. A typical minimum of POX activity appears at the root inductive phase, while the peak of activity within the subsequent increase marks the end of the root initiation phase and the beginning of the protrusion phase (Gaspar et al., 1992). Chen et al. (2002) indicated that a decline in POX activity in NAA-treated tissues was accompanied by a decrease in lignin content during the induction of adventitious roots in soybean hypocotyls. Chao et al. (2001) reported that a decrease in POX activity corresponded with a rise in endogenous IAA levels in IBA-treated tissues during the formation of roots in soybean hypocotyls.

Lignin is a complex phenolic polymer of the cell wall and the second most abundant organic substance in plant. POX isozymes are widely believed to be responsible for the final enzymatic step in lignification. The relationship between POXs and the formation of lignin have been investigated biochemically and cytochemically in many plants, such as zucchini (Carpin et al., 1999), poplar (Christensen et al., 1998), soybean (Chao et al., 2001), and *Ebenus cretica* (Syros et al., 2004). Despite of the importance of *Cinnamomum kanehirae* in Taiwan forestry, we still have little knowledge about the physiological and biochemical properties of *C. kanehirae* during the formation of adventitious roots. In this investigation, we examined the effect of indole-3-butyric acid on the change in lignin content and POX activity during the induction of adventitious roots. In order to understand the regulation of POX by hormones and other environmental signals, we also cloned the POX gene and *C. kanehirae's* potential regulatory regions.

MATERIALS AND METHODS

Plant materials

One-year-old branches of *Cinnamomum kanehirae*, each 15-20 cm in length and with 3-5 leaves were collected for the rooting experiments. After 10 min treatment (2000 ppm, indole-3-butyric acid) and under controlled environmental conditions (25±2°C), the branches were planted in the rooting media in the greenhouse. The rooting media was composed of equal amounts of sphagnum, peat moss and vermiculite. Adventitious rooting was observed and measured on days 10 and 15. The cutting's basal sections, each about 2 cm in length, were analyzed biochemically, primarily for their POX activity and lignin content.

Peroxidase activity assay

Peroxidase (EC 1.11.1.7) activity was determined spectrophotometrically by measuring the increase in absorbance at 470 nm after 20 min incubation at room temperature (Beffa et al., 1990). The reaction mixtures contained 25 μL of 50 mM H_2O_2 , 5 μL of 250 mM guaiacol, 195 μL of 12.5 mM 3,3-dimethyglutaric acid-NaOH (pH 6.0) and 25 μL of enzyme extract.

Isoelectric focusing gel electrophoresis and in situ peroxidase staining

0.3 g basal segments of *Cinnamomum kanehirae* cuttings were homogenized in 1.5 mL of 10 mM phosphate buffer (pH 6.0). The homogenates were centrifuged at 14,000 *g* for 20 min and the supernatant was saved for analysis. Tissue extracts were subjected to analytical flat bed isoelectric focusing on polyacrylamide gels containing ampholines in the pH range 3.5 to 9.5 (Pharmacia LKB, Sweden). The samples were subjected to electrophoresis for 1.5 h at 30 W at 10°C. After that, the gels were soaked in 500 mL of phosphate buffer for 30 min on shaker (70 rpm) to remove ampholines and equalize the pH. The POX isozymes were stained *in situ* by soaking the gel for 10 min in 200 mL of the phosphate buffer containing 0.6 mg/mL 4-chloro-1-naphthol, and 0.16% H_2O_2. Protein concentration was measured according to the Bio-Rad protein assay with micro-title plate. The linear range of the assay is 0 μg mL^{-1} to approximately 20 μg mL^{-1} using bovine serum albumin as a standard. 160 μL of each standard and sample solution was pipetted into separate micro-title plate wells into which 40 μL of dye reagent was added. The sample and reagent were then combined and the reaction mixture was incubated at room temperature for 20 min. The absorbance at 595 nm was measured.

Lignin determination

Lignin was extracted by the Bruce and West method (1989). The sample (0.5 g) was then homogenized in 3 mL of 99.5% ethanol and the extract centrifuged at 10,000 *g* for 30 min. The pellet was transferred to a glass petri dish and air dried. 50 mg of the dried residue was put in a screw-cap tube, then 750 μL of 2 N HCl and 250 μL of thioglycolic acid were added. The sealed tube was heated at 100°C for 4 h. After cooling to 4°C, the contents were centrifuged at 12,000 *g* for 30 min. The pellet was washed once with 2.5 mL of water, then resuspended in 1 mL of 0.5 N NaOH. The solution was agitated gently at 25°C for 18 h. After centrifugation at 12,000 *g* for 30 min, the

supernatant was transferred to a test tube. One mL of concentrated HCl was added to the test tube and the lignin thioglycolate was allowed to precipitate at 4°C for 4 h. After centrifugation at 10,000 *g* for 30 min, the pellets were dissolved in 10 mL of 0.5 N NaOH. The absorbance was measured against a NaOH blank at 280 nm. The amount of lignin was calculated from a linear calibration curve (0-20 µg) with commercial alkali lignin (Cat. -Nr. 37,095-9 Aldrich; Steinheim, Germany) (Müsel et al., 1997).

RNA isolation and reverse transcription

Total RNA was prepared from frozen young adventitous roots (0.2 g) (-80°C) by the plant total RNA miniprep system (Viogene Co. CA. USA) and quantified by UV absorption at 260 nm. There are two reactions to convert total RNA (4 µg): 3′ RACE-ready cDNA library with 3′ CDS primer A (Clontech Co. CA. USA), the other, 5′ RACE-ready cDNA library with 5′ CDS primer and BD SMART II A oligo (Clontech Co.). Both of them were incubated at 70°C for 10 min then quickly chilled on ice. The two mixtures were added individually to obtain a 20 µL reaction volume containing 4 µL 5 × RT buffer, 10 mM DTT, and 0.5 mM each of dNTP and 10 U of SUPERSCRIPTTM II RNase H Reverse Transcriptase (Life Technologies Co. CA. USA). The contents were incubated at 42°C for 50 min, and stopped by heating at 70°C for 15 min. The solutions were stored at -20°C.

Cloning of the *CKPX* full length cDNA

According to the conserved domains found in plant class III peroxidase, with the 3′ RACE-ready cDNA library from *Cinnamomum kanehirae* as a template and the primers pxp6 (5′-CCYGTYCCRTTRAARTCRTA-3′, encoding the reverse of the peptide YDFNGTG) (Christensen et al., 2001) and pxp4 (5′-CAYTTYCATGAYTGTTTYGT-3′, encoding peptide HFHDCFV), a PCR reaction was performed in 10X Advantage 2 PCR buffer, 2.5 mM dNTP, and 50X BD Advantage 2 polymerase mix (Clontech Co.) in a reaction volume of 20 µL. The reaction mixture was subjected to 35 cycles of PCR amplification on a Biometra Tgradient under the following conditions: denaturation at 94°C (30 s), annealing at 45°C (30 s) and extension at 68°C (90 s). A PCR product was excised from an agarose gel, cloned in the pGEM-T vector (Promega Co. WI. USA), and sequenced.

Rapid Amplification of cDNA Ends (RACE)

The 3′ and 5′ ends of cDNA were isolated by SMART™ RACE cDNA amplification kit (Clontech Co.). With the 3′ RACE-ready cDNA library as the template and the primers GSP8 (5′-TCGCCAACAAAGGCCTCAATGTCAC-3′) and UPR (Clontech Co.), annealed at the 3′ end. With 5′ race-ready cDNA library as a template and the primers GSP3 (5′-CCAGCCAACGATAGGTTTGGGATTG-3′) and UPR (Clontech Co.), annealed at the 5′ end. Two mixtures were in PCR reactions 20 µL, composed as described above, except for primers and nucleotides. PCR reactions were performed as follow: 3 cycles of 94°C (30 s), 70°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 66°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 63°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 61°C (45 s), 68°C (1 min); 20 cycles of 94°C (30 s), 59°C (45 s), 68°C (1 min). PCR products were excised from an agarose gel, cloned in the pGEM-T vector (Promega Co.), and sequenced.

According to the sequence of 3′ and 5′ RACE result, the full length cDNA, *CKPX*, were produced by a PCR with 3′ RACE-ready cDNA library as template and primers FCK1-R (5′-TGAACGGTACAAAACGACACA-3′) and FCK1-L (5′-TAGTTAAGAGAGGCTGTTTCCTGAG-3′). PCR reactions were performed as follow: 3 cycles of 94°C (30 s), 64°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 60°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 58°C (45 s), 68°C (1 min); 3 cycles of 94°C (30 s), 56°C (45 s), 68°C (1 min); 20 cycles of 94°C (30 s), 52°C (45 s), 68°C (1 min) with the final extension 68°C (10 min). PCR products were excised from an agarose gel, cloned in the pGEM-T vector (Promega Co.), and sequenced.

PCR cloning of *CKPX3* promoter sequence

Total DNA was prepared from frozen young adventitous roots (0.2 g) (-80°C) using the plant genomic DNA extraction Miniprep System (Viogene, CA. USA). The GenomeWalker method (Clontech Co.) was modified to isolate the promoter sequence. According to the digestion results, restriction endonucleases, *Eco*RV, *Pvu*II, *Stu*I and *Dra*I, were used to generate several DNA fragment libraries, which were separately ligated to corresponding adaptors. Adaptor-ligated genomic DNA fragment libraries were subjected to an initial round of PCR amplification with the outer adaptor primer AP1 and an outer gene-specific primer GSP3 (5′-CCAGCCAACGATAGGTTTGGGATTG-3′) while an inner adaptor primer AP2 and inner gene-specific primer WGSP1 (5′-GCAGCCCCTAACAAAGCAGTCATGGAA-3′) were used for the second round of PCR amplification. PCR products were excised from an agarose gel, cloned in the pGEM-T vector (Promega Co.), and sequenced.

RESULTS

Effect of indole-3-butyric acid on number of roots, peroxidase activity and lignin content during adventitious rooting

As shown in Table 1, adventitious roots were observed in both the control and IBA-treated cuttings of *Cinnamomum kanehirae.* Rootlet emergence was observed on day 15. The rooting ability of IBA-treated cuttings was three times greater than that of the control.

POX activity during adventitious rooting was determined both in the control and IBA-treated cuttings of *Cinnamomum kanehirae.* Figure 1 shows that on day 5, the POX activity in IBA-treated cuttings was much lower than that of the control after treatment. POX activity of both the

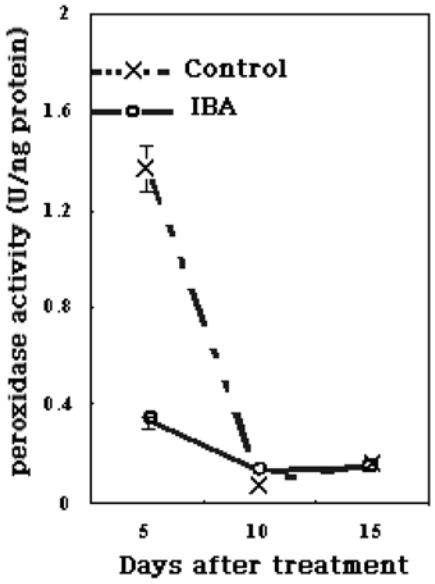

Figure 1. Effect of indole-3-butyric acid on the peroxidase activity during the adventitious root formation in *Cinnamomum kanehirae* cuttings. One unit of peroxidase activity was defined as the amount enzyme that causes the formation of 1 nmole tetraguaiacol per min (extinction coefficient is 26.6 $mM^{-1}cm^{-1}$ at OD. 470). Values are the means ± SE of three replicates.

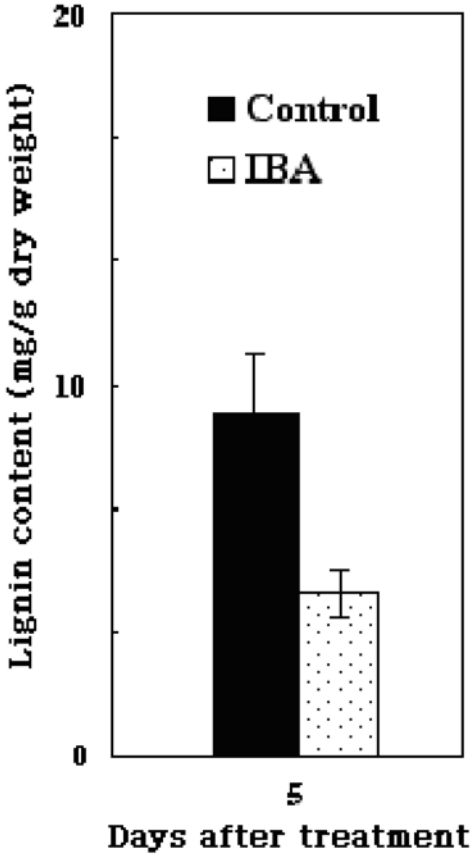

Figure 2. Effect of indole-3-butyric acid on the lignin content during the adventitious root formation in *Cinnamomum kanehirae* cuttings. Values are the means±SE of three replicates.

control and IBA-treated cuttings decreased to minimum levels on day 10, most likely the inductive phase. Meanwhile, in IBA-treated cuttings on day 5, the lignin content was lower than that measured for the control tissues (Figure 2).

2 cm long segments of basal *Cinnamomum kanehirae* cuttings were homogenized, extracted and subjected to polyacrylamide gels for isoelectric focusing electrophoresis. As shown in Figure 3, four isozymes of POX (pI 4.8, pI 4.5, pI 4.0, and pI 3.8) identified by isoelectric focusing were present in the extract. On day 5, the activity of all POX isozymes was lower in IBA-treated tissues than in control tissues (Figure 3), whereas on day 10, only the pI 4.8 POX in the IBA-treated cuttings showed decreased activity. As the duration of reatment was extended, the activity of all POX isozymes increased, performing similarly.

Sequence analysis of *CKPX3*

As shown in Figure 4, *CKPX3* encoded a polypeptide

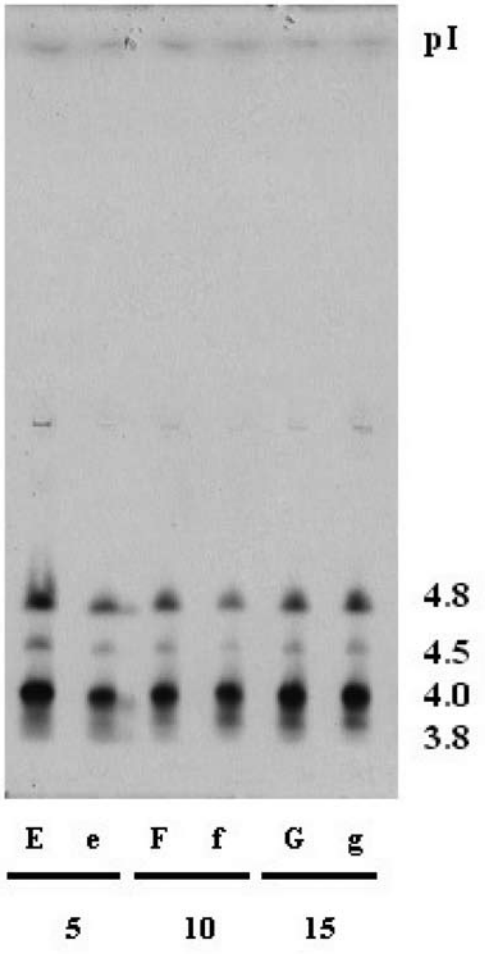

Figure 3. Effect of indole-3-butyric acid on the activities of the peroxidase isozymes during the adventitious root formation of *Cinnamomum kanehirae* cuttings, Lane E (day 5, control), Lane e (day 5, IBA-treated), Lane F (day 10, control), Lane f (day 10, IBA-treated), Lane G (day 15, control), Lane g (day 15, IBA-treated). Isolectric points of peroxidases are marked on the right.

of 325 amino acids. The deduced amino acid sequence of *CKPX3* was aligned to a consensus sequence of class III plant POXs, eight conserved Cys residues for disulfide bridges, a proximal and a distal catalytic His, and all other catalytic residues (Gajhede et al., 1997), then compared with the previously reported POXs using NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) and Bioedit. However, within the same plant species, total amino acid sequence identity among the same class of peroxidase can be lessthan 35% (Welinder et al., 2002). According to this data, *CKPX3* should be considered a member of class III POXs.

The mature *CKPX3* protein had a calculated molecular mass of 35 kDa, but its calculated pI was 6.0. *CKPX3* was also analyzed by Kyte-Doolittle (http://gcat.davidson.edu/rakarnik/kyte-doolittle.htm). The secondary structure of *CKPX3* was predicted through Psipred (http://bioinf.cs.ucl.ac.uk/psipred/). Like all previously isolated sequences of class III POXs, *CKPX3* contained fourteen helix structures which were connected with coil structures. The active sites of *CKPX3* were involved in the third, sixth and ninth helixes. The first helix contained the signal peptide deduced to directly lead *CKPX3* secretion through iPSORT prediction (http://www.psort.org/). *CKPX3* encoded eight putative N-glycosylation sites (NX (S/T) X; X≠P) (Christensen et al., 2001). The *CKPX3* sequence was deposited in the GeneBank database under accession number EF543197.

Table 1. Effect of indole-3-butyric acid on the number of adventitious roots in *Cinnamomum kanehirae* cuttings.

	Control	IBA-treated
Day 10	0	0
Day 15	1.5±0.5	4.5±0.5

Values are the mean±SE of three replicates.

Analysis of the *CKPX3* promoter sequence

Sequence analysis of the *CKPX3* promoter revealed elements common to most plant promoters as well as several potential regulatory elements through PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html), PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and the reports of Klotz et al. (1996) and Ito et al. (2000) (Table 2). The *CKPX3* promoter contained *cis* elements that were related to gibberellin and auxin regulation and two elements related to lignin synthesis Box-P (da Costa e Silva et al., 1993) and maize *P* (Grotewold et al., 1994). The *CKPX3* promoter also contained pathogen defense elements, TC rich repeats and TCA elements. The analysis also revealed that the *CKPX3* promoter may respond to light.

DISCUSSION

Exogenous auxins are reported to stimulate the formation of adventitious roots in many plants. In our investigation, the exogenously applied IBA effectively promoted rooting in *Cinnamomum kanehirae* cuttings in comparison with the control (Table 1). Previous researchers have reported that auxin-induced changes in POX occurred during the rooting process (Chao et al., 2001; Chen et al., 2002). In our studies, POX activity was significantly suppressed

Table 2. Main *cis*-elements present in the upstream of the *CKPX3* gene.

Element	Sequence	Function	Positions for *CKPX3*	Reference
ARE	TAGTNCTGT	Auxin binding element	-389	Klotz et al., 1996
ARE	T/G GTCCAT	Auxin response element	-781, -772	Klotz et al., 1996
Maize P	CCWACC	A *myb*-related transcription factor	-661	Ito et al., 2000
BOX P	ACMWAMC	Part of phenylpropanoid gene promoter	-873	Ito et al., 2000
GARE motif	AAACAGA	Part of GA response complex	-877	PLACE and PlantCARE.
TC rich repeats	GTTTTCTTAC	Cis-acting element involved in defense and stress responsiveness	-998, -952	PLACE and PlantCARE.
TCA element	CAGAAAAGGA	Cis acting element involved in salicylic acid responsiveness	-819	PLACE and PlantCARE.
HSE	AAAAAATTC	Cis acting element involved in heat stress	-725	PLACE and PlantCARE.
CGCG box	VCGCGB	A calmodulin-binding box	-870, -870(-)	PLACE and PlantCARE.
ABRE related sequence	MACGYGB	Ca^{2+} responsive cis elements	-870(-)	PLACE and PlantCARE.
ABRE	ACGTG	Different novel cis acting elements inducing by dehydration stress and dark-induced senescence	-1010, -379	PLACE and PlantCARE.
BOX 4	ATTAAT	Part of a light response element	-1155	PLACE and PlantCARE.
ARE	AGAAACAT	Part of a light response element	-945(-)	PLACE and PlantCARE.

*W=A/T , N=A/T/C/G, M=C/A, V=A/C/G, Y=T/C, B=T/C/G.

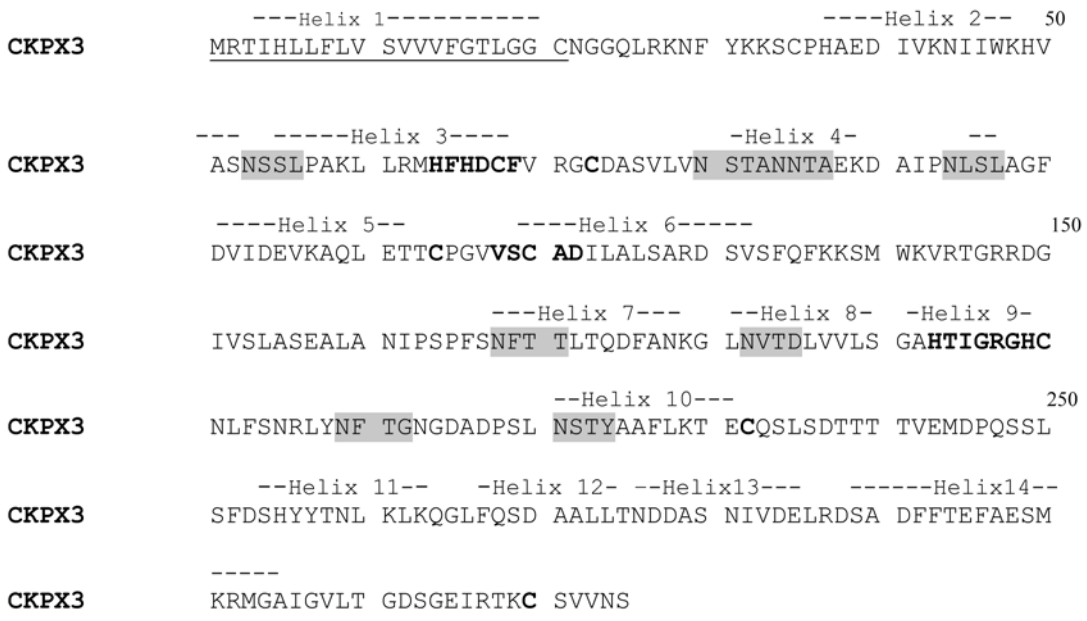

Figure 4. The predicted secondary structure of *CKPX3*. Totally and highly conserved POX residues, eight cysteines and three conserved domains, are indicated in the sequence of *CKPX3* with a dark black. Propetide is underlined. Putative N-glycosylation sites (NX (S/T) X; (X≠P)) are highlight with gray.

by the application of IBA during the induction of adventitious roots on day 5. The POX activity showed a peak after a prior passage through a minimum POX activity on day 10 (Figure 1). Similar results were observed *in vitro* cuttings of grapevine, *Ebenus cretica's* rooting genotype (Gasper et al., 1992; Syros et al., 2004). According to the Gaspar group (Gaspar et al., 1992), the induction period is characterized by a sharp drop in POX activity. In our studies, in IBA-treated cuttings, the lower amounts of lignin correlate with the decline of POX activity (Figures 1 and 2). On day 5, the activity of the four POX isozymes (pI 4.8, pI 4.5, pI 4.0 and pI 3.8) significantly decreased in IBA-treated tissues in comparison with the control tissue (Figure 3). Among the POX isozymes, the anionic POX is most likely involved in lignification (Lagrimini et al., 1997). We thus suggest that the inhibition of lignification in the IBA-treated *Cinnamomum kanehirae* cuttings may be partially due to the decline of POXs activity. Furthermore, the inhibition of POX may lead to the redifferentiation processes induced by IBA, then produce the new emerged roots while the adventitious roots are being formed (Aeschbacher et al., 1994; Chao et al., 2001).

In this investigation, we isolated *Cinnamomum kanehirae* POX cDNA to study the relationship between its POX gene and its rooting. According to the data in Figure 4, *CKPX3* should be a member of class III POX. The deduced amino acid sequence of *CKPX3* contained eight putative N-glycosylation sites suggesting a solubility or stabilizing function (Nigel, 2004). The calculated pI of *CKPX3* is close to the pI 4.8 POX that was induced during adventitious rooting in *Cinnamomum kanehirae*. The pI value of native POX may be different from that of deduced POX due to the number of glycans (Christensen et al., 2001). In this investigation, the pI value of POX shown on IEF gel is not exactly the same as the pI values of POX deduced from the specific POX cDNA.

As far as the regulation of pI 4.8*CKPX3* in *Cinnamomum kanehirae* is concerned, we found that the 5′ upstream of pI 4.8*CKPX3* contained two elements related to lignification and two elements related to auxin response (Table 2). Klotz and Lagrimini (1996), and Chou et al. (2010) reported that auxins suppressed POX gene expression by auxin response factors on regulating the multiple auxin responsive elements within the POX gene promoter. In our studies, we reported that the decrease of POX activity accompanied the lower amounts of lignin content in IBA- treated tissues. Moreover, the Box-P element, a cis-acting element was identified in the *CPKX3*, and this element also existed in other genes that code for enzymes important in the biosynthesis of lignin, lignans and phenylpropanoids including phenylalanine ammonia-lyases, 4-coumarate: CoA ligases, and cinnamyl alcohol dehydrogenases (Ito et al., 2000; Habberer et al., 2006). Therefore, auxin might inhibit lignification in the IBA-treated *C. kanehirae* cuttings by inhibiting the expression of *CKPX3* in the inductive phase.

In addition to the auxin binding and lignification elements, the promoter of *CKPX3* contains several light response elements, GA response elements, cis-acting elements involved in salicylic acid responsiveness, and cis-acting elements involved in defense and stress response (Table 2). According to the analysis of *CKPX3* promoter, we can understand that POXs are involved in a broad range of physiological roles. A few of these are auxin

metabolism, lignin and suberin formation, and defense against pathogens, (Klotz and Lagrimini, 1996; Passardi et al., 2005).

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牛樟不定根誘發過程中吲哚丁酸抑制過氧化酶之活性

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吲哚丁酸（IBA）有效地促進牛樟發根，在 IBA 處理的組織內過氧化酶（POX）的活性明顯比對照組小。IBA 處理的組織內較低的木質素含量和下降的 POX 活性相關。我們認為在不定根的誘發過程，IBA 抑制 POX 活性可能導致組織細胞逆分化，繼而產生新根。我們亦從牛樟幼根選殖並定序一新的 POX cDNA，*CKPX3*，連帶它的調控區域。*CKPX3* 調控區內包括生長素對應元素（auxin response element）、木質化元素、病源入侵及抗逆境元素等。我們的研究認為 *CKPX3* 調控區內的生長素對應元素可能在不定根形成過程中因外加 IBA 的調節，並透過組織細胞內生長素反應因子（auxin response factors）抑制 POX 基因的表現。

關鍵詞：不定根；牛樟；吲哚丁酸；木質化；過氧化酶。