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Lipoxygenase gene expression is modulated in plants by water deficit, wounding, and methyl jasmonate

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Summary. Two classes of lipoxygenase (LOX) cDNAs, designated *lox*A and *lox*B, were isolated from soybean. A third lipoxygenase cDNA, *lox*P1, was isolated from pea. The deduced amino acid sequences of *lox*A and *lox*B show 61–74% identity with those of soybean seed LOXs. *lox*A and *lox*B mRNAs are abundant in roots and non-growing regions of seedling hypocotyls. Lower levels of these mRNAs are found in hypocotyl growing regions. Exposure of soybean seedlings to water deficit causes a rapid increase in *lox*A and *lox*B mRNAs in the elongating hypocotyl region. Similarly, *lox*P1 mRNA levels increase rapidly when pea plants are wilted. *lox*A and *lox*B mRNA levels also increase in wounded soybean leaves, and these mRNAs accumulate in soybean suspension cultures treated with 20 μM methyl jasmonate. These results demonstrate that LOX gene expression is modulated in response to water deficit and wounding and suggest a role for lipoxygenase in plant responses to these stresses.

Key words: Lipoxygenase – Soybean – Pea – Methyl jasmonate – stress responses

Introduction

Lipoxygenases (LOXs) are non-heme iron-containing enzymes which catalyze the hydroperoxidation of fatty acids having *cis, cis*-1,4-pentadiene structures. These enzymes are widely distributed and have been reported in higher plants, mammals, fungi, and algae (Hildebrand et al. 1988). In mammals, LOX catabolizes arachidonic acid to produce a number of biologically active compounds including the leukotrienes, which are potent modulators of smooth muscle contraction and inflammatory responses (Needleman et al. 1986). In plants, LOX has been proposed to play a role in senescence, pathogen and wound responses, biosynthesis of the plant growth regulators abscisic acid and jasmonic acid, and mobilization of lipid reserves during seed germination (Vick and Zimmerman 1987; Hildebrand et al. 1988). The multiple functions ascribed to this enzyme are consistent with heterogeneity in plant LOX isozymes, which vary in isoelectric point, pH optima, heat stability, response to Ca^{2+}, and primary product formed (Vick and Zimmerman 1987). In soybean, three isozymes (LOX1, LOX2, LOX3) which are highly expressed during seed development have been characterized, and the genes encoding these enzymes have been cloned (Shibata et al. 1987, 1988; Yenofsky et al. 1988). A different set of LOXs which appear during germination has been described (Park and Polacco 1989), and characterization of vegetatively expressed genes is beginning (Shibata et al. 1991).

The accumulation of information on LOX activity suggests a role for LOX in plant stress responses. For example, lipoxygenase activity is increased when plants are wounded (Hildebrand et al. 1988). This increase may stimulate the production of traumatic acid and jasmonic acid in wounded tissue because LOX converts linolenic acid into precursors of these compounds (Zimmerman and Coudron 1979; Vick and Zimmerman 1983). Either wounding or exposure to methyl jasmonate increases expression of tomato proteinase inhibitors, which are thought to be involved in plant defense (Graham et al. 1986; Farmer and Ryan 1990). In addition, plant pathogens induce LOX activity and a role for LOX in the plant's hypersensitive response has been proposed (Ocampo et al. 1986). LOX has also been implicated in the synthesis of abscisic acid (ABA), a growth regulator which accumulates when plant tissues are dehydrated (Vick and Zimmerman 1987).

The increases in LOX activity in stressed tissues could be due to activation of pre-existing enzyme or may involve activation of LOX gene expression. In order to examine this latter possibility, we have isolated cDNAs corresponding to two LOX genes expressed in soybean seedlings and one cDNA corresponding to a gene expressed in pea plants. The characterization of these cDNAs and expression of the corresponding genes in

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response to wounding, methyl jasmonate, and water deficit is described.

Materials and methods

Plant material. Soybean (*Glycine max* [L] Merr. cv. Williams) seedlings were grown, treated, and harvested as described by Mason et al. (1988). Three-day-old seedlings were dissected into the following sections: hypocotyl hook (first 5 mm below cotyledon), elongating hypocotyl (15 mm section below hook), mature hypocotyl (remainder of stem), root tip (terminal 15 mm of root) and mature root (remainder of root). Soybean plants (for wounding) were grown for 25 days at 28° C with 18 h light, 6 h dark cycle. Soybean suspension cultures were grown in MS salts (Sigma) plus 0.2 mg/l kinetin, 0.1 mg/l thiamine, 1 mg/l naphthalene acetic acid, and 1% sucrose at pH 5.8 with continuous shaking and illumination. In some experiments 0.5 mM gluathione, 1 mg/ml polygalacturonic acid, 20 μM ABA or 20 μM methyl jasmonate (Bedoukian Research, Danbury, Conn.) was added to the cultures. Pea (*Pisum sativum* Progress No. 9) plants were grown for 4 days in Perlite, then transferred to hydroponic solutions and grown in continuous light for 7 days. Plants were subjected to osmotic stress by immersing roots in 0.6 M mannitol for 4 h with continuous illumination.

DNA amplification. DNA was amplified in 50 mM KCl, 10 mM TRIS-HCl (pH 9), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton-X100, 200 μM each dNTP, 1.5 μg each primer, 10 ng template, and 2.5 units *Taq* DNA polymerase (Promega). A series of 35 cycles of 94° C for 1 min, 41° C for 2 min, and 72° C for 2 min was performed, followed by 10 min at 72° C. Amplified products were separated from unincorporated primers and dNTPs using spin column chromatography prior to radiolabelling.

Nucleic acid analysis. For cDNA screening, plaques were lifted onto BAS85 membranes (Schleicher and Schuell), and hybridized in 6 × SSC, 5 × Denhardt's solution, 0.2% SDS, and 100 μg/ml salmon sperm DNA at 65° C. Filters were washed in 2 × SSC, 0.1% SDS at 23° C, followed by a 65° C wash in 0.1 × SSC, 0.1% SDS. For nucleic acid isolation, 0.2–1.0 gm of frozen tissue was pulverized in liquid N_2 and suspended in 4 ml of 0.2 M TRIS-HCl (pH 8.5), 0.3 M NaCl, 20 mM EDTA, 1% SDS. The solution was extracted once with 2 volumes phenol:chloroform (50:50), followed by two ethanol precipitations. Nucleic acid was fractionated for RNA analysis in agarose gels, transferred to Gene-screen (DuPont), and hybridized as described above. Probes used for all hybridizations were labelled using a random primer kit. The *lox*A probe was the 1408 bp internal *Eco*RI fragment of *lox*A. The *lox*B probe was the 2300 bp *Eco*RI fragment of *lox*B2, the 3′ end of which is shown in Fig. 1. The derived amino acid sequence of *lox*P1 (Fig. 2B) is from the 3′ end of the fragment used as the *lox*P1 probe. The probes con1 and con3 correspond to cDNAs encoding unidentified genes from soybean and pea respectively. The con2 probe encodes a soybean B-tubulin (Creelman and Mullet 1991).

Results

Isolation of LOX cDNAs and sequence analysis

Creelman and Mullet (1991) generated cDNA using mRNA isolated from the elongating hypocotyl region of etiolated soybean seedlings grown in well watered (control) or low water potential (LWP, $\psi_w = -0.3$ MPa) vermiculite for 24 h. We used these cDNAs as templates in polymerase chain reaction (PCR) amplifications using primers which correspond to conserved regions of soybean seed LOX sequences. The sequence of primer 1 is 5′-CACCCAATTTA(T/C)AAGCTTCT, and the sequence of primer 2 is 5′-ATAGTTCTCAAA-TAAGCCTT. The putative annealing site for each primer is indicated in Fig. 1. The cDNAs from control and LWP tissues were also used to construct libraries, which were screened using the LOX PCR products. About 3% of the plaques screened in the LWP library hybridized to the probe made by amplifying LWP cDNA using LOX primers. About 0.03% of the plaques screened from the control library hybridized to the probe made by amplifying control cDNA using LOX primers. Twelve cDNA clones, 10 from the LWP library and 2 from the control library, were selected for further characterization. Based on DNA sequence information (200–300 bp/clone), these 12 cDNAs were placed into two classes designated *lox*A and *lox*B. Seven cDNAs from the LWP library, all with identical sequences, constituted *lox*A. The largest of these was chosen for more extensive sequence analysis. Four cDNAs, two from the LWP library and two from the control library, all with identical sequence, constituted *lox*B1. The remaining cDNA from the LWP library was designated *lox*B2.

The DNA sequences of *lox*A and portions of *lox*B1 and *lox*B2 are shown in Fig. 1. *lox*B1 and *lox*B2 are 96% identical to each other and 64% identical to *lox*A in the region sequenced. At the amino acid level, the *lox*B1 and *lox*B2 segments are 96% identical to each other and 61% identical to *lox*A. The alignment of the deduced amino acid sequences of *lox*A (a partial clone) and the *lox*B2 segment to each other and to published soybean seed LOX sequences is shown in Fig. 2A. At the amino acid level, *lox*A is 70–74% identical and *lox*B2 is 61–70% identical to seed LOXs. While this paper was in preparation, Shibata et al. (1991) published the sequence of a soybean LOX gene whose derived amino acid sequence is identical to the *lox*A sequence shown (Fig. 2A) except for the first three amino acids of *lox*A.

A cDNA library made from RNA isolated from wilted pea shoots (Guerrero and Mullet 1988) was also screened with the LOX PCR probe described above. One cDNA clone, designated *lox*P1, was isolated and partially sequenced. As shown in Fig. 2B, the amino acid sequence deduced from *lox*P1 is similar to the corresponding regions of previously characterized soybean and pea LOX sequences (Shibata et al. 1988; Ealing and Casey 1988).

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A     1 gaattcggattttggtcacttgaagtcatcggatttccttacatacggaatcaaatccttatctcacg
A    69 atgtgatacctttgttcaaatctgcaatttttcaattaagggtcacatcgagtgagtttgaaagcttc
A   137 gaagatgtgcgtagtctctatgagggtggaattaaattgccaacagatatactgagccaaattagccc
A   205 cttaccagccctcaaggaaatcttccgcactgatggtgaaaatgttcttcaatttccaccacctcatg
A   273 tagccaaagttagtaagtctgggtggatgactgatgaggaatttgctagagaggtgattgccggtgta
A   341 aacccaaatgtgatccgtcgtcttcaagaattcccaccaaagagcacacttgatcccacactctatgg
A   409 tgatcaaaccagtaccataacaaaagaacaattggagattaacatgggaggggtcacagtagaagagg
A   477 cacttagtactcagagattattcatattagattaccaagatgcattcatcccatatttgacgaggata
A   545 aacagcctacctactgcaaaagcttatgccacaaggacaatcctgttcttgaaagatgatggtacttt
A   613 gaagccacttgctattgaattaagcaaaccacatccagatggagataatttgggtcctgagagtatag
A   681 tggtgttgcctgcaactgaaggtgttgatagtacaatttggctattggccaaggctcatgttattgtg
A   749 aatgactctggttatcatcagctcgtgagccattggttaaatactcatgcagtgatggaaccatttgc
A   817 catagcaaccaataggcatctcagtgtgctccaccctatttataaacttcttttatcctcactaccgcg
A   885 acaccataaatatcaatggacttgctaggcagtccctgattaatgcagatggcattatagagaaatca
A   953 tttttgcccggaaagtactccattgagatgtcttcatccgtctacaagaattgggttttcactgacca
A  1021 agcattaccagctgatctcgtcaagagaggattggcaattgaagatccctctgcaccccatggccttc
A  1089 gccttgtgatagaggactacccttatgctgttgatggacttgaaatatgggatgctattaagacatgg
A  1157 gttcatgaatatgtctccttgtattacccaacagatgcagcagttcaacaagatactgaactacaagc
A  1225 atggtggaaggaagctgtggagaagggtcatggtgacttaaaagaaaagccttggtggcctaaaatgc
A  1293 agacaactgaagatctcattcaatcatgctctattatcgtttggacagcttcggctctccatgctgct
A  1361 gttaattttggacaatatccttatgggggcttaatcctgaaccgtccaactctagccagaaggtttat
A  1429 cccagctgAAGGaaCcCCaGAgTATGATGaGaTgGtgAAGAAtCCTcAaAAGGctTaTcTGAgaACaA
B2     1         AAGGgtCtCCtGAaTATGATGcGtTaGctAAGAAcCCTgAgAAGGagTtTtTGAagACtA
A  1497 TcACaccCAAGtttGAGACCCTtATTGAtCTTtCAgTgATAGAgATaTTgTCAAGGCAtGCtTCTGAT
B2    61 TtACtggCAAGAAaGAGACCCTCATTGACCTTACAaTTATAGAAATtTTaTCAAGGCACGCATCTGAT
B1     1          AGAAgGAGACCCTCATTGACCTTACAgTTATAGAAATcTTgTCAAGGCACGCATCTGAT
A  1565 GAGaTaTACCTTGG  AGAGAG gGagacTccaaAtTGGACTaCTGATaagaaGgCATTAGAaGCtTT
B2   129 GAGTTCTACCTTGGGCAGAGAGATGGcGGTGACTACTGGACTTCTGATGCCGGGCCATTAGAGGCcTT
B1    60 GAGTTCTACCTTGGGCAGAGAGATGGtGGTGACTACTGGACTTCTGATGCCGGtCCATTAGAGGCaTT
A  1630 cAAaAGGTTTGGAAgcAAaCTgactGgaATTGAAggaAAaaTcAatGcaAgGAACAgTgATccaAgtc
B2   197 TAAGAGGTTTGGAAAGAAtCTTGAAGAGATTGAAAAGAAGCTTATAGAGAAGAACAATaATGAGACAT
B1   128 TAAGAGGTTTGGAAAGAAgCTTGAAGAGATTGAAAAGAAGCTTATAGAGAAGAACAAagATGAGACAT
A  1698 TGAGAAACCGgacTGGGCCaGtTcAgcTGCCaTACACtttGCTCcATCgTTCaAGTGAGGAaGGgTTG
B2   265 TGAGAAACCGCTATGGGCCgGCTAAAATGCCTTACACaaaGCTCTATCCTTCTAGTGAGGAGGGATTG
B1   196 TGAGAAACCGCTATGGGCCaGCTAAAATGCCTTACACtctGCTCTATCCTTCTAGTGAGGAGGGATTG
A  1766 ACTTTCAaAGGAATTCccaacagtatctctatctaattaaaaagagtttgtggtttccttatctatgt
B2   333 ACTTTCAGAGGAATTC
B1   264 ACTTTCAGAGGAATTC
A  1834 tgtacttctgaataacatagactttatgcatgtctatgcgtaattattgcctgaacccgaattc
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Fig. 1. Nucleotide sequence of *lox*A and partial sequences of *lox*B1 and *lox*B2. *Eco*RI sites are *underlined* and putative polymerase chain reaction primer annealing sites are indicated by *double underlining*. These annealing sites correspond to amino acids 521–527 and amino acids 731–737 in the deduced amino acid sequence of soybean seed LOX1 (Shibata et al. 1987)

Expression of vegetative LOX and response to water deficit

Total nucleic acid was isolated from soybean or pea tissues, fractionated in RNA gels, and blotted in order to examine LOX mRNA accumulation under a variety of growth conditions. The estimated sizes of the soybean mRNA species to which *lox*A and *lox*B hybridize are 2.7 and 2.5 kb, respectively, while *lox*P1 hybridizes to a pea mRNA species which is about 2.5 kb. Under the hybridization and wash conditions used, *lox*A and *lox*B DNA do not hybridize to each other and hybridize very weakly to nucleic acid isolated from dry soybean seeds (data not shown).

Examination of LOX mRNA levels in different tissues of either 3-day-old etiolated soybean seedlings or 11-day-old pea plants reveals a non-uniform distribution of these mRNAs. In the soybean seedlings, *lox*A and *lox*B mRNA accumulation is highest in the roots and is also abundant in the mature (non-elongating) hypocotyl, with lower levels detected in other tissues (Fig. 3A). In peas, *lox*P1 mRNA levels are high in the stem, with lower levels in leaves and roots (Fig. 3C).

Exposure of etiolated soybean seedlings to LWP conditions for 24 h results in altered LOX mRNA levels in some tissues. Both *lox*A and *lox*B mRNA levels increase in the elongating hypocotyl section (Fig. 3A). This mRNA increase occurs within 3 h following transfer of seedlings to LWP conditions, and is followed by a slow decline in LOX mRNA levels as LWP treatment continues (Fig. 3B). *lox*B mRNA levels decline in the mature hypocotyl, while levels of both mRNA types remain high in the roots (Fig. 3A). Exposure of pea plants to 0.6 M mannitol, which causes leaf wilting, also leads to a rapid increase in LOX mRNA. Within 4 h a dramatic increase in *lox*P1 mRNA levels is seen in

A

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 A    3  nsdFGHLKSSDFLTYGiKSlShdViPLfkSAiFqLrvTssEFeSFedVrsLYeGGIKLPTDIlSqIS
S3  260 RDEaFGHLKSSDFLTYGlKSvSQnVLPLlqSA FDLNFTPrEFDSFdeVhgLYsGGIKLPTDIISkIS
S2  271 RDENFGHLKSSDFLaYGiKSLSQyVLPAFES vFDLNFTPnEFDSFQDVrDLhEGGIKLPTeVISTIm
S1  242 RDENlGHLKSkDaLeiGtKSLSQiVqPAFES aFDLksTPiEFhSFQDVhDLyEGGIKLPrdVISTIi

 A   68 PLPaLKEIFRTDGEnvLqFPPPhVakVSKSgWMTDEEFAREviAGVNPNvIRrLqEFPPkStLDptlY
S3  327 PLPVLKEIFRTDGEQaLKFPPPkVIQVSKSAWMTDEEFAREMlAGVNPNlIRcLkEFPPrSkLDsqvY
S2  338 PLPVvKELFRTDGEQvLKFPPPHVIQVSKSAWMTDEEFAREMvAGVNPCVIRGLqEFPPKSNLDPtIY
S1  309 PLPViKElyRTDGqhiLKFPqPHVvQVSqSAWMTDEEFAREMiAGVNPCVIRGLeEFPPKSNLDPaIY

 A  136 GDqTStITKEqLEiNmgGvTVeEAlstqRLFiLdyqDafiPYLtRINslptaKAYATRTILFLKdDGT
S3  395 GDhTSqITKEhLEpNLeGlTVDEAiqnkRLFlLghHDpiMPYLRRIN aTstKAYATRTILFLKnDGT
S2  406 GeQTSKIT  ADaLDLDGYTVDEALaSRRLFMLDYHDvFMPYiRRINQ TyAKAYATRTILFLREnGT
S1  377 GdQsSKIT  ADsLDLDGYTmDEALgSRRLFMLDYHDiFMPYvRqINQlnsAKtYATRTILFLREdGT

 A  204 LkPLAIELSkPHPdGDnlGpeSiVvLPAtEGVdStIWLLAKAhViVNDSgYHQLVSHWLNTHAVmEPF
S3  462 LrPLAIELSLPHPqGDqSGAfSQVfLPAdEGVESsIWLLAKAYVVVNDSCYHQLVSHWLNTHAVvEPF
S2  471 LKPVAIELSLPHPAGDLSGAVSQViLPAKEGVESTIWLLAKAYVVVNDSCYHQLMSHWLNTHAViEPF
S1  443 LKPVAIELSLPHsAGDLSaAVSQVvLPAKEGVESTI atsKAYViVNDSCYHQLMSHWLNTHAamEPF

 A  272 aIATNRHLSVlHPIYKLLyPHYRDTiNINGLARqSLiNadGiIEksFLpGkYSiEMSssVYKnWVFTD
S3  530 IIATNRHLSVvHPIYKLLhPHYRDTMNINGLARlSLvNdgGvIEqtFLwGrYSVEMSavVYKdWVFTD
S2  539 IIATNRHLSaLHPIYKLLTPHYRDTMNINALARQSLINAdGIIEksFLPSKhSVEMSSAVYKNWVFTD
S1  510 vIAThRHLSvLHPIYKLLTPHYRnnMNINALARQSLINAnGIIEttFLPSKySVEMSSAVYKNWVFTD

 A  340 QALPADLvKRGlAIEDPSaPHGlRLVIEDYPYAVDGLEIWDAIKTWVHEYVsLYYptDaavqqDtELQ
S3  598 QALPADLIKRGmAIEDPScPHGiRLVIEDYPYAVDGLEIWDAIKTWVHEYVfLYYksDDtlreDpELQ
S2  607 QALPADLIKRGVAIKDPSaPHGlRLLIEDYPYAVDGLEIWAAIKTWVQEYVsLYYARDDDVKpDSELQ
S1  578 QALPADLIKRGVAIKDPStPHGvRLLIEDYPYAaDGLEIWAAIKTWVQEYVpLYYARDDDVKnDSELQ

 A  408 AwWKEaVEkGHGDlKekPWWPKMQTtEdLiqsCsIIvWTASALHAAVNFGQYPYGGLILNRPTLaRRF
S3  666 AcWKElVEvGHGDkKnePWWPKMQTrEELVEaCaIIIWTASALHAAVNFGQYPYGGLILNRPTLSRRF
S2  675 qWWKEAVEKGHGDLKDKPWWPKLQTiEELVEiCtIIIWTASALHAAVNFGQYPYGGfILNRPTsSRRL
S1  646 hWWKEAVEKGHGDLKDKPWWPKLQTlEdLVEvClIIIWiASALHAAVNFGQYPYGGlImNRPTaSRRL

B2    1     GsPEYDalaKNPeKefLkTITgKkETLIDLtiIEILSRHASDEfYLGqrdggdyWTsDagpLEA
 A  476 iPaeGtPEYDEmvKNPQKAYLrTITPKFETLIDLSVIEILSRHASDEiYLG ERetPNWTtDkkALEA
S3  734 mPEKGsaEYEElrKNPQKAYLkTITPKFQTLIDLSVIEILSRHASDEVYLG ERDNPNWTSDtrALEA
S2  743 LPEKGTPEYEEMvKsHQKAYLRTITSKFQTLvDLSVIEILSRHASDEVYLG QRDNPHWTSDSKALQA
S1  714 LPEKGTPEYEEMinnHeKAYLRTITSKlpTLisLSVIEILStHASDEVYLG QRDNPHWTSDSKALQA

con         G pEY    knp K  L TIT K  TLidL  IEILSrHASDE YLG       WTsD   LeA

B2   65 FKRFGknLeeIEkKliekNnnetL RNRyGPakmPYTkLypSSEEGLTFrGI
 A  543 FKRFGsKLtgIEgKinaRNsDpsL RNRtGPVQlPYTLLhrSSEEGLTFkGIPNSISI
S3  801 FKRFGNKLaqIEnKLseRNNDekL RNRcGPVQmPYTLLlpSSkEGLTFRGIPNSISI
S2  810 FQKFGNKLKEIEEKLaRkNNDqSL sNRLGPVQLPYTLL hpnsEGLTcRGIPNSISI
S1  781 FQKFGNKLKEIEEKLvRrNNDpSLqgNRLGPVQLPYTLLypsseEGLTfRGIPNSISI

con      FkrFG  L eIE Kl   Nn   L rNR GP   PYT L psseEGLTfrGI
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B

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pea  750      --VKSpQKAYLkTITpKFQTLiDLSVIEILSRHASDElYLGeRDNPnWTSDkrALeAFkKFGN
              ||| ||||| ||| ||||| ||||||||||||||| ||| |||| |||| || || ||||
 S2  750 --eYeEMVKShQKAYLrTITSKFQTLvDLSVIEILSRHASDEVYLGqRDNPhWTSDSkALQAFqKFGN
         | ||||| ||||| ||| ||||||||||||||||| ||   ||||| | ||| |||
 P1      lqnYdEMVKSpQKAYLkTITSKyQTLiDLSVIEILSRHASDEVYLGeRDskyWTSDSrAvQAFtKFGt

pea  811 KLaEIEkKLtqrNNDekLrN---
         || ||| ||   |||  | |
 S2  816 KLkEIEeKLarkNNDqsLsN---
         || ||| |    | |
 P1      KLaEIEgKihsrNkDs
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Fig. 2A, B. Lipoxygenase (LOX) amino acid sequence comparisons. **A** Deduced amino acid sequences of *lox*A and part of *lox*B2, compared to those of soybean seed LOX1 (S1, Shibata et al. 1987), LOX2 (S2, Shibata et al. 1988), and LOX3 (S3, Yenofsky et al. 1988). *Underlined* regions indicate identity of the *lox*A sequence with at least two seed LOX sequences. In the consensus line, *lower case* indicates identity of the *lox*B2 sequence with at least two other sequences, while *upper case* indicates identity with all other sequences. **B** Alignment of partial deduced amino acid sequence of *lox*P1 with those of soybean LOX2 (Shibata et al. 1988) and pea seed LOX (Ealing and Casey 1988)

both leaf and stem tissue, while levels in root tissue increase to a lesser extent (Fig. 3C).

LOX mRNAs are induced by wounding and methyl jasmonate

LOX activity has been reported to be induced as part of a plant's response to wounding (Hildebrand et al. 1988), so we examined the level of *lox*A and *lox*B transcripts in wounded soybean plants. Plants were wounded by crushing the tip end of the middle leaflet of the oldest trifoliate leaf (leaf 1) repeatedly with a hemostat. The plants were then incubated either in the light or dark for 24 h. Tissue from the wounded and unwounded portions of the middle leaflet, a lateral leaflet of the same leaf, and the middle leaflet of the next leaf up the stem (leaf 2) was collected and nucleic acid isolated. As shown in Fig. 4A, some increase in LOX mRNA levels is seen in the wounded tissue (lane D). This increase is also seen in the unwounded section of the wounded leaflet (lane E), but not in the lateral leaflet of the same leaf (lane F) or in the leaf above (lane G). It is interesting to note that all tissues from the plants incubated in the dark

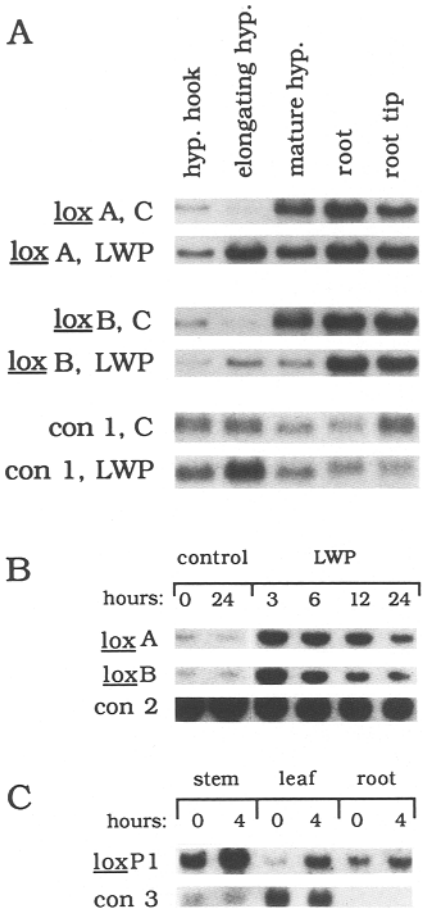

Fig. 3A–C. Accumulation of LOX mRNAs under specific growth conditions in specific tissues. Blots of total nucleic acid were hybridized with the probes indicated. **A** Tissue specificity of LOX mRNA in etiolated soybean seedlings grown under well watered (C) or low water potential (LWP) conditions. Total nucleic acid (4 μg/lane) was isolated from hook, elongating, and mature hypocotyl, root, and root tip sections (see Mason et al. 1988). **B** Time course of LOX mRNA accumulation in the elongating hypocotyl section of etiolated soybean seedlings transferred to well watered (control) or low water potential (LWP) vermiculite. Times shown are hours after transfer. Nucleic acid was loaded at 5 μg/lane. **C** Induction of *lox*P1 mRNA accumulation by osmotic stress in different pea tissues. Times shown are hours following transfer of plants to 0.6 M mannitol. Nucleic acid was loaded at 5 μg/lane

for 24 h contain lower levels of LOX transcripts than those incubated in the light, although a similar wound response is observed (Fig. 4A). In order to determine how rapidly LOX mRNA accumulates in wounded leaves, the middle leaflet of leaf 1 was wounded in several plants. The plants were then incubated in the light, and wounded leaflets collected at different times. As shown in Fig. 4B, most of the LOX mRNA accumulation occurs at least 8 h following the wound treatment.

We also examined the effect of three compounds which induce plant defense responses (methyl jasmonate, polygalacturonic acid, and glutathione) on the levels of LOX mRNAs in soybean suspension cultures. Cultures were grown for 6 or 36 h in the presence of one of the compounds before harvest. Cells treated with methyl jasmonate show a dramatic increase in *lox*A and *lox*B

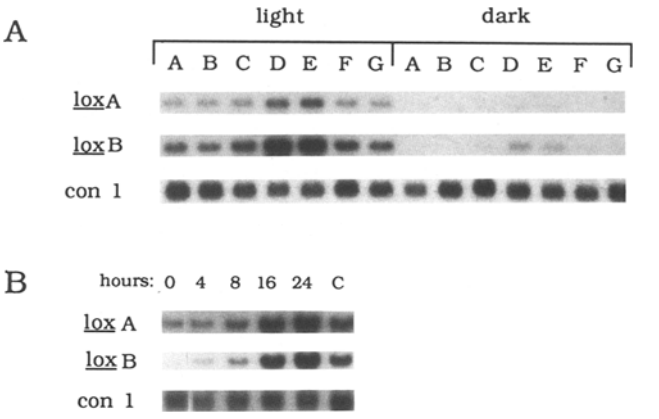

Fig. 4A, B. Induction of LOX mRNA accumulation by wounding. Blots of total nucleic acid were hybridized with the probes indicated. **A** Nucleic acid was isolated from unwounded (A–C) or wounded (D–F) plants incubated in the light or dark for 24 h. Samples A, D, and E are from the middle leaflet of leaf 1. Sample D tissue was wounded directly, with sample E tissue adjacent to the wound site. Samples B and F are from the lateral leaflet of leaf 1, and samples C and G are from the middle leaflet of leaf 2. Nucleic acid was loaded at 3.8 μg/lane. **B** Time course of LOX mRNA accumulation in the middle leaflet of leaf 1 following wounding of this tissue. Control (C) is from an unwounded plant at 24 h. Nucleic acid was loaded at 5 μg/lane

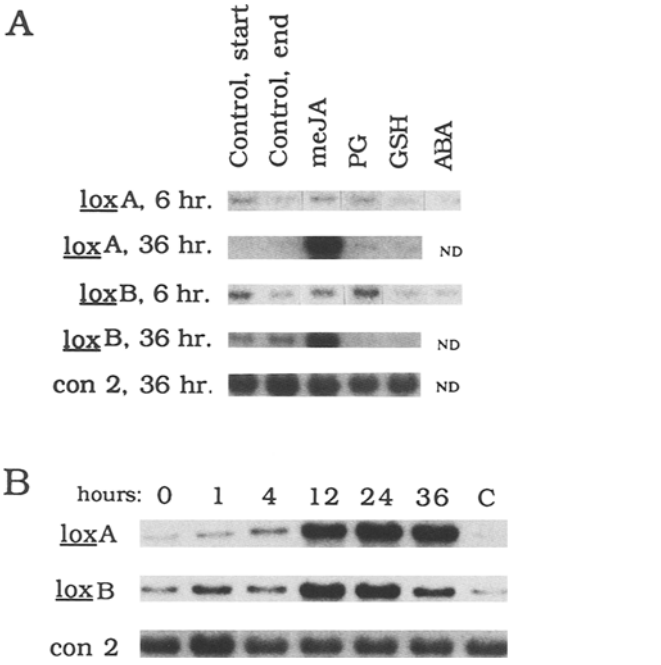

Fig. 5A, B. LOX mRNA levels in soybean cell cultures. Blots of total nucleic acid were hybridized with the probes indicated. **A** Effect of specific compounds on LOX mRNA accumulation. Soybean cell cultures were treated with 20 μM methyl jasmonate (meJA), 1 mg/ml polygalacturonic acid (PG), 0.5 mM glutathione (GSH), 20 μM abscisic acid (ABA), or nothing (control) for the times indicated. Nucleic acid was loaded at 5 μg/lane. ND indicates that the treatment was not done. **B** Time course of LOX mRNA accumulation in response to methyl jasmonate (20 μM) treatment of soybean cell cultures. Times shown are hours following methyl jasmonate addition. Control (C) is from an untreated culture at 36 h. Nucleic acid was loaded at 4.1 μg/lane

mRNA levels by 36 h (Fig. 5A). In contrast, treatment with polygalacturonic acid, which has elicitor activity (Jin and West 1984), or glutathione, which induces mRNA encoding two proteins involved in defense responses (Wingate et al. 1988), has little effect on *lox*A and *lox*B mRNA levels (Fig. 5A). Treatment with ABA, a plant growth regulator which mediates plant responses to water deficit, has little effect on *lox*A and *lox*B mRNA levels after 6 h (Fig. 5A). A time course of methyl jasmonate treatment of soybean cultures indicates that LOX mRNA accumulates within 12 h after addition of this compound (Fig. 5B).

Discussion

We report here the isolation of three cDNAs (*lox*A, *lox*B1, *lox*B2) encoding vegetatively expressed LOXs in soybean. In etiolated 3-day-old soybean seedlings, *lox*A and *lox*B mRNAs show a similar tissue distribution, with the highest levels found in the non-growing region of the hypocotyl and in all parts of the root. Park and Polacco (1989) have reported the appearance of at least two new LOX isozymes in soybean seedlings grown in near dark for 2–5 days. They identified these LOXs as distinct from seed LOXs based upon their pIs. Our DNA sequence information confirms the existence of at least two classes of LOX mRNAs in vegetative tissue of soybean which are encoded by genes that differ from the seed LOX genes previously identified (Shibata et al. 1987, 1988; Yenofsky et al. 1988). We have also isolated a pea LOX cDNA, *lox*P1, which encodes a vegetatively expressed LOX. Based on amino acid sequence comparison, *lox*P1 is different from the pea seed LOX cDNA previously reported (Ealing and Casey 1988). *lox*P1 mRNA levels are highest in stems and found at lower levels in leaves and roots.

Exposure of etiolated soybean seedlings to water deficit results in a shift in *lox*A and *lox*B mRNA levels relative to well watered control tissue. The levels of *lox*A and *lox*B mRNA in the elongating hypocotyl section increase dramatically within 3 h and then decline gradually but remain elevated over control levels for at least 24 h. *lox*B mRNA levels decline in the mature hypocotyl of seedlings exposed to LWP conditions, while *lox*A and *lox*B mRNA levels in the roots are similar to levels in well watered seedlings. Previous studies showed that transfer of well watered soybean seedlings to LWP Vermiculite results in ABA accumulation, a rapid decline in hypocotyl elongation, and changes in the mRNA population of the elongating hypocotyl section (Bensen et al. 1988). The rapid increase in abundance of LOX mRNA in this tissue could be a consequence of the decreased growth rate or a specific response to water deficit.

Previous studies showed that alterations occur in the mRNA population of pea shoots when the plants are wilted (Guerrero and Mullet 1988). We imposed a rapid change in the water status of pea seedlings by transfer of their roots to 0.6 M mannitol. Under these conditions leaf wilting is visible within 30 min and the level of *lox*P1 mRNA in shoot tissue increases within 90 min (data not shown), with a dramatic increase seen within 4 h. These results indicate that LOX mRNA accumulation can be induced by water deficit.

The rapid changes in LOX mRNA levels seen under water deficit may be related to increased synthesis of ABA in these plants. Earlier studies using excised pea shoots demonstrated that dehydration of these tissues leads to the accumulation of ABA, beginning approximately 1 h after dehydration (Guerrero and Mullet 1988). Likewise, in soybean seedlings exposed to LWP conditions ABA accumulates within 2 h (Bensen et al. 1988). The possible involvement of LOX in ABA biosynthesis has been previously suggested, based in part on work by Firn and Friend (1972), who showed that LOX could convert violaxanthin to xanthoxin in the presence of linoleate. Xanthoxin is a precursor of ABA, and conversion of an oxygenated carotenoid to xanthoxin may be the rate-determining step in ABA biosynthesis in stressed plants (Sindhu and Walton 1987). Accumulation of ABA in dehydrated pea or wheat leaves requires transcription and translation (Quarrie and Lister 1984; Guerrero and Mullet 1986), indicating the need for induction of one or more proteins. Takeo and Tsushida (1980) found that dehydration of tea leaves increased LOX activity, although the basis for this increase was not determined. Our data showing an increase in LOX mRNAs in water stressed plants provide further correlation between modulation of LOX levels and ABA levels in tissues exposed to water deficit. It should be noted, however, that ABA accumulates throughout seedlings exposed to LWP conditions (Creelman et al. 1990), while *lox*A and *lox*B mRNA levels remain constant in some tissues.

Hildebrand and coworkers (1988) reported that wounding results in localized and systemic increases in LOX activity in soybean leaves. Our experiments show that LOX mRNA levels also increase in wounded soybean leaves. However, at least in the time frame examined, wounding did not induce a systemic increase in LOX mRNA.

Genes encoding the vegetative storage proteins of soybean are induced in response to wounding and to methyl jasmonate treatment (Mason and Mullet 1990). Likewise, the proteinase inhibitors of tomato accumulate in response to wounding and to methyl jasmonate (Farmer and Ryan 1990). LOX mRNA levels are dramatically increased in cell cultures treated with methyl jasmonate, providing another example of genes induced both by wounding and methyl jasmonate treatment and suggesting that methyl jasmonate (or jasmonic acid) may mediate gene expression in wounded tissue. Recent work has demonstrated that jasmonate levels start to increase within 2 h following wounding of soybean hypocotyls (Dr. Robert Creelman, personal communication), supporting a role for jasmonate in plant wound responses. Based on its timing, *lox*A and *lox*B mRNA accumulation in wounded tissue may be a secondary response to wounding, possibly induced by increased jasmonate levels, the synthesis of which is accomplished through other regulatory mechanisms.

These results demonstrate two different patterns of LOX mRNA accumulation in response to wounding

and water deficit. Water deficit elicits a relatively rapid (<4 h) accumulation of LOX mRNA, while wounding or methyl jasmonate treatment induces a slower (>6 h) increase. Further study of these responses may help explain the roles of lipoxygenase in vegetative tissue.

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