Analysis of Lipoxygenase mRNA Accumulation in the Common Bean (*Phaseolus vulgaris* L.) during Development and under Stress Conditions

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Plant lipoxygenases (LOX, EC 1.13.11.12) have been involved in processes such as stress responses and development. The levels of these enzymes and their corresponding mRNAs are modulated during these processes as well as by different effectors such as jasmonic acid (JA), its methyl ester (MeJA) or abscisic acid (ABA). A new lipoxygenase (LOX) cDNA clone, PvLOX2, was isolated from a *Phaseolus vulgaris* nodule library and used to study the LOX mRNA accumulation pattern in some developmental stages and in plants subjected to hormone and stress treatments. In nodules, LOX mRNA reaches a maximum level around day 14 to 16 after *Rhizobium tropici* inoculation, as compared with LOX mRNA present in uninoculated and inoculated roots at the same days. LOX antigen is detected in the nodule parenchyma and in the uninfected cells. During germination, bean LOX transcripts start to accumulate 48 h after imbibition, remains at the same level until 72 h after imbibition and then declines. In hypocotyl, LOX mRNA is abundant in the growing region and almost absent in the mature region. After water stress or ABA treatment, this mRNA increases in the mature region and decreases in the growing region. In bean seedlings, LOX mRNA is accumulated in response to some types of stresses such as cold and desiccation. Wounding, MeJA or ABA treatment of mature leaves also induces LOX mRNA accumulation. These results indicate that in common bean plants LOX is required during development and stress conditions.

Key words: Abscisic acid — Jasmonic acid — Lipoxygenase (EC 1.13.11.12) — Nodule — *Phaseolus vulgaris* — Wounding.

The enzyme lipoxygenase (LOX, EC 1.13.11.12), catalyzes the hydroperoxidation of fatty acids containing a *cis, cis*-1–4 pentadiene structure. In plants, products of the LOX pathways include mediators of the stress response such as jasmonic acid (JA) (Sembdner and Parthier 1993), traumatin (Zimmerman and Coudron 1979) or six-carbon volatiles (Bate and Rothstein 1998). These six-carbon volatiles also have bactericidal activity (Croft et al. 1993). In addition, LOX has been associated with some developmental stages since high levels of the enzyme are found in rapidly growing tissues (Siedow 1991) and it has been shown that during germination a LOX activity initiates the mobilization of storage lipids (Feussner et al. 1995). There is also data indicating that LOX is used as temporary storage of nitrogen during vegetative growth (Tranbarger et al. 1991).

LOX gene expression is regulated by different effectors such as nitrogen (Staswick et al. 1991), phosphate (Sadka et al. 1994), JA (Bell and Mullet 1991), ABA (Melan et al. 1993); or different forms of stress as wounding (Royo et al. 1996, Saravitz and Siedow 1996), touch stimulation (Mauch et al. 1997), water deficiency (Bell and Mullet 1991) or pathogen attack (Meier et al. 1993, Melan et al. 1993). Several genomic or cDNA clones have been isolated from different plant species showing differential organ-specific expression (Melan et al. 1993, Eiben and Slusarenko 1994, Royo et al. 1996, Saravitz and Siedow 1996).

LOX mRNA or protein has been found in nodules of *Vicia faba* (Perlick et al. 1996), *Pisum sativum* (Gardner et al. 1996) and *Lotus japonicus* (Szczyglowski et al. 1997). The *V. faba*, VfLOX1, gene is expressed at high levels in nodules and epicotyls and very weakly in roots. Tissue print experiments show that in the nodule VfLOX1 transcripts are localized outside the infected tissues, in the nodule parenchyma and around the root stele (Perlick et al. 1996). In the pea, LOX antigen was found in the lumen of the infection threads (Gardner et al. 1996). These localization results allow the proposition, that LOX activity could be involved in a defense mechanism or that the protein could function as nitrogen store.

In bean plants, the expression of two LOX genes has been analyzed. *LOX1* transcripts are found in young leaves, flowers, embryonic axes and 5-d old hypocotyls, but do not accumulate in nodules, or in leaves treated with MeJA or after pathogen infection (Eiben and Slusarenko

1994). pLOX3 is regulated by the presence of pathogens, both in a compatible or incompatible interaction (Meier et al. 1993).

To have a wider understanding of the role of LOX in the common bean, a new *Phaseolus vulgaris* LOX cDNA clone was characterized and the expression of the LOX gene during nodule, root development, germination, and seedling establishment as well as in response to stress conditions and treatments with MeJA and ABA was examined.

Materials and Methods

Plant material and growth conditions—Bean seeds, *Phaseolus vulgaris* L. cv. Negro Jamapa; (PRONASE, México) were surface sterilized in a commercial solution of sodium hypochloride at 15% (v/v) for 10 min, rinsed in running tap water for 2 h, sown on water saturated paper towels and germinated in the dark at 25°C and 100% relative humidity. Common bean plants were grown in vermiculite or in a hydroponic system supplemented with 50% nutrient solution (Somerville and Ogren 1982) in a growth chamber under 16 h light and 8 h dark cycles and 12,000 lux at 25°C, until 21 d after germination when their two trifoliate leaves were expanded.

P. vulgaris seedlings were inoculated 3 d after germination with the *Rhizobium tropici* strain CIAT 899 (Martinez-Romero et al. 1991) and grown in a growth chamber with 16 h photoperiod at 24°C. Plants were watered every other day alternatively with nitrogen-free nutrient solution and water.

cDNA isolation—A cDNA library from 21 d nodules constructed in the λZap vector (Stratagene) was screened with a PCR fragment, obtained using the primers reported by (Bell and Mullet 1991), primer 1 is 5′-CACCCAATTTA(T/C)AAGCTTCT and primer 2 is 5′-ATAGTTCTCAAATAAGCCTT, and total common bean DNA as template. To obtain the full-length cDNA clone, a 15 d nodule cDNA library in λZap vector was screened using a probe derived from the longest clone obtained in the first screening (Probe A, Fig. 2).

Experimental treatments—Methyl jasmonate (MeJA) from Apex Organics, Leicester, U.K. (>90% pure), was dissolved in *N,N*-dimethylformamide to prepare a 100 mM stock solution. Racemic *cis-trans* abscisic acid (ABA, 99% pure) from Sigma, U.S.A., obtained through the Mexican distributor, was dissolved in ethanol (100 mM stock solution). Both phytohormones were subsequently diluted in nutrient solution to obtain 50 μM final concentration for treatment of plants in hydroponic cultures. MeJA and ABA (100 μM added to the irrigating solution) treatments were done for 24 h on bean seedlings grown in the dark for 4 d.

Twenty-one d-old plants were wounded in one leaflet and after 24 h the wounded leaflet and the two other leaflets in the trifolium were collected separately. To test the systemic accumulation of LOX mRNA, we also collected the upper, unwounded trifolium. In a leaf, the levels of induction are the same for the wounded leaflet and the other two leaflets, therefore in Fig. 6B only the result corresponding to the wounded leaflet is presented.

For cold treatment, dark grown 4 d-old bean seedlings were transferred to 4°C and maintained at this temperature for 24 h. For the drought treatment, 5 d-old seedlings were transplanted to vermiculite containing different amounts of water. The control growth condition contained 5 ml of water per gram of vermiculite ($\Psi_w = -0.074$ MPa). The water deficit conditions corresponded to $\Psi_w = -0.35$ MPa, 1/12 × the amount of water in the control. Twenty-four h later, seedlings were collected. Vermiculite was maintained at a constant water potential throughout the experiment. Bean hypocotyls were divided into discrete regions: growing 1 and growing 2 correspond to the elongating regions, growing 1 being the region closest to the insertion of the cotyledons and growing 2, the section with the highest elongation rate. Mature corresponds to the most basal region (for a detailed description see Fig. 4a in Colmenero-Flores, et al. 1999).

In all cases plant material was frozen immediately in liquid N_2 after harvesting, and stored at −80°C until used for RNA extraction.

DNA sequencing and sequence analysis—DNA was sequenced by the dideoxy chain termination method (Sanger et al. 1977) using a Sequenase II kit (Amersham Pharmacia Biotech, U.S.A., obtained through the Mexican distributor) following the instructions of the manufacturer. PvLOX2-5 was sequenced using fluorescence dideoxynucleotides and analyzed on a model 377-18 automated sequencer (PE Applied Biosystems, U.S.A.). Computer analysis was performed with the Wisconsin Package Version 9.1, Genetics Computer Group (GCG), Madison, Wisc.

DNA extraction and gel blot analysis—Genomic DNA was extracted from embrionary leaves of seven d-old plants according to Saghai-Maroof et al. (Saghai-Maroof et al. 1984). Approximately 20 μg of DNA were cleaved with *Eco*RV, *Eco*RI, *Bgl*II and *Bam*HI (Boerhinger-Mannheim, Germany, obtained through the Mexican distributor). After separation on a 0.7% (w/v) agarose gel, DNA was denatured and transferred to Hybond-N^+ membrane (Amersham Pharmacia Biotech). The membrane was prehybridized in 7% SDS, 0.3 M of NaH_2PO_4 pH 7.2 and 1 mM EDTA and hybridized in the same buffer with the random-primed ^{32}P-labeled 1.7 kb *Bgl*II fragment of the PvLOX2 cDNA clone. After hybridization the membrane was washed twice with 0.4× SSC, 0.1% SDS at 65°C for 15 min and autoradiographed.

RNA extraction and analysis—RNA was extracted following the protocol reported by Logemann et al. (Logemann et al. 1987). Northern blot analysis was performed with 50 μg of total RNA per lane, electrophoresed on 1% agarose-formaldehyde gels, transferred to Hybond-N^+ membrane, and fixed both in a gel drier at 80°C for 2 h and with 0.05 M NaOH for 5 min. Hybridization and washing was done the same way as the Southern blot.

Immunocytolocalization of LOX protein in bean nodule—*R. tropici* induced nodules were fixed overnight in glutaraldehyde-formaldehyde solution (0.5–4%). Samples were dehydrated in graded ethanol and ethanol-xylol series and embedded in paraffin. Two μm sections were used. Immunochemistry was done with the Histostain SAP Kit (Zymed, U.S.A., obtained through the Mexican distributor) and performed according to the manufacturer instructions. Primary antibody against soybean lipoxygenase-2 (Peterman and Siedow 1985) diluted 1 : 100 in T-PBS was incubated overnight at 4°C. The blocking agent, levamisol (Zymed), was included to inhibit the endogenous alkaline phosphatase. The chromogen-substrate used was AP-red, creating an intense red deposit in the antigen-antibody-enzyme complex formation site.

Results

LOX cDNA clones isolation and analysis—To understand the role of LOX during developmental and stress responses, we started by analyzing LOX gene expression in

P. vulgaris. A LOX-specific PCR fragment was synthesized from bean total DNA as described in Materials and Methods. The sequence of this fragment showed high homology (>99%) to *LOX1* (Eiben and Slusarenko 1994), including the last whole intron of this gene. Therefore, this fragment must correspond to the *LOX1* gene in the cv. Negro Jamapa. A 21 d-old nodule cDNA library was screened using this PCR fragment as probe. Six cDNA clones of different sizes were obtained. Restriction mapping and partial sequence indicated that all of them derived from the same gene. However, three of these clones seem to use the same polyadenylation signal and the other three use alternative signals. The clone with the longest 5′-end, PvLOX2-2, was chosen for further characterization. This clone was completely sequenced showing that the cDNA length was 2,112 bp (Fig. 1).

According to the cDNA sizes of the LOX genes reported, the PvLOX2-2 clone lacked around 700–800 bp in the 5′-end. To obtain a complete LOX cDNA clone, a 15 d-old nodule cDNA library was screened. This time a 320 bp fragment of the most 5′-end region of PvLOX2-2 was used as a probe (Fig. 2B, Probe A). From this screening, a complete cDNA clone, named PvLOX2-5, was isolated (Fig. 1). PvLOX2-5 contains the PvLOX2-2 clone with a 100% of identity. PvLOX2 encodes a protein of M_r 96.359 kDa. The comparison of the PvLOX2 derived amino acid sequence with other *P. vulgaris* lipoxygenases revealed a 89.2% similarity and 85.5% of identity with the LOX1 protein (Eiben and Slusarenko 1994) and 75.8% similarity and 68.1% identity with the pLOX3 protein (Meier et al. 1993). The differences in identity among these three sequences indicate that PvLOX2 is a new gene.

Southern analysis—Southern blot analysis was performed to determine how many copies of the LOX gene were present in the *P. vulgaris* genome. Common bean DNA was isolated and digested with either *Eco*RV, *Eco*RI, *Bgl*II and *Bam*HI, and probed with a 1.7 kb *Bgl*II fragment of PvLOX2 (Fig. 2B, Probe B). The blot was washed at high stringency. The results showed a major band most probably corresponding to PvLOX2. In addition, weaker

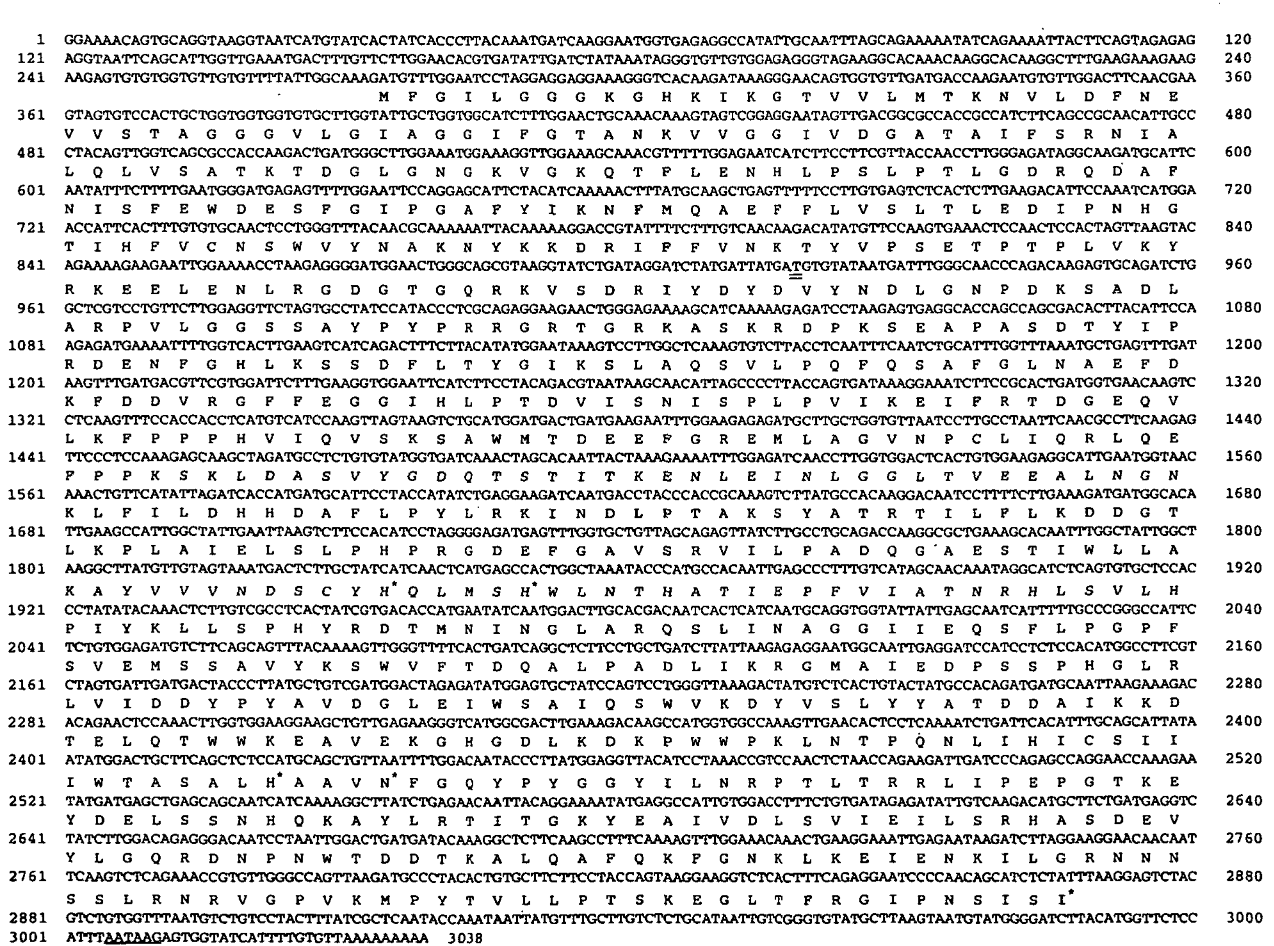
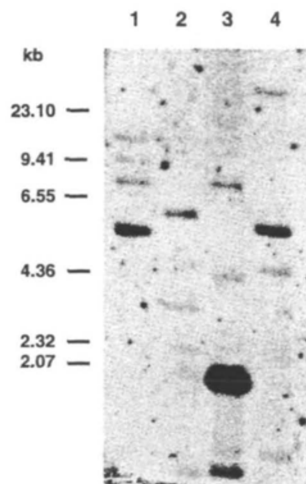


Fig. 1 Nucleotide and deduced amino acid sequence of PvLOX2. Numbers in the left and right margins refer to nucleotides residues. A double underline shows the beginning of clone PvLOX2-2. (*) indicates residues involved in iron atom binding (Minor et al. 1993). The putative polyadenylation site is underlined.



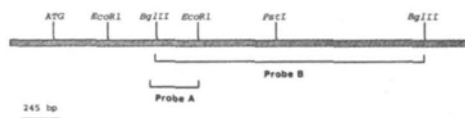


Fig. 2 Southern blot analysis of bean lipoxygenase gene family. Bean DNA was digested with the following restriction enzymes: (1) *Eco*RV, (2) *Eco*RI, (3) *Bgl*II, and (4) *Bam*HI. Hybridization was carried out using the 1.7 kb *Bgl*II PvLOX2 fragment (Probe B). The filter was washed at 65°C with 0.2×SSC, 0.1% SDS. (B) The figure shows the restriction sites of the PvLOX2-5 full length clone. Probe A was used to screen the 15 dai nodule cDNA library to obtain the full length PvLOX2 clone. Probe B, was used for the Southern and northern analysis.

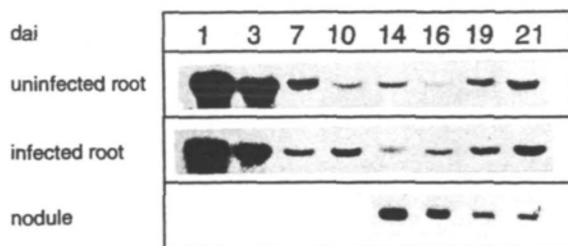


Fig. 3 LOX mRNA accumulation during nodulation. Total RNA was extracted from uninfected roots, *R. etli*-infected roots and the detached nodules at the days shown in the figure. RNA was electrophoresed, transferred to nylon membrane, and hybridized to the 1.7 kb *Bgl*II PvLOX2 fragment. Washing was as in indicated Fig. 2.

hybridization bands were also detected, suggesting the presence of 2–3 LOX genes in *P. vulgaris* with low homology to PvLOX2 (Fig. 2A). This result may indicate that the expression analysis done in this work mainly represents the accumulation of PvLOX2 mRNA. However, the detection of transcripts from a different LOX gene could not be discarded. Therefore, we prefer using LOX instead of PvLOX2 to refer to these products.

Expression of lipoxygenase during nodule and root development—It has been suggested that LOX could participate in nodule growth and development, as part of the defense mechanism and as a nitrogen store (Gardner et al. 1996, Perlick et al. 1996). To analyze the expression pattern of LOX during nodule biogenesis, total mRNA isolated from *R. tropici* infected roots, nodules, and uninfected roots were examined. From dai 1 to 10, when no visible or very small nodules are present, the whole root system was collected. From dai 14 to 21, nodules were detached from the root. Results in Fig. 3 show that, in uninfected roots, LOX mRNA was very abundant in young tissue between 1–3 dai and progressively decreased with root age until 16 dai, increasing again between 19–21 dai. A similar transcript accumulation pattern was observed for RNA obtained from infected roots. Whereas an increase was detected around 14 and 16 dai nodules, as compared with infected or uninfected roots of the same age (Fig. 3).

Tissue immunolocalization of LOX during nodule development—To explore the accumulation and localization of LOX protein during nodule development, immunolocalization experiments were carried out using 14, 16, 19 and 21 dai bean nodules, and lipoxygenase-2 antibodies (Peterman and Siedow 1985). At 14–19 dai LOX protein was detected in parenchyma and non-infected cells of the central nodule tissue (Fig. 4A–C). At 21 dai, a decrease of LOX protein accumulation in both types of cells was observed (Fig. 4D). No reaction was detected with the second antibody alone (Fig. 4E).

Expression of LOX during germination—The modulation of LOX transcript accumulation during seed germination and seedling establishment, when a high nutrient mobilization and turnover of cell structures occur, was investigated. Seeds were germinated and material was collected at different times. Total RNA was isolated from the axis of germinating seeds and seedlings. LOX mRNA began to accumulate within 48 h after imbibition, was maintained until 72 h, and declining after that time (Fig. 5A). Higher levels of LOX and JA have been detected in actively growing tissue (Siedow 1991), therefore LOX mRNA accumulation in different regions of the hypocotyl was analyzed. Bean hypocotyls were divided into discrete regions: growing 1 and growing 2 correspond to the elongating regions, growing 1 being the region closest to the insertion of the cotyledons, and growing 2 the section with the highest elongation rate. Mature corresponds to the most basal region (Colmenero-Flores et al. 1999). In the germinating hypocotyl, the LOX transcript is present in the

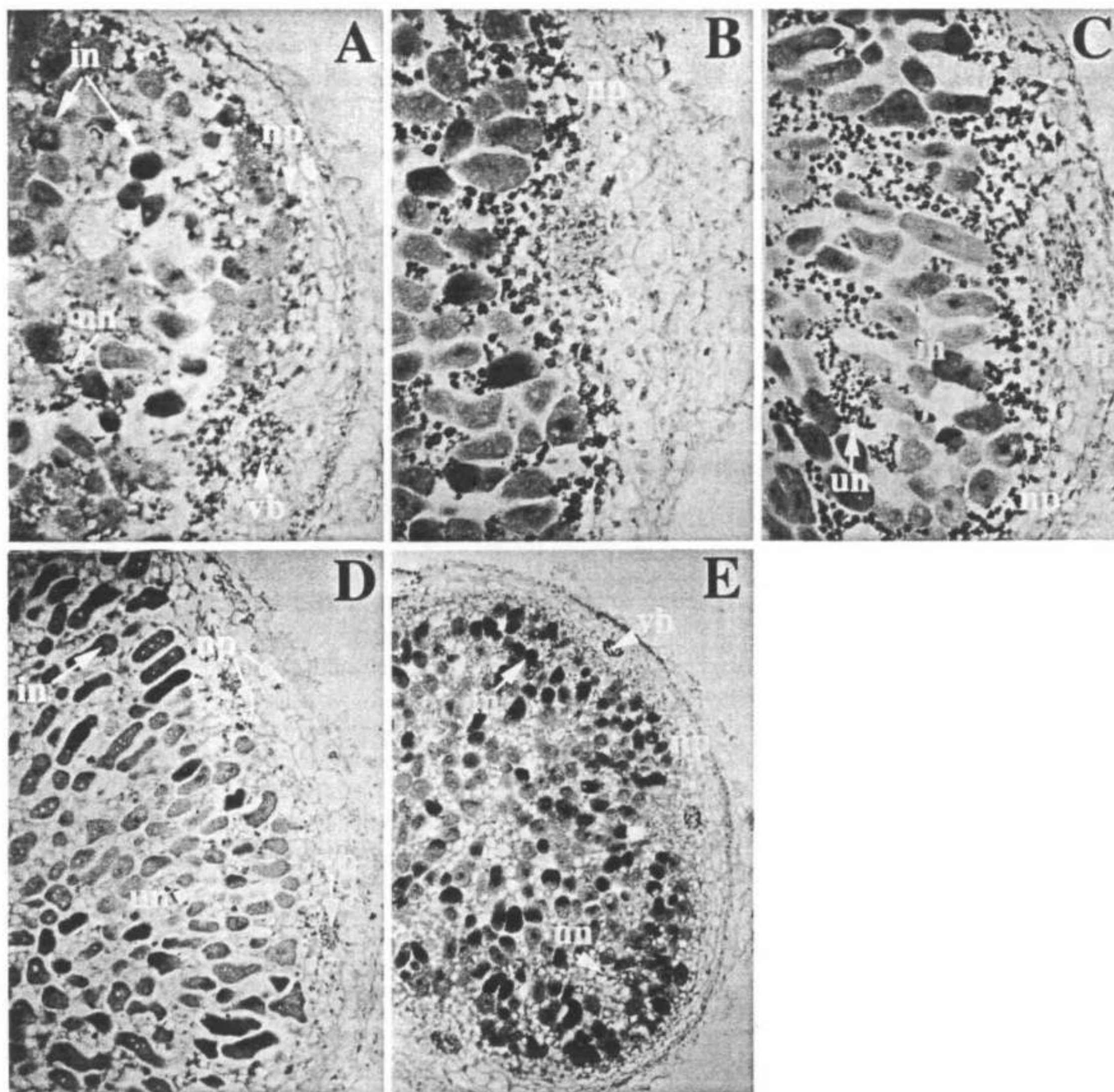


Fig. 4 Light micrographs showing localization of LOX antigen in longitudinal sections of common bean nodules. A, B, C and D correspond to 14, 16, 19 and 21 dai respectively. AP-red complex formation is observed in the parenchyma and non infected cells of 14,16, and 19 dai. At 21 dai de AP-red complex is scarcely detected. E, shows a 19 dai nodule control developed with the second antibody alone. np, nodule parenchyma; vb, vascular bundle; in, infected cells; un, uninfected cells. Magnification of A, B, C and D, 20×. Magnification of E, 10×.

growing regions, with a higher abundance in region 1. In contrast, almost no LOX transcript was detected in the mature region (Fig. 5B, lanes C).

Stress induction of LOX mRNA accumulation in bean seedlings and leaves—Since LOX has been involved in the stress plant response, we asked whether the LOX transcript accumulation in the common bean was affected by different stress conditions such as wounding, drought or cold. When seedlings were subjected to drought or ABA treatment, the pattern of accumulation in hypocotyls changed dramatically compared with normal growing conditions (described above). LOX mRNA decreased substantially in growing region 1 and increased in the mature zone (see Fig. 5B, lanes A, ABA and D, drought).

Stress conditions producing water stress such as drought and low temperature were analyzed. The results in Fig. 6A show that LOX mRNA accumulates in bean seedlings in response to water deficit, as well as low temperatures (4°C), although to a lesser extent.

LOX mRNA accumulation in response to wounding was analyzed in mature leaves by injuring only one leaflet in a trifolium with forceps, as described in Materials and Methods. Fig. 6B shows that LOX mRNA is induced in the wounded leaflet as well as in the systemic trifolium. LOX

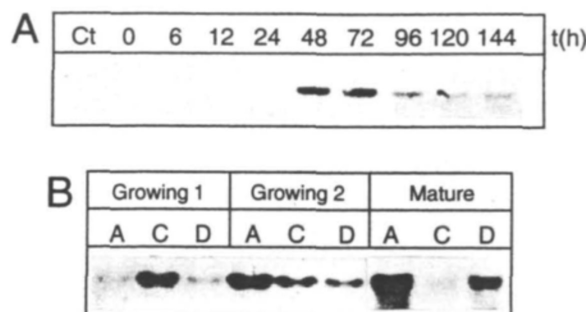


Fig. 5 LOX mRNA accumulation in germinating seedlings. (A) RNA extracted from common bean seeds germinated at the times shown, was electrophoresed, transferred to nylon membrane and hybridized to the 1.7 kb *Bgl*II PvLOX2 fragment. Washing was as indicated in Fig. 2. (B) Bean hypocotyls were divided into discrete regions: growing 1 and growing 2 correspond to the elongating regions, growing 1 being the region closest to the insertion of the cotyledons and growing 2, the section with the highest elongation rate. Mature corresponds to the most basal region. RNA was extracted from untreated (C), ABA (A) or drought (D) treated common bean hypocotyls, electrophoresed, transferred to nylon membrane, and hybridized to the 1.7 kb *Bgl*II PvLOX2 fragment. Washing was as indicated in Fig. 2.

transcript was not detected in unwounded leaves.

We asked whether LOX mRNA accumulation responds to treatment with growth regulators that have been involved in stress responses such as MeJA and ABA. To answer that question, hydroponic cultures of common bean plants were treated with 50 μM MeJA or 50 μM ABA and total mRNA was extracted from leaves. The time-courses of the LOX mRNA accumulation in response to

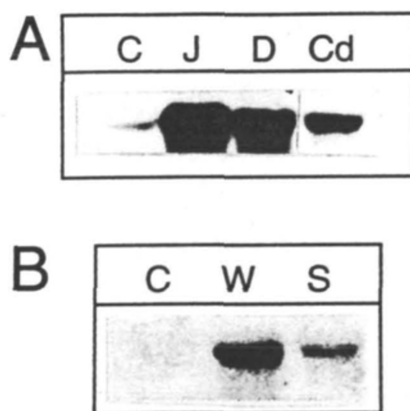


Fig. 6 LOX mRNA accumulation after stress treatment. Common bean plants (A) or seedlings (B), were subjected to different stresses as described in Materials and Methods. Total RNA was extracted, electrophoresed, transferred to nylon membrane, and hybridized to the 1.7 kb *Bgl*II PvLOX2 fragment. Washing was as indicated in Fig. 2. (A) C, untreated seedling; D, drought; J, 50 μM methyl jasmonate; Cd, cold. (B) W = wounded leaflet; S = systemic leaf.

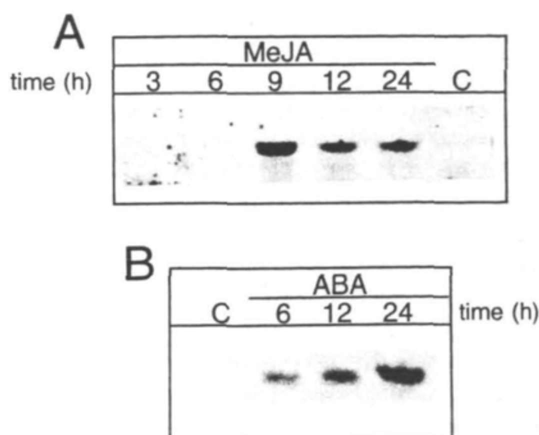


Fig. 7 LOX mRNA accumulation in response to MeJA and ABA. Common bean plants grown in hydroponic conditions were treated with (A) 50 μM MeJA or (B) 50 μM ABA by adding these compounds to the nutritive solution. Leaves were collected at the times shown in the figure. Total RNA was extracted, electrophoresed, transferred to nylon membrane, and hybridized to the 1.7 kb *Bgl*II PvLOX2 fragment. Washing was as indicated in Fig. 2.

MeJA or to ABA is different. In response to MeJA a transient LOX transcript accumulation was detected within the first 6 h of treatment. This accumulation reached a maximum level at 9 h and declined thereafter (Fig. 7A). In the case of the ABA treatments, a progressive accumulation of LOX mRNA was detected after 6 h (Fig. 7B).

Discussion

In the present work, the isolation of a new LOX cDNA clone (PvLOX2) from a common bean nodule cDNA library, is reported. This cDNA was used to further analyze LOX gene expression during normal development and stress conditions. Two additional LOX DNA sequences have been reported for the common bean, *LOX*1 (Eiben and Slusarenko 1994) and pLOX3 (Meier et al. 1993) that show 84.2% and 72.7% homology, respectively, to PvLOX2. Southern blot experiments carried out at high stringency shows that a PvLOX2 probe detects one main band and additional bands with less homology (Fig. 2A). Although mainly PvLOX2 transcripts and antigen may be detected in the analysis presented in this work, we decided to use LOX to refer to these products, because it is still possible that mRNAs or proteins coming from another LOX gene could be detected by the probes and antibodies used.

The analysis of the LOX transcript accumulation pattern during bean-*Rhizobium* interaction shows that LOX mRNA is present at all times tested. A maximum accumulation is detected in young tissue between 1–3 dai, suggesting that LOX is required in actively growing tissues, in

agreement with data already reported (see (Siedow 1991) and references therein). Further, a progressive decrease of LOX mRNA levels occurs until 16 dai, followed by an increase between 19–21 dai (Fig. 3). Although we can conclude that this increase is not a consequence of *Rhizobium* infection, since it is observed in infected and uninfected roots, the factors involved in the re-induction of LOX transcript accumulation are unknown. In nodules, LOX mRNA (Fig. 3) increases around 14 dai, as compared with what is found in roots. Also, LOX antigen is detected at 14–19 dai and is almost undetectable at 21 dai. Most probably this pattern is associated with cell elongation, because 14–19 dai nodules, but not 21 dai nodules, are in the growing stage. In common bean nodules, nitrogenase acetylene-reducing activity is low around 14 dai and very high at 21 dai (Padilla et al. 1987). Therefore it is not likely that nitrogen levels regulate LOX mRNA accumulation in the nodule as it has been suggested (Gardner et al. 1996, Perlick et al. 1996). The localization of LOX in nodule parenchyma (Fig. 4A–C) suggests a defensive role for this enzyme against possible pathogen invasion, as it has been proposed (Perlick et al. 1996).

We do not know if soybean lipoxygenase-2 antibodies used in the immunolocalization experiments may detect PvLOX2 and/or other LOX proteins. The time course of LOX protein accumulation in a western blot analysis of nodule protein extracts, using the same antibodies, parallels the results of the immunolocalization experiments. Only one band, with M_r around 97 kDa was observed (data not shown). Furthermore, only PvLOX2 cDNA clones were found after the screening of two nodule libraries with two different probes (see Results). In addition, RT-PCR experiments with gene-specific primers, revealed the presence of PvLOX2 transcripts in nodule total RNA. Neither *LOX1* (Eiben and Slusarenko 1994) mRNA nor transcripts belonging to the clone pLOX3 (Meier et al. 1993) were detected in these RT-PCR experiments (H. Porta, in preparation). All these results suggest that PvLOX2 is the main isoform in nodule. Nevertheless, we can not discard the existence of another isoform in this organ.

A LOX mRNA transient accumulation during germination was found in common beans (Fig. 5A). Diverse roles for LOX throughout this process have been suggested: lipid mobilization, defense, and JA synthesis for growth regulation. However, none of these have been clearly proven (Kato et al. 1992, Melan et al. 1994, Park et al. 1994).

The presence in common beans of LOX mRNA in actively growing tissue was examined in different hypocotyl growing regions. The results indicate a clear correlation between LOX mRNA levels and the developmental stage of the tissue. Higher LOX transcript levels were detected in the growing region than in the mature region (Fig. 5B). Common bean LOX mRNA levels in the different hypocotyl regions resemble those reported for the LOX antigen (Eiben and Slusarenko 1994). A similar distribution was observed for JA levels: hook > elongating region > mature region (Creelman and Mullet 1995).

When common bean seedlings are subjected to drought or treated with ABA, the pattern of mRNA accumulation changes radically. LOX mRNA levels are higher in the mature region than in the growing region. This contrasting expression pattern could be the result of a decrease in the seedlings growth rate provoked by drought or ABA treatment (Creelman et al. 1990, Colmenero-Flores, et al. 1999). In addition, the increase in LOX transcript levels in the mature region, suggest that LOX mRNA increase is responding to the water status in this hypocotyl region, which after the water stress treatment shows the highest lost of turgor ($\Delta \Psi p_{mature} = 2.05$; $\Delta \Psi p_{growing} = 0.63$) (Colmenero-Flores, J.M. Ph.D. thesis). Since ABA treatment induces a similar LOX transcript accumulation pattern, it could be hypothesized that ABA acts as a mediator in this response. Our results are dissimilar to those reported by Bell and Mullet (Bell and Mullet 1991), since they found a higher level of LOX transcript in the mature region than in the growing region, and this relationship inverts during water deficit. These results do not agree with the observation that LOX protein and activity are abundant in actively growing tissue (Siedow 1991). It is possible that different lipoxygenases could have specific functions under distinct physiological situations. Therefore, PvLOX2 could have a different role than that detected by Bell and Mullet (Bell and Mullet 1991). Further experimentation should be performed to clarify the function of the different LOX proteins in the plant cell.

In common bean seedlings, drought and cold induce LOX mRNA accumulation (Fig. 6A). This increase could be related to the membrane deterioration occurring during water deficit. It has been reported that water deficit induces a rise in the content of hydroperoxides in the membrane lipids and in the LOX activity that leads to membrane damage (Maccarrone et al. 1995). Alternatively, a LOX mRNA increase in *P. vulgaris* could be required for JA synthesis in response to drought stress (Creelman and Mullet 1995). A different requirement of LOX during diverse types of stress can be suggested, since lower LOX transcript levels are accumulated after drought or cold stress than with MeJA application or wounding.

This work shows that in *P. vulgaris* wounding of mature leaves triggers a LOX mRNA increase (Fig. 6B). This increase could also be related to several factors, such as the formation of JA, a mediator in this response (Farmer 1992), membrane damage (Siedow 1991), or to the synthesis of six-carbon volatiles (Croft et al. 1993, Bate and Rothstein 1998). Additionally, a systemic wound-induced LOX mRNA accumulation was observed (Fig. 6B). In

Solanaceae, the systemic response is regulated by systemin, a 18 aa peptide, synthesized after wounding, which activates this response by inducing JA synthesis (Pearce et al. 1991, Peña-Cortés et al. 1995). In common beans, systemin has not been found. However, the systemic response could be mediated by a molecule with an equivalent function.

Finally, in agreement with the role of JA and ABA as mediators of abiotic and biotic stress responses, LOX mRNA accumulation after application of these regulators was analyzed. The results of these experiments show that MeJA and ABA activate LOX mRNA accumulation (Fig. 7), suggesting their involvement in the response of common beans to adverse conditions, such as cold, drought or wounding.

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