BIOCHEMICAL EVALUATION OF LIPOXYGENASE PATHWAY OF SOYBEAN PLANTS SUBMITTED TO WOUNDING

ANGÉLICA APARECIDA VIEIRA[1], MARIA GORETI DE ALMEIDA OLIVEIRA[2], INES CHAMEL JOSÉ[1], NEWTON DENIZ PIOVESAN[1], SEBASTIÃO TAVARES DE REZENDE[2], MAURILIO ALVES MOREIRA[2] AND EVERALDO GONÇALVES DE BARROS[3,*]

BIOAGRO, Universidade Federal de Viçosa (UFV), 36571-000, Viçosa, MG, Brazil

ABSTRACT - Leaf lipoxygenases (LOX) are involved with important physiological processes such as plant growth and development, senescence, biosynthesis of regulatory molecules, and response to pathogens and insects. We did a biochemical evaluation of the LOX pathway of soybean leaves submitted to wounding in a normal genotype (IAC-100) and its counterpart lacking seed LOX (IAC-100 TN). Our results indicate that LOX activities in the different pHs and temperatures tended to be higher in the wounded plants compared to their respective controls. The $K_{M\ app}$ values at 168 h after wounding reached a minimum in both genotypes indicating that the plants respond by changing the leaf LOX pool. There was an increase on protease inhibitor levels in all time points after wounding, for both cultivars. The levels of hexanal and total aldehydes are similar for the wounded plants at different times after wounding and their respective controls for both genotypes. Our results strongly suggest that the LOX pathway is activated during the wound response leading to jasmonate by the initial action of hydroperoxide cyclase. In addition, the results show that the genetic removal of seed LOX does not interfere with the plant's ability to respond to wound via the LOX pathway.

ADDITIONAL INDEX TERMS: Lipoxygenases, soybean, mechanical wounding.

AVALIAÇÃO BIOQUÍMICA DA VIA DAS LIPOXIGENASES EM PLANTAS DE SOJA SUBMETIDAS A FERIMENTO

RESUMO - Lipoxigenases (LOX) de folhas estão envolvidas em importantes processos fisiológicos, tais como: crescimento e desenvolvimento, senescência, biossíntese de moléculas regulatórias e resposta a patógenos e a insetos. Neste trabalho, foi feita uma avaliação da via bioquímica das lipoxigenases em folhas de soja submetidas a ferimento em um genótipo normal (IAC-100) e em uma linhagem avançada derivada de IAC-100, mas isenta de LOX na semente (IAC-100 TN). De um modo geral, as atividades de LOX em diferentes valores de pH e temperaturas foram maiores em plantas feridas com relação a seus respectivos controles. Os valores de $K_{M\ app}$, 168h após ferimento, atingiram o seu mínimo em ambos os genótipos, indicando que as plantas responderam ao ferimento por meio da alteração no "pool" de LOX da folha. Em ambos os genótipos houve um aumento nos níveis de inibidores de proteases após o ferimento. Os níveis de hexanal e de aldeídos totais foram similares em plantas feridas em diferentes tempos, após ferimento, com relação a seus respectivos controles para ambos os genótipos.

Received: 25/10/99 - Accepted: 20/9/2000

1. BIOAGRO
2. Depto. de Bioquímica e Biologia Molecular/BIOAGRO, UFV, 36571-000, Viçosa, MG, Brazil
3. Depto. de Biologia Geral/BIOAGRO, UFV, 36571-000, Viçosa, MG, Brazil.
*Author to whom correspondence should be addressed (email: ebarros@mail.ufv.br)

Pelos resultados, infere-se fortemente que a via das lipoxigenases é ativada durante a resposta a ferimento, levando à síntese de jasmonato pela ativação inicial da hidroperóxido ciclase. Além disso, por esses mesmos resultados, constata-se que a remoção genética de LOX das sementes não interfere com a capacidade de a planta responder a ferimentos por meio da via bioquímica das lipoxigenases.

TERMOS ADICIONAIS PARA INDEXAÇÃO: Lipoxigenases, soja, ferimento mecânico.

INTRODUCTION

Lipoxygenases (LOX) (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12) are dioxygenases that catalyze the addition of molecular oxygen to polyunsaturated fatty acids containing the group *cis, cis* -1,4-pentadiene. Linolenic acid ($C_{18:3}$) contains such group and is the most abundant fatty present in the majority of plant tissues. Linoleic acid ($C_{18:2}$) which also contains the *cis, cis*-1,4-pentadiene group is found in high concentrations in seeds and embryos (Hildebrand *et al*., 1988).

Several studies have been conducted aiming to elucidate the physiological role of LOX in plant tissues. LOX have been studied in soybeans (Axelrod *et al*., 1981; Peterman and Siedow, 1985; Park and Pollaco, 1989; Grayburn *et al*., 1991; Saravitz and Siedow, 1995; Saravitz and Siedow, 1996), in wheat (Bohland *et al*., 1997), barley (Reinbothe *et al*., 1997), tobacco (Rickauear *et al*., 1997), tomato (Farmer and Ryan, 1992), and potato (Bell and Mullet, 1993), among others. These studies suggest that LOX are related to plant growth and development, senescence, plant defense against pathogens and insects, and biosynthesis of regulatory molecules.

In soybeans, LOX have been studied mostly in seeds and leaves. It has been suggested that 10 to 12 genes encoding LOX are present in the soybean genome. Three LOX isozymes have been extensively characterized by Axelrod et al. (1981) in soybean seeds. Despite the extensive characterization, the physiological roles of these enzymes have not been fully understood. However, recent data show that these enzymes affect the levels of protease inhibitors in the seed suggesting that they have an important function during seed development (Carvalho *et al*., 1999). In leaves, it has been observed that LOX expression increases considerably as a result of reproductive sink removal (Kato *et al*., 1990), pod removal (Bunker *et al*., 1995), and mechanical wounding (Saravitz and Siedow, 1996), indicating that LOX can be considered a vegetative storage protein as well as an important component of the plant response system to pathogen and insect attack.

The main goal of this work was to biochemically characterize the LOX pool present in leaves of soybean plants submitted to wounding. Wild type genotypes and mutant lines lacking seed LOX were used to determine if these two genetic materials respond to wounding by activating the LOX pathway.

MATERIAL AND METHODS

Genetic material

Soybean (*Glycine max* L.) leaves at development stage V3 (Fehr *et al*., 1971) were obtained from commercial cultivar IAC-100 and from an advanced line derived from IAC-100 but lacking seed LOX (IAC-100 TN). Seeds from IAC-100 were obtained from the Germplasm Bank of the Department of Plant Sciences of the Federal University of Viçosa (UFV), MG, Brazil. IAC-100 TN was developed by conventional breeding after five cycles of backcrossing in our Breeding Program conducted at the UFV Biotechnology Institute (BIOAGRO). The high genetic simarility between IAC-100 and IAC-100 TN was confirmed by analysis of DNA fingerprinting with random amplified polymorphic DNA (RAPD) markers and phenotypic characteristics. The plants were cultivated in the greenhouse in 4 kg pots containing three plants each.

Mechanical wounding

Seven mechanical wounds were inflicted in each of the three leaflets of the first trifoliolate leaf with the aid of a pincer. After 0, 6, 12, 24, 48, and 168 h the wounded leaflets (local response) and the leaflets of the second fully expanded trifoliolate leaf (systemic response) were collected, immediately immersed in liquid nitrogen and kept at –80^{o} C for the biochemical analyses. Leaves from control plants, not submitted to wounding, were also collected in all time points.

Leaf extract

Leaf extract for biochemical determinations were obtained according to Ohta *et al*. (1986) with the following modifications: the extraction buffer was 0.05 mol.L^{-1} sodium phosphate pH 6.5 with no Triton X-100. Protein determination was by the bicinchoninic acid assay (Smith *et al*., 1985).

Lipoxygenase activity

LOX activity in the leaf extracts was determined using linolenic acid as substrate according to Axelrod *et al*. (1981). This method determines the increase on absorbance at 234 nm as a result of the formation of conjugate double bonds in the linoleic acid hydroperoxide. For optimum pH determination different buffer systems at a final concentration of 50 mmol.L^{-1} were used covering the range of pH 2 to 10. For determination of optimum temperature enzyme activity was determined in 50 mmol.L^{-1} sodium phosphate pH 6.0 in the range of 20 to 50 ^{o}C. All determinations were done in triplicate.

Kinetic parameters

LOX activity was determined in 50 mmol.L^{-1} phosphate buffer pH 6.0, at 25 ^{o}C, using linoleic acid in the following concentrations: 10, 20, 40, 80, 160, 320, 640, and 1,280 μmol.L^{-1}. The kinetic parameters, in the steady state, were obtained by non-linear regression analyses using the program Enzifitter (Leatherbarrow, 1987). All determinations were done in triplicate.

Protease Inhibitors

Determination of protease inhibitor contents in the leaf extracts was based on the inhibition of trypsin activity upon N-benzoyl-D,L-arginine-p-nitroanilide (D,L-BapNA) (Kakade *et al*., 1974).

Hexanal determination

Hexanal production by leaf extracts was determined by gas chromatography based on the procedure developed by Utumi *et al*. (1998). The column used was a carbowax (25 m x 0.53 mm). The initial column temperature of 45 ^{o}C was raised 7 ^{o}C per minute until 70 ^{o}C and kept at this temperature for 5 min. Then it was raised 15 ^{o}C per minute until 200 ^{o}C. The temperatures of the injector and detector were 200 ^{o}C and 250 ^{o}C, respectively. Nitrogen was used as the carrier gas at a flow rate of 5.7 ml per min.

Total aldehyde determination

Total aldehyde content in leaf extracts was determined according to Wilson and McDonald (1986) using 3-methyl-2-benzothiozolinone hydrazone (MBTH).

RESULTS AND DISCUSSION

To biochemically characterize the LOX pool present in soybean leaves submitted to mechanical wounding, we first determined pH and temperature effects on the activity of these enzymes. Figure 1 shows the pH profiles of enzyme activities (systemic response) analyzed in extracts from cultivar IAC-100 and respective control at 168 h after wounding. At this time point the $K_{M\ app}$ value reached a minimum in relation to the control (Table 1). Two prominent activity peaks are seen around pHs 4.5 and 6.0, with and without wounding, indicating the existence of at least two LOX isoenzymes. The activity profiles for all time points analyzed for IAC-100 and IAC-100 TN, local and systemic response, followed the same trend observed in this time point (data not shown). Our data are similar to

those observed for leaf LOX from other soybean cultivars (Lanna *et al*., 1996), from potato tubers (Galliard *et al*., 1971), sunflower seeds (Leoni *et al*., 1985), and green pepper (Minguez-Mosqueira *et al*., 1993).

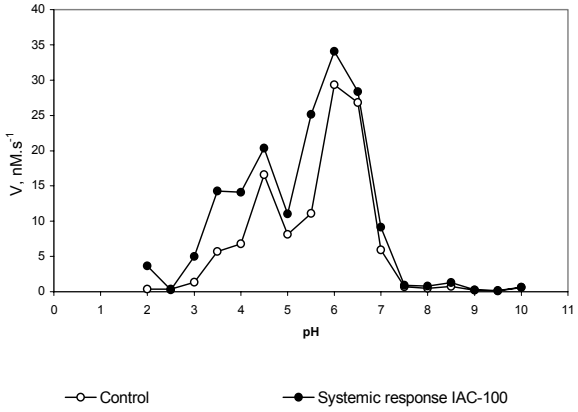

FIGURE 1 - Effect of pH on the activity of soybean leaf lipoxygenases (cultivar IAC-100 – systemic response). Experimental conditions: temperature: 25 °C; linoleic acid concentration: 40 μmol.L^{-1}; amount of leaf extract: 1 μl. Protein concentration in leaf extract: control – 1.82 mg/ml, treatment – 1.58 mg/ml.

Figure 2 shows the temperature profiles of enzyme activities (local response) analyzed in extracts from cultivar IAC-100 and respective control at 168 h after wounding. Optimum temperature was around 25 °C, with or without wounding. The activity profiles for all time points analyzed for IAC-100 and IAC-100 TN, local and systemic responses, followed the same trend observed in this time point (data not shown). Lanna *et al*. (1996), working with cultivars IAC-100 and Cristalina, both wild type and LOX minus, at vegetative stage V4, found the same temperature optimum for leaf LOX.

As a whole, considering that the protein concentrations in all extracts analyzed were very close to each other, our data suggest that LOX activities in the different pHs and temperatures tended to be higher in the wounded plants compared to their respective controls. This indicates that both genotypes (wild type and LOX minus) respond to wounding by activating the LOX pathway.

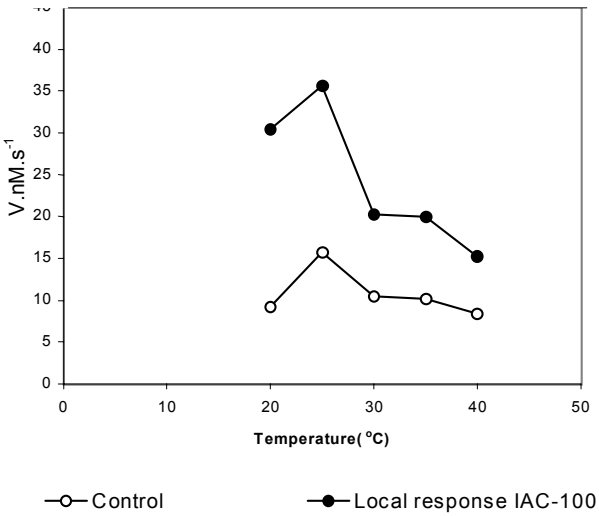

FIGURE 2 - Effect of temperature on the activity of soybean leaf lipoxygenases (cultivar IAC-100 – linoleic acid concentration: 40 μmol.L^{-1}; amount of leaf extract: 1 μl. Protein concentration in leaf extract: control – 1.09 mg/ml, treatment – 1.13 mg/ml.

TABLE 1 - $K_{M\ app}$ values of soybean leaf lipoxygenases upon linoleic acid.

Time (hours)	Local Response $K_{M\ app}$ (μmol.L^{-1})		Systemic Response $K_{M\ app}$ (μmol.L^{-1})	
	IAC-100	IAC-100 TN	IAC-100	IAC-100 TN
0	288.00	234.70	375.10	140.62
6	335.04	379.99	302.04	288.48
12	275.94	234.72	197.67	276.12
24	234.68	282.08	219.19	388.60
48	275.28	241.66	198.82	291.66
168	186.24	173.05	102.05	142.28

The leaf LOX pool activities present in the two genotypes analyzed using the linoleic acid concentrations defined in our experiments, at pH 6.0, follow the Michaelis-Menten kinetics. Figure 3 shows the Michaelis-Menten for LOX activity 48 h after wounding using IAC-100 TN extract (local response). The activity profiles for all time points analyzed for both cultivars, wild type and LOX minus, local and systemic response, followed the same trend observed in this time point (data not shown). Table 1 presents the $K_{M\ app}$ values obtained for IAC-100 and IAC-100 TN, respectively, local and systemic responses, at different times after wounding. The local response shows that $K_{M\ app}$ values increased 6 h after wounding for both genotypes in relation to the control plants (not wounded). The $K_{M\ app}$ values at 12, 24, and 48 h after wounding are similar to the respective controls. However, at 168 h after wounding the $K_{M\ app}$ values reached a minimum in both genotypes.

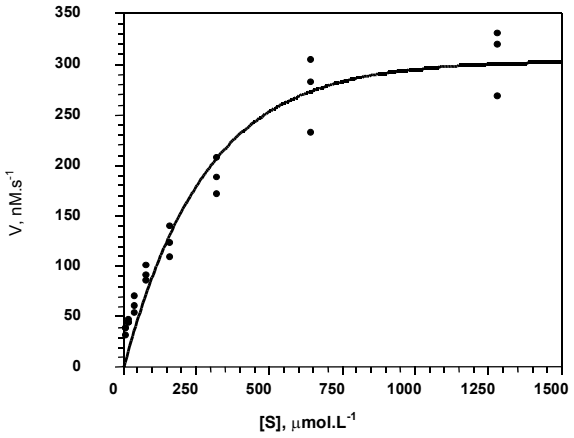

FIGURE 3 - Michaelis-Menten plot of soybean leaf lipoxygenases activity (cultivar IAC-100 TN – local response, 48 h after wounding). Experimental conditions: temperature: 25 ^{o}C; pH 6.0; amount of leaf extract: 1 µl. Protein concentration in leaf extract: 1.32 mg/ml.

The LOX pool in the systemic response does not show the same profile for the two genotypes. In cultivar IAC-100, $K_{M\ app}$ values decreased 12 h after wounding reaching its minimum after 168 h in relation to the controls. In the case of cultivar IAC-100 TN, $K_{M\ app}$ values were greater than the controls from 6 to 48 h after wounding, but dropped again to a value similar to the control 168 h after wounding. These data suggest an alteration on the leaf LOX pool at 168 h after wounding, indicating that the plant responds to wounding by activating the LOX pathway.

Saravitz and Siedow (1996) determined the local and systemic responses of soybean plants to wounding in the same vegetative stage analyzed in this work. In that work, they analyzed the variation in transcript levels for LOX 7 and 8 in the first (local response) and the second trifoliate leaves (systemic response). Transcripts for LOX 7 were detected only transiently at 8 h after wounding in both types of responses while LOX 8 transcripts were detected from 8 to 72 h only in the local response. The changes in $K_{M\ app}$ values observed in our work at 6 h after wounding in the local responses of IAC-100 and IAC-100 TN and in the systemic response of IAC-100 (Table 1) coincide with the alterations observed for LOX 7 by Saravitz and Siedow (1996).

Although the $K_{M\ app}$ values vary differently during the systemic response when cultivar IAC-100 is compared to IAC-100 TN, as a whole these values are very close to each other in both types of genotypes, indicating that the genetic removal of seed LOX does not interfere with the plant response to wounding. Consequently, the expression of seed LOX genes in the cultivar analyzed does not interfere with the expression of genes coding for the LOX pool present in the leaves.

Tables 2 and 3 depict the protease inhibitor levels (expressed in mg of trypsin inhibited per mg of protein) of IAC-100 and IAC-100 TN submitted to wounding, local and systemic responses. The data show that there was an increase on protease inhibitor levels in all time points after wounding, for both cultivars, in both types of responses. These data indicate that the plant respond to wounding by activating transcription of genes encoding protease inhibitors, most probably through the LOX pathway. This suggestion is in accordance to the model proposed

by Farmer and Ryan (1992) working with tomato leaves. In this model, hydroperoxides derived from LOX activity are converted into jasmonic acid by a series of enzymatic steps. This plant hormone would finally cause the activation of genes coding for protease inhibitors.

The LOX pathway can have two alternative branches, one starting with the hydroperoxide cyclase leading to jasmonates, and the other starting with hydroperoxide lyase leading to aldehydes. To test if there is a major branch used during the wound response, we determined the levels of hexanal and total aldehydes in IAC-100 and IAC-100 TN. However, our data showed that the levels of these compounds are similar for the wounded plants at different times after wounding and their respective controls for both genotypes (data not shown). This result suggests that the wound response through the LOX pathway does not occur via aldehyde production.

TABLE 2 – Protease inhibitor in extracts of soybean leaves submitted to wounding (cultivar IAC-100).

Time (hours)	Mg Trypsin Inhibited/G Protein In Leaf Extract[1]			
	Control	Local Response	Control	Systemic Response
0	1.835 ± 0.072	1.830 ± 0.406	2.786 ± 0.111	2.621 ± 0.131
6	1.795 ± 0.050	3.152 ± 0.354	1.914 ± 0.513	3.829 ± 0.580
12	1.883 ± 0.879	6.825 ± 0.630	2.756 ± 0.315	7.223 ± 0.558
24	1.929 ± 0.940	6.240 ± 0.594	3.821 ± 0.576	7.171 ± 0.158
48	1.539 ± 0.913	5.924 ± 0.857	3.773 ± 0.602	7.322 ± 0.670
168	1.199 ± 0.070	5.530 ± 0.218	4.622 ± 0.005	6.470 ± 0.225

[1] Values correspond to the average of three separate determinations ± standard deviation.

TABLE 3 – Protease inhibitor in extracts of soybean leaves submitted to wounding (cultivar IAC-100 TN).

Time (hours)	mg trypsin inhibited/g protein in leaf extract[1]			
	Control	Local Response	Control	Systemic Response
0	1.794 ± 0.262	1.561 ± 0.189	4.240 ± 0.189	4.030 ± 0.223
6	1.528 ± 0.871	3.332 ± 0.182	1.603 ± 0.125	5.462 ± 0.456
12	1.158 ± 0.100	4.276 ± 0.276	2.365 ± 0.085	3.621 ± 0.039
24	2.184 ± 0.236	3.355 ± 0.171	2.150 ± 0.146	4.155 ± 0.346
48	2.870 ± 0.151	4.079 ± 0.354	2.223 ± 0.115	5.005 ± 0.258
168	1.777 ± 0.332	4.094 ± 0.387	2.369 ± 0.203	4.539 ± 0.337

[1] Values correspond to the average of three separate determinations ± standard deviation.

Our results involving the determination of LOX enzymatic parameters, and products of the LOX pathway in soybean leaves submitted to mechanical wounding strongly suggest that this pathway is activated during the wound response leading to jasmonate by the initial action of hydroperoxide cyclase. In addition, the results show that the genetic removal of seed LOX does not interfere with the plant's ability to respond to wound via the LOX pathway.

ACKNOWLEDGEMENTS

This work was supported by BID/FINEP and FAPEMIG.

REFERENCES

AXELROD, B.; CHEESBROUGH, T. M. & LAAKSO, S. Lipoxygenases in soybean. **Meth. Enzymol.,** 71:441-451, 1981.

BELL, E. & MULLET, J. E. Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding. **Plant Physiology,** 103:1133-1137, 1993.

BOHLAND, C.; BALKENHOHL, T.; LOERS, G.; FEUSSNER, I. & GRAMBOW, H. J. Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharides, chitosan, and methyl jasmonate. **Plant Physiology,** 114: 679-685, 1997.

BUNKER, T. W.; KOETJE, D. S.; STEPHENSON, I. C.; CREELMAN, R. A.; MULLET, J. E. & GRIMES, H. D. Sink limitation induces the expression of multiple soybean lipoxygenase mRNAs while the endogenous jasmonic acid level remains low. **Plant Cell**, 7:1319-1331, 1995.

CARVALHO, W. L.; OLIVEIRA, M. G. A.; BARROS, E. G. & MOREIRA, M. A. Lipoxygenases affect protease inhibitor levels in soybean seeds. **Plant Physiology and Biochemistry,** 37(6): 497-501, 1999.

FARMER, E. E. & RYAN, C. A. Octadenoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. **Plant Cell,** 4:129-134, 1992.

FEHR, W. R.; CAVINESS, C. E.; BURMOOD, D. T. & PENNINGTOON, J. S. Stages of development descriptions for soybeans, *Glycine max* (L.) Merrill. **Crop Science,** 11:91-93, 1971.

GALLIARD, T. & PHILLIPS, D. R. Lipoxygenases from potato tubers. Partial purification and properties of na enzyme that specifically oxygenates the 9-positon of linoleic acid. **Biochemistry Journal ,** 124:431-438, 1971.

GRAYBURN, W. S.; SCHNEIDER, G. R.; HAMILTON-KEMP, T. R.; BOOKJANS, G.; ALI, K. & HILDEBRAND, D.F. Soybean leaves countain multiple lipoxygenases. **Plant Physiology,** 95:1214-1218, 1991.

HILDEBRAND, D. F.; HAMILTON-KEMP, T. R.; LEGG, C. S. & BOOKJANS, G. Plant lipoxygenase: occurrence, properties and possible functions. **Current Topics In Plants Biochemistry and Physiology,** 7:201-219, 1988.

KAKADE, M. L.; RACKIS, J. J.; McGHEE, J. E. & PUSKI, G. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. **Cereal Chemistry,** 51:376-382, 1974.

KATO, T.; OTHA, H.; TANAKA, K. & SHIBATA, D. Appearance of new lipoxygenases in soybean cotyledons after germination and evidence for expression of a major new lipoxygenase gene. **Plant Physiology,** 98:324-330, 1990.

LANNA, A. C.; OLIVEIRA, M. G. A.; BARROS, E. G. & MOREIRA, M. A. Kinetic parameters of leaf lipoxygenase pools from normal soybean genotypes and a line devoid of seed lipoxygenases. **Brazilian Journal Plant Physiology,** 8:87-92, 1996.

LEATHERBARROW, R. J. **Enziffiter manual**. Biosoft. London, pp. 13-42, 1987.

LEONI, O.; IORI, R. & PALMIERI, S. Purification and properties of lipoxygenase in germinating sunflower seeds. **Journal of Food Science,** 50:88-92, 1985.

MINGUEZ-MOSQUEIRA, M. I.; JAREN-GALAN, M. & GARRIDO-FERNANDEZ, J. Lipoxygenase activity during pepper ripening and processing of paprika. **Phytochemistry,** 32:1103-08, 1993.

OHTA, H.; IDA, S.; MIKAMI, B. & MORITA, Y. Changes in lipoxygenase components of rice seedling during germination. **Plant Cell Physiology,** 22:911-918, 1986.

PARK, T. K. & POLLACO, J. C. Distinct lipoxygenase species appear in leaves hypocotiles/radicles of germinating soybean. **Plant Physiology,** 90:285-290, 1989.

PETERMAN, T. K. & SIEDOW, J. N. Behavior of lipoxygenase during establishment, senescence, and rejuvenation of soybean cotyledons. **Plant Physiology,** 31:313-340, 1985.

REINBOTHE, C.; PARTHIER, B. & REINBOTHE, S. Temporal pattern of jasmonte-induced alterations in gene expression of barley leaves. **Planta,** 201:281-287, 1997.

RICKAUEAR, M.; BRODSCHELM, W.; BOTTIN, A.; VÉRONÉSI, C.; GRIMAL, H. & ESQUERRÉ-TUGYÉ, M. T. The jasmonate pathway is involved differentially in the regulation of different defence responses in tobacco cells. **Planta,** 202:155-162, 1997.

SARAVITZ, D. M. & SIEDOW, J. N. The lipoxygenase isozymes in soybean [*Glycine max* (L.) Merr.]. Changes during leaf development, after wounding, and following reproductive sink removal. **Plant Physiology,** 107:535-543, 1995.

SARAVITZ, D. M. & SIEDOW, J. N. The differential expression of wound-inducible lipoxygenase genes in soybean leaves. **Plant Physiology,** 110:287-299, 1996.

SMITH, P. K.; KROHN, R. I.; HERMANSON, G. T.; MALLIA, A. K.; GARTNER, F. H.; PROVENZANO, M. D.; FUJIMOTO, E. K.; GOEKE, N. M.; OLSO, B. J. & KLENK, C. Measurement of protein using bicinchoninic acid. **Analog Biochemistry,** 150:76-85, 1985.

UTUMI, M. M.; BARROS, E. G.; OLIVEIRA, M. G. A.; SEDIYAMA, C. S. & MOREIRA, M. A. Efeito da eliminação genética de lipoxigenases e peptídeos de reserva na qualidade da proteína de soja. **Brazilian Journal Plant Physiology,** 10:203-212, 1998.

WILSON, D. O. Jr & McDONALD, M. B. Jr. A convenient volatile aldehyde assay for measuring seed vigour. **Seed Science Technology** 14:259-268, 1986.