RESISTANCE TO STEM CANKER, FROGEYE LEAF SPOT AND POWDERY MILDEW OF SOYBEAN LINES LACKING LIPOXIGENASES IN THE SEEDS

Carlos Alberto Osório Martins[1,3]; Carlos Sigueyuki Sediyama[1]*; Maria Goreti de Almeida Oliveira[2]; Múcio Silva Reis[1]; Valterley Soares Rocha[1]; Maurílio Alves Moreira[2]; José Luiz Lopes Gomes[1]

[1]*Depto. de Fitotecnia - UFV - CEP: 36571-000 - Viçosa, MG.*
[2]*Depto. de Bioquímica e Biologia Molecular - UFV/BIOAGRO.*
[3]*CNPq Fellow.*
**Corresponding author <csediyama@ufv.br>*

ABSTRACT: The soybean [*Glycine max* (L.) Merrill] crop holds a prominent position in the Brazilian economy because of the extension of the planted area and volume of grain production, but the beany flavor has been a limiting factor for soybean derivatives consumption by western population. This flavor is produced mainly by action of lipoxygenase enzymes (Lox1, Lox2 and Lox3), present in some commercial varieties. The genetic elimination of the alleles that codify these enzymes is the most appropriate way to avoid problems associated to this deleterious flavor. To elucidate the effect of seed lipoxygenase elimination on the resistance to plant pathogens, normal varieties of soybean (FT-Cristalina RCH, Doko RC and IAC-12) and their backcross-derived lines, both with the three lipoxygenases present in their seeds (triple-positive, TP) and without the three lipoxygenases (triple-null, TN), were tested for their resistance to stem canker (*Diaporthe phaseolorum* f.sp. *meridionalis*), frogeye leaf spot (*Cercospora sojina* Hara), and powdery mildew (*Microsphaera diffusa* Cke. & Pk.). All genetic materials studied were resistant to stem canker. FT-Cristalina RCH and Doko-RC and their TP and TN lines were resistant to frogeye leaf spot. IAC-12 and its derived lines not only presented a higher disease index, but also the derived lines, TP and TN, were more susceptible, indicating the loss of genes for disease resistance in the backcrosses. There was no association between the elimination of lipoxygenases from the seeds with the resistance to frogeye leaf spot. In relation to the powdery mildew, TP or TN lines presented similar or higher resistance than their respective recurrent parents whose susceptibility appeared in the following order: IAC-12, less susceptible, Doko-RC, intermediate and FT-Cristalina RCH, more susceptible.
Key words: Glycine max, breeding, genetics

RESISTÊNCIA AO CANCRO-DA-HASTE, À CERCOSPORIOSE E AO OÍDIO DE LINHAGENS DE SOJA SEM LIPOXIGENASES NAS SEMENTES

RESUMO: A cultura da soja [*Glycine max* (L.) Merrill] ocupa lugar de destaque na economia brasileira, tanto em termos de área plantada, quanto de produção de grãos. A presença de gosto de feijão cru tem sido limitante para o consumo de derivados de soja pelos povos ocidentais. Esse sabor característico é proporcionado pelas enzimas lipoxigenases (Lox1, Lox2 e Lox3). A eliminação dessas enzimas, pela manipulação genética dos alelos que as codificam, é a maneira mais adequada de contornar os problemas associados ao sabor desagradável. Visando elucidar a participação das lipoxigenases, no processo de resistência da soja a patógenos, variedades normais de soja (FT-Cristalina RCH, Doko RC e IAC-12) e suas respectivas linhagens obtidas por retrocruzamentos, sem as três lipoxigenases nas sementes (triplo-nulas - TN) e com as três lipoxigenases (triplo-positivas - TP), foram testadas quanto às suas resistências ao cancro-da-haste (*Diaporthe phaseolorum* f.sp. *meridionalis*), à cercosporiose (*Cercospora sojina* Hara) e ao oídio (*Microsphaera diffusa* Cke. & Pk.). Todos os materiais genéticos foram resistentes ao cancro-da-haste. Com relação à cercosporiose, FT-Cristalina RCH e Doko-RC e suas respectivas linhagens com ou sem lipoxigenases mostraram-se resistentes, enquanto IAC-12 e suas linhagens derivadas mostraram índices de doença mais elevados, sendo que as linhagens IAC-12 TP e TN foram mais suscetíveis, indicando perda de gene de resistência nos retrocruzamentos. Não houve relação entre retirada dos genes que codificam lipoxigenases nas sementes com a resistência à cercosporiose. No caso do oídio, as linhagens TP ou TN apresentaram-se similares ou pouco mais resistentes que seus respectivos progenitores recorrentes, os quais se mostraram suscetíveis na seguinte ordem: IAC-12, menos suscetível, Doko-RC, intermediário e FT-Cristalina RCH, mais suscetível.
Palavras-chave: Glycine max, melhoramento, genética

INTRODUCTION

The soybean crop holds a prominent position in the Brazilian economy, both in terms of planted area and in terms of grain production. This status was obtained due to the availability of improved varieties adapted to cultivation, resistant to diseases and productive and, especially, due to the potential of the grains as a source of raw material for several products.

However, a greater use of soybean in the food composition has been difficult due to the characteristic beany flavor of the commercially cultivated soybean seeds. The genetic elimination of lipoxygenases by means of backcrosses with progenitors that present genes for its absence has been used as an alternative for the suppression of beany flavor in these commercial varieties (Oliveira et al., 1998). On the other hand, an increased expression of leaf lipoxygenases has been detected in plants during or after submission to varied types of stress, including mechanical wounds (Saravitz & Siedow, 1995; Wang et al., 2000; Vieira et al., 2001), insect attack (Hildebrand et al., 1988; Silva, 1999) and infection by pathogens (Melan et al., 1993).

The physiological relevance of lipoxygenase pathway induction in plants under biotic and abiotic stress conditions has been established and some proposals have been suggested, including membrane degradation during the hypersensitivity reaction (Croft et al., 1990), production of antimicrobian molecules and fat acid derivatives (Croft et al., 1993) and synthesis of substances related to plant growth, as jasmonic acid, which is involved in the activation of genes that express protease inhibitors (Farmer & Ryan, 1992).

Among the main factors that limit the attainment of high income of soybean crops are the diseases that, in general, are difficult to control. Approximately 40 diseases caused by fungi, bacteria, nematodes and viruses have been already identified in Brazil. The economic importance of each of them varies from year to year and from region to region, depending upon the climatic condition of each season (Embrapa, 2000). During the 1999/2000 growing season, diseases were responsible for losses estimated in US$ 1.39 billion (Yorinori, 2000). Because soybean is cultivated over large areas, all over Brazil, diseases are spread over the whole country, and the possibility of appearing new kinds of diseases exists.

Under favorable conditions, end-of-cycle leaf diseases, like frogeye leaf spot, can reduce the income in more than 20%. Losses will be greater if the damages from other diseases, for example, stem canker, are added. Powdery mildew is another disease that, in more infected crops, can cause losses estimated between 30% and 40% (Embrapa, 2000).

To elucidate the participation of lipoxygenase in the process of soybean resistance to pathogens, this work was carried out to estimate the infection index in the tests for resistance to stem canker, frogeye leaf spot and powdery mildew, using normal varieties of soybean that present lipoxygenases in seeds, and lines presenting complete absence of lipoxygenases, obtained by backcrosses.

MATERIAL AND METHODS

A greenhouse experiment was carried out in Viçosa, MG, Brazil, from August to October 1999.

The TN soybean lines available in the Soybean Germplasm Bank, of the Federal University of Viçosa, were obtained from the cross of the CR2,3 line with a line without all the three lipoxygenase isozymes (triple-null), obtained at the “National Agriculture Research Center”, in Tsukuba, Japan, by Dr. Keisuke Kitamura, who donated F_1 seeds to BIOAGRO.

Initially, the CR2,3 line was obtained and characterized by the absence of lipoxygenases (Lox) 2 and 3. This line is originated from the cross between the commercial FT-Cristalina variety with Ichigowase (variety without Lox 3) and with PI 86023 (line without Lox 2). Later, lines without lipoxygenases 2 and 3 were selected, called FT-Cristalina 2,3 (CR2,3), identified in the segregating population by the colorimetric method proposed by Suda et al. (1995). Selections were carried out from F_2 to F_6 generation.

The F_1 seeds were sown in a greenhouse and the F_2 were selected for the absence of all three lipoxygenases. They were crossed with the CR2,3 line, and the first backcross was obtained. Later, this material was backcrossed with FT-Cristalina RCH, Doko-RC and IAC-12 varieties. In the forth backcross with these varieties, the triple-null seeds having the three pairs of recessive genes that promote the absence of the three lipoxygenases, and the triple-positive, carrying the dominant genes, were selected.

Triple-null and triple-positive seeds were selected by colorimetric tests (Suda et al., 1995). The genotypes were confirmed by lipoxygenase activity determination (Oliveira et al., 1998). The treatments were called “Commercial Cristalina”, “Triple-Null Cristalina” ($lx_1lx_1lx_2lx_2lx_3lx_3$) and “Triple-Positive Cristalina” ($Lx_1Lx_1Lx_2Lx_2Lx_3Lx_3$), with analogous denominations for materials derived from Doko-RC and IAC-12 varieties. The selected seeds were sown, in a greenhouse, from May to June of 1998, to multiply the seeds for the assays. The seeds were harvested manually during September and October of the same year.

Disease resistance tests were carried out using four lines of each type of genetic material (Commercial, TP and TN) of each variety. The seeds were sown in 5L pots, with soil fertilized with 10 g dm^{-3} of P, in the form of simple super phosphate, 20 g dm^{-3} of K, as potassium chloride and 5 g dm^{-3} of N, as dress-applied ammonium sulphate. Five plants per pot were grown, with 30 inoculated plants per treatment. Maximum and minimum temperatures were 35 and 22°C, respectively. The seedlings emerged four days after sowing.

Diaporthe phaseolorum f.sp. *meridionalis* isolate CH 08, supplied by the National Center of Soybean Research (Embrapa - Soybean) and collected from Davis soybean variety stems, from Palmeiras, PR, Brazil, was used in the inoculations. The isolate was multiplied and supplied by the Soybean Breeding Program, of the Plant Sciences Department of the Federal University of Viçosa, MG, Brazil.

The toothpick colonized with fungus mycelium inoculation method was used, according to the technique described by Crall (1952) and Keeling (1982), modified by Yorinori (1991). The original FT-Cristalina variety, susceptible to stem canker, was used as the susceptible control, while in the breeding program FT-Cristalina RCH, resistant to stem canker, was used.

Seedlings were inoculated in the morning, at the V_1 (Fehr et al., 1977) developmental stage, 15 days after sowing, introducing the fungus colonized toothpick into the main stem of the seedlings, 10 mm below the unifoliate leaves. The inoculated plants were kept in a mist chamber, for 72 hours, at 20-22°C, and 100% relative humidity; after that, were moved to a greenhouse. The evaluations, carried out 50 days after inoculations, consisted of visual grading of the presence of injury at the inoculation point. Plants that did not show injuries were considered resistant and those injured and subsequently dead, as susceptible.

Artificial inoculation of *Cercospora sojina* Hara was carried out following Cordeiro (1986), using race 4 inoculum, one of the most virulent of the prevailing races in Central Brazil, also supplied by the Plant Sciences Department of the Federal University of Viçosa. The inoculation of the pathogen was carried out when the plants showed the first trifoliate leaves completely expanded, about 20 days after emergence. The leaves were sprayed with 10 mL of a suspension of conidia, on both, upper and lower sides. The calibration of the suspension was made with an hemacitometer, for the concentration of 40,000 conidia per milliliter, according to Casela et al. (1979).

Based on the more infected foliole of the plant, 20 days after the inoculation, in the greenhouse, the severity of the disease was evaluated using the scale used by Ross (1968) to determine the infection level: 1 – without disease symptom; 2 – presence of disease traces; 3 – injuries of small size; 4 – injuries of average size; and 5 – large injuries. The scheme presented by Cordeiro (1986), which shows folioles with several degrees of infection, served as standard for the evaluations. Degrees 1 to 3 were considered to be resistance reaction and degrees 4 and 5 to be susceptibility reaction (Casela et al., 1981).

Inoculation for powdery mildew was carried out using susceptible plants of FT-Cristalina RCH variety, infected with *Microsphaera diffusa* Cke. & Pk.; presenting fungus on the leaves, placed among the plants to be tested, 20 days after their emergence, so that the inoculi should spread over their leaves and cause infection.

Three evaluations were made, 7, 21 and 45 days after the introduction of the infected plants. The infection level (IL) was visually estimated on the basis of the infected foliar area (IFA), following the scale described by Yorinori (1997). In this scale, IL = 0 (without infection); IL = 1 (traces - 10% of the IFA); IL = 2 (11 - 25% of the IFA); IL = 3 (26 - 50% of the IFA); IL = 4 (51 - 75% of the IFA); and IL = 5 (76 - 100% of the IFA). The following classification criterion of reaction was adopted: R = resistant (IL = 0 to 2); MR = moderately resistant (IL = 2 to 3); S = susceptible (IL = 3 to 4); and HS = highly susceptible (IL = 4 to 5).

Analysis of variance was carried out according to the completely randomized design, considering the following sources of variation: types of genetic material (commercial variety and lines with or without lipoxygenases in the seeds), lines within genetic materials, and plants within lines within genetic materials. Means were compared by the Tukey test at the 5% level.

RESULTS AND DISCUSSION

In the three varieties (FT-Cristalina RCH, Doko-RC and IAC-12) and in their respective lines with or without lipoxygenases, stem canker symptoms were absent on plants grown in the greenhouse, while the control plants showed susceptibility and died. Therefore, all inoculated materials showed resistance to the isolate CH 08 of *Diaporthe phaseolorum* f.sp. *meridionalis*, confirming the maintenance of the resistance during the backcrosses, given that the recurrent ancestors were chosen because they were resistant in previous tests.

Two dominant genes confer resistance in Tracy-M variety (Kilen & Hartwig, 1987; Bowers et al., 1993). Siviero (1992), Oliveiras (1993), Carvalho (1995) and Tyler (1996) detected the presence of a gene controlling the resistance to stem canker. IAC-12 soybean is resistant to stem canker, and this resistance is governed by two independent genes (Silva, 1998).

The small number of genes involved in the resistance to a given isolate facilitates their maintenance in the process of selection monitored by molecular markers, which was confirmed by the results obtained in the present work. Therefore, varieties without lipoxygenases in the seeds, derived from the lines under study, should be resistant to the CH 08 isolate. The varieties used in this study and their derived lines with and without lipoxygenases in seeds still have other lipoxygenases in leaves which may be involved in soybean reactions to damages caused by insects or by disease injuries.

For frogeye leaf spot, there were effects of variety and type of genetic material (commercial material; triple-positive, TP; or triple-null, TN), as well as of their interaction (Table 1). However there were no differences among the four lines within each type of genetic material. Inoculations with *Cercospora sojina* caused different reactions of the varieties (Table 1). FT-Cristalina RCH as well as TP and TN lines revealed high resistance to frogeye leaf spot. Doko-RC can be considered resistant, although it showed some infection symptoms, the same was observed on TP and TN derived lines. IAC-12 variety and its derived TP and TN lines had higher disease indexes, the TP and TN derived lines being more susceptible, which indicates loss of some resistance genes in the process of transference of the null alleles for lipoxygenases in this variety. In this case, it is necessary to carry out additional backcrosses to recover the resistance to frogeye leaf spot. On the other hand, these results do not indicate relation between the elimination of the genes that codify for lipoxygenases in the seeds and the resistance to *C. sojina* race 4 isolate.

The development of resistant varieties, or the introduction of resistance genes into susceptible varieties, is a priority in many breeding programs. However, for higher effectiveness in the process of incorporation of resistance genes, a better understanding of the mechanism of inheritance of the resistance to *Cercospora* is needed (Martins Filho, 1999). Additionally, the biotechnological tools, like the use of DNA markers in the mapping of resistance genes, have facilitated the breeding programs seeking the control of diseases (Kelly et al., 1994).

For powdery mildew incidence, there were effects of genetic material types and variety versus line contrasts, but no effect of lines within genetic materials was observed (Table 1). TP and TN lines presented similar results or indicated to be a little more resistant than their respective recurrent progenitors, which had shown to be susceptible in the following order: IAC-12, less susceptible, Doko-RC, intermediate and FT-Cristalina RCH, more susceptible.

Soybean resistance to *Microsphaera diffusa,* the causal organism of powdery mildew disease, is controlled by only one dominant gene, (Grau & Laurence, 1975, Buzzell & Haas, 1978, and Lohnes & Nickell, 1994). The presence of three levels of infection in the present work, points out to the presence of more genes involved in the resistance.

Juliatti et al. (1999), analyzing the level of infection in ten soybean varieties with the purpose of estimating the losses caused by powdery mildew, found an interaction between varieties and time of sowing and confirmed that the UFV-16 variety has high resistance to this disease. Silva & Seganfredo (1999) verified that powdery mildew is responsible for economic losses in soybean crops, despite the application of fungicides.

The improvement of soybean flavor, by means of genetically eliminating the lipoxygenases from the seeds, by backcrosses, can concomitantly be carried out with the maintenance of the resistance to stem canker, frogeye leaf spot and powdery mildew diseases, presented by the recurrent progenitors.

Table 1 - Estimated means of infection indexes obtained from frogeye leaf spot and powdery mildew resistance tests of FT-Cristalina RCH, Doko-RC and IAC-12 commercial soybean varieties and their lines with (triple-positive, TP) or without (triple-null, TN) the three lipoxygenases in the seeds. Viçosa, MG[1].

Genetic material	Frogeye leaf spot	Powdery mildew
FT-Cristalina RCH Commercial	1.00 a	2.96 b
FT-Cristalina RCH TP	1.00 a	2.70 a
FT-Cristalina RCH TN	1.00 a	2.93 ab
Mean	1.00 A	2.86 C
Doko-RC Commercial	1.20 a	2.59 b
Doko-RC TP	1.15 a	2.17 a
Doko-RC TN	1.08 a	2.26 a
Mean	1.14 B	2.34 B
IAC-12 Commercial	2.64 a	2.03 a
IAC-12 TP	3.47 b	1.92 a
IAC-12 TN	3.58 b	2.04 a
Mean	3.23 C	2.00 A
CV (%)	7.80	32.51

[1]In the columns, variety means followed by the same higher case letters, or genetic material type means followed by the same lower case letters, do not significantly differ by the Tukey´s test at 5 % level.

ACKNOWLEDGMENTS

To CNPq and FINEP for the financial support.

REFERENCES

BOWERS JR., G.R.; NGELEKA, K.; SMITH. O.D. Inheritance of stem canker in soybean cultivars Crockett and Dowling. **Crop Science**, v.33, p.67-70, 1993.

BUZZELL, R.I.; HASS, J.H. Inheritance of adult plant resistance to powdery mildew in soybeans. **Canadian Journal of Genetics and Cytology,** v.20, p.151-153, 1978.

CARVALHO, G.A. Marcadores RAPD ligados a genes de resistência ao cancro da haste da soja. Viçosa, 1995. 51p. Dissertação (M.S.) – Universidade Federal de Viçosa.

CASELA, C.R.; BRANCÃO, N.; GASTAL, M.F.C. Mancha "olho-de-rã" (*Cercospora sojina* Hara) em soja (*Glycine max* (L.) Merrill). In: SEMINÁRIO NACIONAL DE PESQUISA DE SOJA, 1., Brasília, 1979. **Anais.** Londrina: EMBRAPA, CNPSo, 1979. v.2, p.139-143.

CASELA, C.R.; BRANCÃO, N.; GASTAL, M.F.C. Raças fisiológicas de *Cercospora sojina* Hara, agente causal da mancha "olho-de-rã" em soja (*Glycine max* (L.) Merrill). In: SEMINÁRIO NACIONAL DE PESQUISA DE SOJA, 2., Brasília, 1981. **Anais.** Londrina: EMBRAPA, CNPSo, 1981. v.2, p.255-258.

CORDEIRO, A.C.C. Herança da resistência da soja (*Glycine max* (L.) Merrill) à *Cercospora sojina* Hara, isolado de São Gotardo, Minas Gerais. Viçosa, 1986. 61p. Dissertação (M.S.) – Universidade Federal de Viçosa.

CRALL, J. M. A toothpick method of inoculation. **Phytopathology**, v.42, p.5-6, 1952.

CROFT, K.P.C.; JÜTTNER, F.; SLUSARENKO, A.J. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv *phaseolicola*. **Plant Physiology,** v.101, p.13-24, 1993.

CROFT, K.P.C.; VOISER, C.R.; SLUSARENKO, A.J. Mechanism of hypersensitive cell collapse: Correlation of increased lipoxygenase activity with membrane damage in leaves of *Phaseolus vulgaris* (L) inoculated with *Pseudomonas syringae* pv *phaseolicola*. **Molecular Plant Physiology,** v.36, p.49-62, 1990.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Doenças e medidas de controle. In: EMBRAPA. **Recomendações técnicas para a cultura da soja na região central do Brasil 2000/2001**. Londrina: Embrapa Soja; Fundação MT, 2000. p.171-219.

FARMER, E.E.; RYAN, C.A. Octadecanoid precursors of jasmonic acid activates the synthesis of wound-inducible proteinase inhibitors. **The Plant Cell**, v.4, p.129-134, 1992.

FEHR, R.W.; CAVINESS, C.E.; BURMOOD, D.T.; PENNINGTON, J.S. **Stage of soybean development**. Ames: Iowa State University, Cooperative Extension Service, 1977. 11p. (Special Report 80).

GRAU, C.R.; LAURENCE, J.A. Observations on resistance and heritable resistance to powdery mildew of soybean. **Plant Disease Reporter,** v.59, p.458-460, 1975.

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

JULIATTI, F.C.; LINS, P.Z.; SANTOS, V.L.M.; HAMAWAKI, O.T. Avaliação de variedades de soja a Oídio (*Microsphaera diffusa*) no inverno em Uberlândia – MG. In: CONGRESSO BRASILEIRO DE SOJA, Londrina, 1999. **Resumos**. Londrina: Embrapa Soja, 1999. p.446.

KEELING, B.L. A seedling test for resistance to soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora*. **Phytopathology**, v.77, p.807-809, 1982.

KELLY, J.; HALEY, S.; AFANADOR, L. Application of RAPD markers for disease resistance breeding in beans. **Annual Report of the Bean Improvement Cooperative**, v.37, p.15-16, 1994.

KILEN, T.C.; HARTWIG, E.E. Identification of single gene controlling resistance to stem canker in soybean. **Crop Science**, v.27, p.863-864, 1987.

LOHNES, D.G.; NICKELL, C.D. Effects of powdery mildew alleles Rmd-c, Rmd, and rmd on yield and other characteristics in soybean. **Plant Disease**, v.78, p.299-301, 1994.

MARTINS FILHO, S. Mancha olho-de-rã da soja: Análise genética da resistência e identificação de marcadores moleculares. Viçosa, 1999. 59p. Tese (Doutorado) – Universidade Federal de Viçosa.

MELAN, M.A.; DONG, X.; ENDARA, M.E.; DAVIS, K.R. AUSUBEL, F.M.; PETTERMAN, T.K. An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. **Plant Physiology,** v.101, p.441-450, 1993.

OLIVEIRA, A.L. Herança da resistência ao cancro da haste *Diaporthe phaseolorum* (Cke. & Ell) Sacc. f.sp. *meridionalis* Morgan-Jones em soja **(***Glycine max* (L.) Merrill**)**. Jaboticabal, 1993. 40p. Tese (Livre Docente) – Universidade Estadual Paulista "Júlio de Mesquita Filho".

OLIVEIRA, D.A.; PIOVESAN, N.D.; MORAES, R.M.A. de; ROCHEBOIS, G.B.; OLIVEIRA, M.G.A.; BARROS, E.G. de; MOREIRA, M.A. Identification of three genotypic classes of soybean lipoxygenases 1 and 3 based on enzymatic activity. **Biotechnology Techniques**, v.12, p.71-74, 1998.

ROSS, J.P. Additional physiological races of *Cercospora sojina* on soybean in North Carolina. **Phytopathology**, v.58, p.708-709, 1968.

SARAVITZ, D.M.; SIEDOW, J.N. Changes during leaf development, after wounding, and following reproductive sink removal. **Plant Physiology,** v.107, p.535-543, 1995.

SILVA, F.B. Caracterização da "via das lipoxigenases" de folhas de soja submetidas a insetos-pragas. Viçosa, 1999. 67p. Dissertação (M.S.) – Universidade Federal de Viçosa.

SILVA, M.A. Resistência ao cancro da haste da soja: Herança e identificação de marcadores moleculares. Viçosa, 1998. 43p. Dissertação (M.S.) – Universidade Federal de Viçosa.

SILVA, O.C.; SEGANFREDO, R. Quantificação de danos ocasionados por doenças de final de ciclo e oídio, em duas variedades de soja. In: CONGRESSO BRASILEIRO DE SOJA, Londrina, 1999. **Resumos**. Londrina: Embrapa Soja, 1999. p.460.

SIVIERO, A. Herança da resistência da soja a *Diaporthe phaseolorum* f.sp. *meridionalis*. Piracicaba, 1992. 39p. Tese (Livre Docente) – Escola Superior de Agricultura "Luíz de Queiroz", Universidade de São Paulo.

SUDA, I.; HAJIKA, M.; NISHIBA, Y.; FURUTA; E.; IGITA, K. Simple and rapid method for the selective detection of individual lipoxygenase isozymes in soybean seeds. **Journal of Agriculture and Food Chemistry**, v.43, p.742-747, 1995.

TYLER, J.M. Characterization of stem canker resistance in Hutcheson soybean. **Crop Science**, v.36, p.591-593, 1996

VIEIRA, A.A.; OLIVEIRA, M.G.A.; JOSÉ, I.C.; PIOVESAN, N.D.; REZENDE, S.T.; MOREIRA, M.A.; BARROS, E.G. Biochemical evaluation of lipoxygenase pathway of soybean plant submitted to wounding. **Revista Brasileira de Fisiologia Vegetal**. v.13, p.1-12, 2001.

WANG, C.; ZIEN, C.A.; AFITLHILE, M.; WELTI, R.; HILDEBRAND, D.F. WANG, X. Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in Arabidopsis. **The Plant Cell**, v.2, p.2237-2246, 2000.

YORINORI, J.T. Metodologia de produção de *Diaporthe phaseolorum* f. sp. *meridionalis*. **Fitopatologia Brasileira**, v.16, p.58, 1991. Suplemento.

YORINORI, J.T. Variedades de soja resistentes à *Microsphaera diffusa*. **Fitopatologia Brasileira**, v.22, p.320-321, 1997. Supplemento.

YORINORI, J.T. Riscos de surgimento de novas doenças na cultura da soja. In: CONGRESSO DE TECNOLOGIA E COMPETITIVIDADE DA SOJA NO MERCADO GLOBAL, Cuiabá, 2000. **Anais.** Cuiabá: Fundação Mato Grosso, 2000. p.165-169.

Received September 25, 2001