BIOLOGIA PLANTARUM 55 (3): 522-527, 2011

Thiamine seed treatment enhances *LOX* expression, promotes growth and induces downy mildew disease resistance in pearl millet

H.G. PUSHPALATHA[1,2], J. SUDISHA[1], N.P. GEETHA[1], K.N. AMRUTHESH[3] and H. SHEKAR SHETTY[1]*

Department of Studies in Biotechnology, University of Mysore, Mysore-570006, Karnataka, India[1]
Maharani's Science College for Women, Bangalore-560001, Karnataka, India[2]
Department of Studies in Botany, University of Mysore, Mysore-570006, Karnataka, India[3]

Abstract

Seeds of pearl millet [*Pennisetum glaucum* (L). R.Br.] susceptible cv. 7042S were treated with thiamine at 5, 10, 15, 20 and 25 mM concentrations and growth promotion and downy mildew resistance were tested. Seed treatment with 20 mM thiamine resulted in 72 and 70 % disease protection under greenhouse and field conditions, respectively, and enhanced vegetative and reproductive growth parameters. Analysis of lipoxygenase (LOX) activity in inoculated pearl millet seedlings at different time intervals indicated that increased LOX activity was initiated at 3 h after inoculation (hai) and maximum activity was observed at 24 hai. Northern analysis showed that *LOX* mRNA transcript accumulation was higher in the resistant seedlings (cv. IP18292) than in susceptible seedlings. Thiamine seed treatment induces rapid *LOX* gene expression and results in significant disease protection against downy mildew disease.

Additional key words: induced systemic resistance, lipoxygenase, *Pennisetum glaucum*, *Sclerospora graminicola*, transcript accumulation.

Introduction

Unlike traditional pesticides, induced systemic resistance (ISR) provides a way to control disease without exerting direct pressure on pathogen population. Such strategies are environmentally safe and do not affect the appearance of chemical tolerant strains and induces long lasting efficient resistance against a broad spectrum of pathogens (Tanabe *et al*. 2006). In recent years, the importance of vitamins as plant disease control agents has been emphasized. Several vitamins such as riboflavin (Dong and Beer 2000), menadione sodium bisulphite (Borges *et al*. 2004), thiamine (Ahn *et al*. 2005) have been utilized as disease resistance inducers. Thiamine treated rice, *Arabidopsis*, vegetables and crops showed resistance to fungal, bacterial and viral diseases. Thiamine treatment also induced the transient expression of pathogenesis-related genes in rice after infection with *Magnaporthe grisea* (Ahn *et al*. 2005, 2007).

Pearl millet is an important grain, forage crop mainly in the arid and subtropical regions of the world. A major biotic constraint in pearl millet production is downy mildew disease caused by *Sclerospora graminicola*, an oomycete pathogen resistant to many fungicides (Tyler 2001).

The interaction between the pathogen and host induces challenges in cell metabolism primarily in the enzyme activities including lipoxygenase (Porta *et al*. 2008). Plant lipoxygenases (LOXs; linoleate oxygen oxidoreductase, EC 1.13.11.12) are a family of non-heme, iron containing enzymes that catalyze the oxygenation of linoleic and linolenic acids, polyunsaturated fatty acids most likely derived from membrane lipids (Siedow 1991, Gao *et al*. 2008). In plants, products of the LOX pathway have several diverse functions including the mobilization of storage lipids and proteins during germination (Feussner *et al*. 2001). Contribution of the oxylipin pathway to plant defense can

Received 9 November 2009, *accepted* 13 August 2010.
Abbreviations: ISR - induced systemic resistance; hai - hours after inoculation; HPL - hydroperoxide lyase; HR - hypersensitive response; LOX - lipoxygenase.
Acknowledgements: The lipoxygenase probe was a kind gift from Dr. D.J. Hannapel, Iowa University, Iowa, USA.
* Corresponding author; fax: (+91) 0821 2515126, e-mail: hss_uom@hotmail.com

also proceed from production of signal molecules inducing defense gene expression leading to a powerful plant defense mechanism against pathogens known as the hypersensitive response (La Camera *et al*. 2004, Avanci *et al*. 2010). The fatty acid hydroperoxide produced by LOX can be converted into two aldehydes by hydroperoxide lyase (HPL) and HPL products may have a role in oxidation of octadecanoid pathway by lipid-hydrolyzing activity (Howe and Schilmiller 2002).

Hence, the present study focuses on the expression and activity of LOX during induction of pearl millet downy mildew disease resistance by thiamine seed treatment. Also the effect of thiamine in the growth promotion of pearl millet has been evaluated.

Materials and Methods

Pearl millet [*Pennisetum glaucum* (L.) R.Br.] seeds of highly resistant (IP18292) and highly susceptible (7042S) to downy mildew disease (Sudisha *et al*. 2008) were used throughout the study. The downy mildew pathogen of pearl millet *Sclerospora graminicola* isolated from the susceptible pearl millet cultivar 7042S and maintained in the same cultivar under greenhouse conditions (temperature of 22 ± 2 ºC and relative humidity of 80 %) was used for all inoculation experiments. The concentration of the *S. graminicola* zoospores was adjusted to 4×10^4 zoospores cm^{-3} with sterile distilled water using haemocytometer (Safeeulla 1976).

Seeds of this susceptible cultivar were treated with different concentrations of thiamine (vitamin B_1, *Hi media*, Mumbai, India; 5, 10, 15, 20 and 25 mM) and kept in a rotary shaker for 6 h. Two-day-old seedlings of resistant (IP18292), susceptible (7042S) and thiamine treated 7042S were inoculated by dipping roots into solution with 4×10^4 zoospores cm^{-3} and incubated in dark at 25 ± 2 °C (Safeeulla 1976). Inoculated and uninoculated seedlings were harvested at 0, 3, 6, 9, 12, 24, 48 and 72 h after inoculation (hai) and immediately wrapped in an aluminum foil and stored at -20 ºC for LOX enzyme assay and Northern blot hybridization experiments. Seedlings mock inoculated with distilled water were taken as control.

Thiamine treated seeds, distilled water treated seeds (control) and seeds treated with metalaxyl (*Apron 35SD* at 6 g kg^{-1}), which is used as standard control, were grown separately in clay pots containing 2:1:1 soil, sand and farm yard manure. Two-day-old-seedlings were whorl-inoculated with the zoospore suspension of *S. graminicola* at the above mentioned concentration following the procedure of Singh and Gopinath (1985). The experiment was carried out in four replicates of 100 seedlings and repeated twice. These pots were arranged in a completely randomized block design and maintained under greenhouse conditions (25 ± 2 ºC, 95 % relative humidity).

Another set of experiment was conducted under field conditions using thiamine treatments at the same concentrations with control sets at the downy mildew sick plot (Department of Biotechnology, University of Mysore, Mysore, Karnataka, India) in completely randomized block design in 8 × 5 m plots in four replications consisting of four rows of 35 plants and repeated twice. Following the normal agronomical practices, the plants were raised and disease screening was done using the infector row system.

Disease observations were done at 15-d intervals after inoculation. The plants were rated for disease when they showed typical downy mildew symptoms such as sporulation on the abaxial leaf surface, chlorosis or stunted growth. At the end of 60 d, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. The scoring system consists of four-level scale described as 0 - 5 % = highly resistant, 5.1 - 10 % = resistant, 10.1 - 25 % = susceptible and 25.1 - 100 % = highly susceptible (Sudisha *et al*. 2008).

To test the role of thiamine in promoting vegetative growth and reproductive parameters, thiamine treated seeds at 5, 10, 15, 20 and 25 mM concentrations along with the control sets were sown in the field as explained earlier. The effects on growth parameters (plant height, length and girth of ear head) were measured at 60 d. The experiment was conducted with four replicates of 100 plants each and repeated twice.

LOX enzyme extracts were prepared from frozen seedlings (2 g) homogenized with 5 cm^3 of ice-cold 0.2 M sodium phosphate buffer [pH 6.5, 1 % (m/v) polyvinylpyrrolidone (PVP), 0.1 % *Triton X-100* and 0.04 % (m/v) sodium meta-bisulfite]. The homogenate was centrifuged at 9 000 *g* for 20 min at 4 ºC and the supernatant was used as the enzyme source. Protein content in the supernatant was quantified according to Bradford (1976). Enzyme activity was measured by monitoring the appearance of the conjugated dienehydroperoxide at 234 nm. Linoleic acid was used as substrate, which was prepared according to the standard method (Axelrod *et al*. 1981). Activity was recorded for 3 min using a *Hitachi U-200* (Tokyo, Japan) spectro-photometer.

Total RNA was extracted using the phenol-chloroform method (Sambrook *et al*.1989). For hybridization analysis, 20 µg of total RNA were separated electrophoretically in formaldehyde agarose gel and blotted on *Hybond-N*$^+$ membrane (*Amersham Pharmacia Biotech*, Aylesbury, Buckinghamshire, UK) by capillary transfer method in 20× SSC and fixed to the membrane by baking for 90 min at 80 ºC. RNA gel blots were pre-hybridized in a solution containing 50 % (v/v)

formamide, 0.25 M sodium phosphate (pH 7.2), 0.25 M NaCl, 7 % (m/v) sodiumdodecyl sulphate (SDS) and 1 mM EDTA at 42 ºC for 3 h and hybridized with (α-^{32}P) LOX probe in the same solution overnight. The membranes were washed at 42 ºC twice for 20 min each in 20× standard saline citrate and 0.1 % SDS. The blots were exposed to image plates of phosphor imager for 2 - 3 h and the plates were scanned with red laser using *FLA* (*Fuji film*, Tokyo, Japan). After the images were obtained, transcript intensity was analyzed with the *Bioprofile Image Analysis System* (*Vilber*, Lourmat, France).

Data from three replicates were analyzed separately for each experiment and subjected to arcsine transformation and analysis of variance (*ANOVA*) using *SPSS Inc. 16.0*. Significant effects of treatments were determined by *F* values ($P < 0.05$). Treatment means were separated by Tukey's Honestly Significant Differences (HSD) test.

Results and discussion

Under greenhouse conditions, thiamine seed treatment induced significantly higher downy mildew disease protection in all the tested concentrations over the untreated control. Among the five different concentrations, the best result was noticed in 20 mM thiamine, which recorded 72 % protection, followed by 25 mM concentration, which offered disease protection of 65.5 %, whereas other concentrations of 15, 10 and 5 mM recorded disease protection of 63, 59 and 42 %. On the other hand *Apron 35SD* treated seedlings recorded 91 % disease protection. Under field conditions, highest downy mildew protection of 70 % was also recorded with 20 mM thiamine treatment, whereas, disease protection of 63, 62, 56 and 40 % was noticed within 25, 15, 10 and 5 mM

Table 1. Effect of seed treatment with thiamine on growth parameters of pearl millet plants under field conditions. Values are means of 4 independent replicates ± SE. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD.

Thiamine [mM]	Plant height [cm]	Time to 50 % flowering [d]	Number of reproductive tillers	Length of ears [cm]	Girth of ears [cm]
0	58.4 ± 0.2d	40 ± 0.7d	2.1 ± 0.5b	9.4 ± 0.8d	3.6 ± 0.9c
5	58.4 ± 0.3d	41 ± 0.7c	2.6 ± 0.0b	10.3 ± 0.1c	3.7 ± 0.1c
10	61.0 ± 0.1c	41 ± 0.4c	3.7 ± 0.2a	11.9 ± 0.6b	3.8 ± 0.0bc
15	67.2 ± 0.3b	42 ± 0.2bc	3.4 ± 0.5a	12.1 ± 0.3ab	3.8 ± 0.0bc
20	72.0 ± 1.1a	44 ± 1.4a	3.5 ± 0.3a	12.4 ± 0.7a	4.3 ± 0.3a
25	67.7 ± 0.1b	43 ± 1.1ab	3.5 ± 0.1a	12.1 ± 0.2ab	4.0 ± 0.1ab

Table 2. Lipoxygenase activity [nmol mg^{-1}(protein) min^{-1}] in pearl millet seedlings measured at different hai. STU - susceptible thiamine-treated uninoculated, STI - susceptible thiamine-treated inoculated, SU - susceptible uninoculated, SI - susceptible inoculated, RU - resistant uninoculated, RI - resistant inoculated. Values are means of four independent replicates ± SE. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD.

Plants	Thiamine [mM]	0 h	3 h	6 h	12 h	24 h	48 h	72 h
STU	5	10.9±0.1d	12.0±0.3c	13.2±0.2c	16.1±0.4b	21.0±0.4a	17.7±0.2b	16.0±0.4b
STI	5	12.8±0.4d	14.0±0.4c	15.2±0.5c	19.2±0.2b	24.3±0.1a	23.1±0.9a	21.1±0.2ab
STU	10	11.2±0.6f	12.4±0.5e	14.3±0.4cd	16.5±0.1c	22.0±0.1a	19.1±0.2b	16.5±0.1c
STI	10	13.9±0.1d	16.1±0.1c	16.9±0.4c	20.0±0.3b	25.7±0.1a	25.0±0.4a	19.2±0.6b
STU	15	11.9±0.1f	15.2±0.1e	17.7±0.3d	21.4±0.3b	23.3±0.7a	23.1±0.6a	20.0±0.5c
STI	15	15.0±0.4ef	16.9±0.3e	20.4±0.5cd	26.1±0.3b	29.7±0.5a	27.2±0.8ab	23.7±0.1bc
STU	20	13.5±0.2e	29.5±0.6d	37.6±0.1bc	39.7±0.9b	43.8±0.3a	42.6±1.0a	30.8±0.2c
STI	20	15.0±0.5e	32.6±0.5d	43.6±0.8bc	45.2±0.3b	49.7±0.4a	49.0±0.7a	38.2±0.2c
STU	25	12.1±0.2d	15.3±0.5c	17.5±0.1c	21.9±0.1ab	24.0±0.1a	23.6±0.4a	20.5±0.8b
STI	25	15.0±0.2de	18.2±0.8d	25.8±0.4c	30.7±0.4b	33.9±0.2a	33.5±0.4a	31.7±0.4b
SU		10.6±0.6e	12.0±0.2de	13.6±1.0d	15.5±0.5c	20.8±0.4a	18.4±0.2b	15.3±0.2c
SI		12.3±0.1	13.8±0.2	14.6±0.1	18.9±0.3	24.5±0.1	22.0±0.7	20.9±0.9
RU		19.0±0.8e	35.7±0.6d	46.0±0.5bc	50.0±0.4b	57.6±0.5a	52.5±0.3ab	49.3±1.4b
RI		21.0±0.5e	43.2±0.9d	49.0±0.7d	55.3±1.4c	69.2±1.2a	63.0±0.4ab	60.1±1.1b

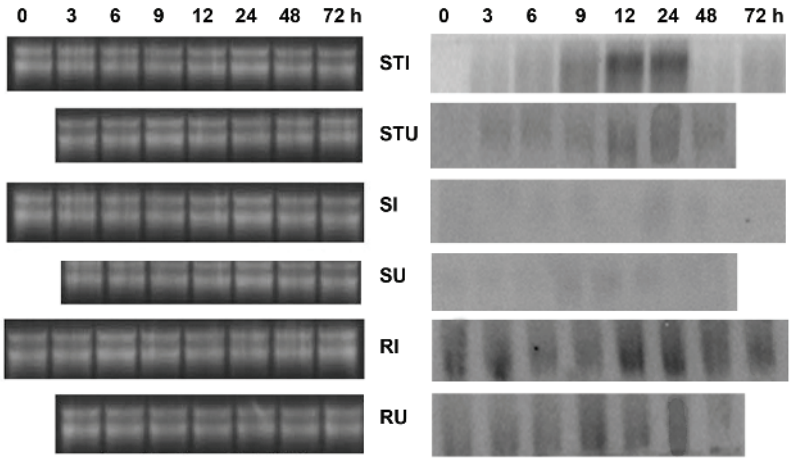

Fig. 1. Northern blot analysis. Temporal pattern of transcript accumulation hybridizes with (α.32P) LOX probe in pearl millet seedlings. STI - susceptible seedlings treated and inoculated, STU - susceptible seedlings treated and uninoculated, SI - susceptible seedlings inoculated, SU - susceptible seedlings uninoculated, RI - resistant seedlings inoculated, RU - resistant seedlings uninoculated. Equal loading of total RNA is *on the left.*

concentrations of thiamine, respectively. Advancing from greenhouse trials to field trial is an important step towards the goal of practical application of induced resistance elicited by any biotic or abiotic agents. The mechanisms involved in thiamine induced resistance are unknown and have not been attempted. However, the induction of resistance by thiamine application has been previously reported. The disease progress inhibiting activities of thiamine against *Xanthomonas oryzae* in rice, pepper mild mottle virus infection in tobacco plants, *Colletotrichum lagenarium* in cucumber, *Pseudomonas syringe* in *Arabidopsis* plants have been proved (Ahn *et al*. 2005). Subsequent investigations indicated that the resistance induced by thiamine is systemic, broad-spectrum, and long-lasting (Ahn *et al*. 2005). Malamy *et al*. (1996) demonstrated that thiamine stimulate resistance to tomato mosaic virus in a salicylic acid dependent manner. In *Arabidopsis*, thiamine primes the pathogen-induced expression of pathogenesis-related 1 (PR-1) proteins and phenylalanine ammonia lyase as well as callose deposition and an oxidative burst associated with the hypersensitive response (HR) (Ahn *et al*. 2007). From other vitamins, riboflavin application was shown to protect various hosts from viral, bacterial, fungal, and oomycete pathogens, with little or no phytotoxicity (Averyanov *et al*. 2000). Dong and Beer (2000) have reported that foliar spray of riboflavin effectively protected *Arabidopsis thaliana* from *Perenospora parasitica*. Recent research demonstrated that riboflavin induced resistance in rice against *Rhizoctonia solani via* jasmonate-mediated priming of phenylpropanoid pathway indicating the efficiency of riboflavin in LOX expression (Taheri and Tarighi 2010).

Among five different concentrations used, treatment with 20 mM thiamine revealed significant ($P < 0.005$) enhancement of plant growth parameters followed by 25, 15, 10 and 5 mM thiamine over the untreated control (Table 1). Earlier studies from our laboratory showed that application of the plant growth promoter *Vitazyme* at the lowest concentration (2 %) improve field emergence and yield especially when an additional foliar spray was applied. Application of thiamine and ascorbic acid enhanced the vegetative growth and flowering in *Gladiolus* (Nahed *et al*. 2009). Youssef and Talaat (2003) reported a pronounced increase in vegetative growth and chemical constituents of rosemary plants by foliar application of thiamine. In another research, Karima (2005) found that application of ascorbic acid on sunflower plant led to significant increase in plant height, number of leaves, fresh and dry mass of leaves.

In inoculated resistant and thiamine-treated seedlings the LOX activity was evident at 0 hai and maximum was reached at 24 hai. Increase (13.4 %) in LOX activity was observed in inoculated seedlings treated with 20 mM thiamine at 24 hai. Increase in LOX activity was also noticed in 25, 15, 10 and 5 mM thiamine treated inoculated samples (Table 2).

In this study we report the enhancement of lipoxygenase enzyme during pearl millet - downy mildew disease interaction. The constitutive expression of *LOX* was seen in all the categories of seedlings. But there was marked difference in the transcript accumulation in resistant, and thiamine-treated seedlings (Fig. 1). The *LOX* transcript abundance increased from 3 hai and reached its maximum at 24 hai in resistant and thiamine treated seedlings, whereas in susceptible cultivar the expression of *LOX* was less in both inoculated and uninoculated seedlings. LOXs have several functions in plants; response to wounding, stress and pathogen attack (Kolomiets *et al*. 2001, Gao *et al*. 2008). In our study, the distilled water-treated controls of both resistant and susceptible pearl millet seedlings exhibited a constant

increase in constitutive LOX activity, even without elicitor-treatment or pathogen inoculation. This is because, LOXs are known to have a central role in the regulation of the biosynthesis of several secondary metabolites, including terpenoids, phenylpropanoids and antioxidants and usually the constitutive activation of these metabolites are occurring around a few days after germination (Avanci *et al*. 2010). The study of transgenic lines and of the physiological role of different oxylipins have made clear that LOX is not only important for the synthesis of jasmonates, but also of a number of other products that have specific constitutive roles in development and in responses to stress (Porta and Rocha-Sosa 2002). During germination, new LOXs are synthesized in the seedling and the cotyledons. Maximal accumulation of constitutive LOX protein and the corresponding mRNAs lasts from a few hours to a few days after germination due to lipid mobilization. The constitutive mRNAs synthesized during germination could also be found in the mature plant (Porta and Rocha-Sosa 2002).

An increase in LOX activity in response to infection has been reported for several plant-pathogen systems and LOX activity has been correlated with plant resistance against pathogens (Kolomiets *et al*. 2001, Porta *et al*. 2008). In resistant tobacco plants inoculated with *Phytophthora parasitica nicotianae* LOX activity peaked at 3 d, whereas in the susceptible plants the activity peaked one day later (Veronesi *et al*. 1996). The transcriptional activation of LOX enzyme was also increased in soybean roots upon infection with cyst nematode (Alkharouf *et al*. 2006). Our results also demonstrated a similar pattern, in the time interval of LOX transcripts accumulation of examined samples by Northern blot analysis. This study clearly demonstrates that LOX is involved during development of downy mildew disease resistance in pearl millet and can be used as a marker for screening disease resistance.

The present investigation provides a novel paradigm for developing alternative strategies for growth promotion and control of downy mildew disease in pearl millet. Thiamine treatment potentiates stronger and higher protection and also rapidity in *LOX* gene expression and the up-regulation of LOX activity.

References

Ahn, I.P., Kim, S., Lee, Y.H.: Vitamin B_1 functions as an activator of plant disease resistance. - Plant Physiol. **138**: 1505-1515, 2005.

Ahn, I.P., Kim, S., Lee, Y.H., Suh, S.C.: Vitamin B_1 induced priming is dependent on hydrogen and the *NPR1* gene in *Arabidopsis*. - Plant Physiol. **143**: 838-848, 2007.

Alkharouf, N.W., Klink, V.P., Chouikha, I.B., Beard, H.S., Macdonald, M.H., Meyer, S., Knap, R., Matthews, B.F.: Time course microarray analyses reveal global changes in gene expression of susceptible *Glycine max* (soybean) roots during infection by soybean cyst nematode. - Planta **224**: 838-852, 2006.

Avanci, N.C., Luche, D.D., Goldman, G.H., Goldman, M.H.S.: Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. - Genet. mol. Res. **9**: 484-505. 2010.

Averyanov, A.A., Lapikova, V.P., Nikolaev, O.N., Stepanov, A.I.: Active oxygen-associated control of rice blast disease by riboflavin and roseoflavin. - Biochemistry **65**: 1292-1298, 2000.

Axelrod, B., Cheesbrough, T.M., Laakso, S.: Lipoxygenase from soybeans. - Methods Enzymol. **71**: 441-451, 1981.

Borges, A.A., Perez, A.B., Falcon, M.F.: Induced resistance to fusarial wilt of banana by menadione sodium bisulphite treatments. - Crop Protect. **23**: 1245-1247, 2004.

Bradford, M.M.: A rapid and sensitive method for the quantification microgram quantities of protein utilizing the principle of protein-dye binding. - Ann. Biochem. **72**: 248-254, 1976.

Dong, H., Beer, S.V.: Riboflavin induces disease resistance in plants by activating a novel signal transduction pathway. - Phytopathology **90**: 801-811, 2000.

Feussner, I., Kühn, H., Wasternack, C.: Lipoxygenase-dependent degradation of storage lipids. - Trends Plant Sci. **6**: 268-273, 2001.

Gao, X., Starr, J., Gobel, C., Engelberth, J., Feussner, I., Tumlison, J., Kolomiets, M.: Maize 9-lipoxygeanse ZmLOX3 controls development root specific expression of defense genes and resistance to root-knot nematodes. - Mol. Plant Microbe Interact. **21**: 98-109, 2008.

Howe, G.A., Schilmiller, A.L.: Oxylipins metabolism in response to stress. - Curr. Opin. Plant Biol. **5**: 230-236, 2002.

Karima, M., El-Din, G., Abdel-Wahed, M.S.A.: Effect of some amino acids on growth and essential oil content of chamomile plant. - Int. J. agr. Biol. **7**: 376-380, 2005.

Kolomiets, M.V., Hannapel, D.J., Chen, H., Tymeson, M., Gladon, R.J.: Lipoxygenase is involved in the control of potato tuber development. - Plant Cell **13**: 613-626, 2001.

La Camera, S., Gouzerh, G., Dondt, S., Hoffmann, L., Fritig, B., Legrand, M., Heitz, T.: Metabolic reprogramming in plant innate immunity the contributions of phenylpropanoid and oxylipin pathways. - Immunol. Rev. **198**: 267-284, 2004.

Malamy, J., Sanchez, C.P., Hennig, J., Guo, A. L., Klessig, D.F.: Dissection of the salicylic acid signaling pathway in tobacco. - Mol. Plant Microbe Interact. **9**: 474-482, 1996.

Nahed, G.A.A., Taha Lobna, S., Ibrahim Soad, M.M.: Some studies on the effect of putrescine, ascorbic acid and thiamine on growth, flowering and some chemical constituents of *Gladiolus* plants at Nubaria. - Ozean J. appl. Sci. **2**: 169-179, 2009.

Porta, H., Figueroa-Balderas, R.E., Rocha-Sosa, M.: Wounding and pathogen infection induce a chloroplast-targeted lipoxygenase in the common bean *(Phaseolus vulgaris* L.*)*. - Planta **227**: 363-373, 2008.

Porta, H., Rocha-Sosa, M.: Plant lipoxygenases physiological

and molecular features. - Plant Physiol. **130**: 15-21, 2002.

Safeeulla, K.M.: Biology and Control of the Downy Mildews of Pearl Millet, Sorghum and Finger Millet. - Wesley Press, Mysore 1976.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: a Laboratory Manual. 2nd Ed. - Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989.

Siedow, J.N.: Plant lipoxygenase – structure and function. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 145-188, 1991.

Singh, S.D., Gopinath, R.: A seedling inoculation technique for detecting downy mildew resistance in pearl millet. - Plant Dis. **69**: 582-584, 1985.

Sudisha, J., Ananda Kumar, S., Shekar Shetty, H.: Characterization of downy mildew isolates of *Sclerospora graminicola* by using differential cultivars and molecular markers. - J. cell. mol. Biol. **7**: 41-55, 2008.

Taheri, P., Tarighi, S.: Riboflavin induces resistance in rice against *Rhizoctonia solani* via jasmonate-mediated priming of phenylpropanoid pathway. - J. Plant Physiol. **167**: 201-208, 2010.

Tanabe, S., Okada, M., Jikumaru, Y., Yamane, H., Kaku, H., Shibuya, N., Minami, E.: Induction of resistance against rice blast fungus in rice plants treated with a potent elicitor, *N*-acetylchitooligosaccharide. - Biosci. Biotech. Biochem. **70**: 1599-1605, 2006.

Tyler, BM.: Genetics and genomics of the oomycete-host interface. - Trends Genet. **17**: 611-614, 2001.

Veronesi, C., Rickauer, M., Fournier, J., Pouenat, M.L., Esquerre-Tugaye, M.T.: Lipoxygenase gene expression in the tobacco *Phytophthora parasitica* interaction. - Plant Physiol. **112**: 997-1004, 1996.

Youssef, A.A., Talaat, I.M.: Physiological response of rosemary plants to some vitamins. - Egypt Pharm. J. **1**: 81-93, 2003.