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Biotic Induction of Resistance in Pearl Millet against *Sclerospora graminicola* and Its Biochemical Aspects

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ABSTRACT

Two varieties of pearl millet viz., 7042S and IP18294, highly susceptible and highly resistant to virulent pathotype 1 of S. graminicola, and a virulent pathotype 1 of S. graminicola was used in the present investigation. The commonly available herbs, viz., Alternaris sessilus, Curcuma longa, Cuminum cyminum, Cinnamon camphora and Cucurbita maxima were homogenised with distilled water, acetone, chloroform and methanol at 4°C and the extract was used as inducer of resistance. After preliminary studies like seedling vigor, vegetative and reproductive growth parameters, selected inducer in solvent was used to induce the resistance in plants by seed treatment. In the experimental sets, the biochemical parameters like qualitative analysis of peroxidase, superoxide dismutase and esterases were tested. The test results clearly indicated that Cucurbita extract induced resistance. Experimental sets showed remarkable variation when compared to control sets.

Keywords: *Induced resistance, Pennisetum glaucum, Sclerospora graminicola, Peroxidase, SOD, Esterase.*

INTRODUCTION

Plant diseases in general are controlled by humans to suit their requirement for feeding the exploding population. Of the control measures employed, the concept of induced resistance is ecofriendly and biocompatible and has been known for many years. Induced resistance may be expressed locally (LAR-Local Acquired Resistance) at the site of infection as well as systemically (SAR-Systemic Acquired Resistance). Although different defense responses in plants are known, only a few correlate with induced resistance. However, defense responses associated with induced resistance at cellular, biochemical and molecular levels are well established in a number of host-pathogen interactions[8]. The physiological changes associated with the induction of resistance and also challenge inoculation with the pathogen has resulted in commitment activation of various defense related enzymes which play a major role in limiting the pathogen development in the host tissues.

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The first resistance response that is triggered in inducer treated plants is the enhanced activity of peroxidases and several of its isozymes is reported in cucumber *C. lagenarium* and tobacco *Peronospora tabacina* and TMV interaction[28]. Ye *et al.,* [29] have reported specificity of induced systemic resistance against blue mold elicited by ethephon and tobacco mosaic virus in tobacco. Role of pathogenesis related protein (PR) is also recorded. Local and systemic accumulation of proteins in plants infected with *Phytophthora infestans* and *Fulvia fulva* and in plants treated with ultra violet light, indole acetic acid or ethophan has been reported. Apart from inducing defense activity, collective set of PRs are found to effective in inhibiting pathogen growth, multiplication and/or spread, and is known to be responsible for the state of SAR. Peroxidases represent another component of an early response system in plants to pathogen attack. The products of these enzymes, in the presence of a suitable hydrogen donor and hydrogen peroxide can inactivate fungi, bacteria and viruses[26]. Both the timing and the localization of increased peroxidase activity may be important in limiting pathogen infections. The extra cellular location of peroxidase isozymes stimulated during pathogen attack, and their affinity for substrates involved in lignification, as well as the capacity of peroxidases to form hydrogen peroxide, suggest that peroxidase isozymes may be involved in the formation of barrier substances confining the pathogen to the site of penetration. Superoxide dismutases (SOD) are a family of metalloenzymes that catalyze the disproportionate superoxide (O_2^-) radicals, and they play an important role in protecting cells against the toxic effects of superoxide radicals produced in different cellular loci[11]. SOD activity increased 2-3 folds in resistant seedlings upon inoculation[6]. The role of the enzyme superoxide dismutase in imparting resistance to downy mildew in pearl millet has been established by the authors[5]. Esterases are group of enzymes which catalyses the hydrolysis of various types of acetyl esters. The study of such polymorphic enzymes is an important tool during the selection for economically important traits[10]. Hence the present investigation was undertaken in an attempt to acquire information on the effects of induced resistance on peroxidases, superoxide dismutase and non specific esterases.

MATERIALS AND METHODS

Host Plant: Cultivars of Pearl Millet *viz.*, 7042S and IP18294, highly susceptible and highly resistant to virulent pathotype 1 of *S. graminicola,* obtained from International Crop Research Institute for Semi- Arid Tropics, Hyderabad, India, were used for the study.

Pathogen: A virulent pathotype 1 of *S. graminicola* isolated from and maintained on the Pearl Millet cultivar (7042 S) under green house conditions was used.

Solvents: Total components of the test biotic inducers were extracted with distilled water, acetone, chloroform and methanol at 4^{o}C.

Biotic inducer: Biotic inducers used in the present study include leaf materials of the commonly available herbs *viz*., *Alternaris sessilus*, *Curcuma longa*, *Cuminum cyminum*, *Cinnamon camphora* and *Cucurbita maxima*. Twenty five grams of each sample was ground into fine paste with test solvents at 4^{o}C. The samples were centrifuged at 10,000 g for 15 minutes. Later the supernatant solution was air dried to obtain fine powder. This powder was dissolved in distilled water (25 ml) and appropriate dilutions made and used as a source of inducer. Initially the test inducers were tested for their effect on germination at a concentration of 10 to 100% w/v. Those concentrations, which did not affect the germination, were selected to test their effect on the zoospore release of *S. graminicola*.

Test for antifungal activity: Antifungal activity of the test inducers were tested according to Shetty *et al.*

Seed treatment with inducers and their effect on germination: Seeds of pearl millet surface sterilized with 0.01% sodium azide for 5 minutes followed by thorough washing in sterile distilled water to remove the traces of sodium azide were immersed in different

concentrations *viz.*, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% inducer in distilled water (w/v) at 20^{o}C for 1-8 hours. After treatment the seeds were washed in tap water for 20-30 seconds to remove excess adhering inducer and then air dried in laminar airflow for 3-4 hours until the seeds regained their original weight. Germination tests were done according to ISTA, 2005 specification by placing the seeds between sheets of moistened paper towels at 25^{o}C. The control seeds received distilled water treatment only.

Inducer treatment: Concentration of the inducer in distilled water, which did not affect the germination, was used to test for its efficacy to induce resistance in seeds of pearl millet against downy mildew disease in the present study. These treated seeds were sown onto earthen pots consisting of soil, sand and manure mix of 1:1:1 and watered regularly and maintained under green house condition. Corresponding control sets were also maintained with seeds treated in sterile distilled water.

Collection of sporangia and preparation of inoculum: Collection of sporangia and release of zoospores was as per Safeeulla. For challenge inoculation; the zoospore inoculum at a concentration of 3x10^{4} ml^{-1} was used.

Test for induced resistance: The treated as well as the control seedlings on germination i.e. five days continuously from single leaf stage (~4 days after sowing) onwards were challenge inoculated by whorl inoculation with 3x10^{4} zoospores ml^{-1} [22].

Maintenance of plants: The plants after inoculation were maintained under green house conditions and assessed for disease expression. The plants were watered regularly and further supplemented with NPK every fortnight. Seedlings treated with sterile distilled water alone served as control.

Assessment of disease: Careful observations were made for the appearance disease symptoms in plants inoculated with zoospores of *S. graminicola*. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. Biotic inducer cucurbita extract in acetone at concentration of 40% w/v protected pearl millet plants against downy mildew. Hence plants induced for resistance at this concentration were further analyzed for biochemical changes in host plant due to host-pathogen interaction.

Effect of induced resistance on Seedling vigor: Seeds treated with 40% w/v of acetone extract of Cucurbita was tested for germination following the standard blotter method (Anon, 1995). The seeds were placed between sheets of moistened paper towel and incubated at 25 $\pm$ 2^{o}C. Seeds treated with distilled water alone and those treated with distilled water were used as controls. Four replicates of 100 seeds were taken for each sample. Germination percentages, seedling root and shoot lengths were determined and seedling vigor calculated using the formula formulated by Abdul Baki and Anderson[1].

Vigor index = Mean root length (MRL) + Mean shoot length (MSL) x Percentage of germination.

Sample Preparation for Biochemical Studies: The seeds were plated on moistened blotters and incubated in BOD incubator at 25$\pm$2^{o}C. For each test three replicates of 25 seeds each were used. For histochemical analysis the seedlings were challenge inoculated with 3x10^{4} zoospores ml^{-1} by root dip method according to Safeeulla[17]. Seeds that received distilled water treatment and resistant cultivars, which were subjected to germination and pathogen inoculation as above, were used as controls.

The total protein present in tissues was determined by the dye binding method[7]. Bovine serum albumin was used as standard protein. The results were expressed as µg of protein/ µg of tissue.

The qualitative analysis of isozymes of three enzymes *viz.*, peroxidase, superoxide dismutase and esterase was carried out in Native Poly Acrylamide Gel Electrophoresis (PAGE) with the discontinuous buffer system. A uniform quantity of protein (150 µg) from each batch was loaded to each slot.

Activity staining of peroxidase (EC 1.11.1.7): The gels, soon after the removal,

washed in running distilled water followed by the incubation in staining solution. The staining solution was prepared by dissolving 50 mg Benzidine in 0.5 ml ethanol and then, 30 ml of distilled water and 0.4 ml of glacial acetic acid was added. The suspension was filtered through cotton and 50 µl of hydrogen peroxide was added. The gels were stained 1-2 minutes or until blue colored bands appeared. The enzyme reaction was stopped by soaking the gel in 7% acetic acid.

Activity staining of Superoxide dismutase (SOD, EC 1.15.1.1): The gels, soon after the removal, were washed in running distilled water followed by the incubation in 100 ml 50 mM sodium phosphate buffer pH 7.8 containing 50 mg NBT, 1 mg riboflavin and 0.326 ml TEMED for 30 min in dark. Then the solution was poured off, gels were placed in distilled water and illuminated under a fluorescent lamp for 15 min or until the desirable transparent (achromatic) bands clearly appeared in a dark blue background[25].

Activity staining of non specific esterase (Acetylesterases: EC 3.1.1.6): The gels, soon after the removal, washed in running distilled water and incubated in the staining solution comprised of 100ml 50mM Acetic buffer at pH 4.0, 2ml acetone/water (1:1 v/v) containing 20mg alpha and 20mg beta naphthyl acetate, 80mg of fast blue B salts in a rotary shaker at 37ºC in dark for 20 min or until the bands appeared. After the appearance of bands the reaction was stopped by the addition of 2-3 ml glacial acetic acid.

After the appearance of bands, the gels were scanned, analyzed and photographed in a gel scanner (Vilber Laurmat Bioprofil image analysis system). All experiments were repeated twice with three replicates. Data were subjected to Duncan multiple range test (DMRT) wherever necessary and the level of significance compared at 0.05 level. Data were analyzed using statistical program system software.

RESULTS AND DISCUSSION

Seeds of pearl millet showed varied germination percentage on treatment with different extracts of the medicinal plants for various time intervals. Maximum germination was obtained when the seeds were treated with 40 % w/v of the test inducer for 6 hours. Accordingly, on identifying the best concentration of the solvent extract which does not affect the germination and the duration of treatment, the seeds were treated with varying concentration of chemical inducer listed for identification of efficient resistance inducer against downy mildew. The results of antifungal activity test concluded that the test inducer had no direct antifungal activity.

Fig. 1: Pearl Millet plants protected against downy mildew disease by seed treatment with Cucurbita extract in acetone at 40% w/v: Unprotected plants infected with downy mildew is also shown (left)

The test inducer used for identification of resistance induction in pearl millet is listed in Table 1. Cucurbita extract in acetone at 40% w/v gave maximum protection of 56 % at the end of 60 days of growth. Hence this concentration was selected to evaluate the effect on seedling vigor. The results are presented in Table 2 and Figure 2. The statistical analysis exhibited significant increase (p = 0.05) in root and shoot length along with increased germination when compared with control and the test plants. The difference in percentage of germination between the inducer treated samples and control was not significantly high (p=0.05) as recorded on the seventh day of incubation. However marked difference in percentage germination with reference to increased seedling vigor in inducer treated plants was observed during the first 3 days of incubation.

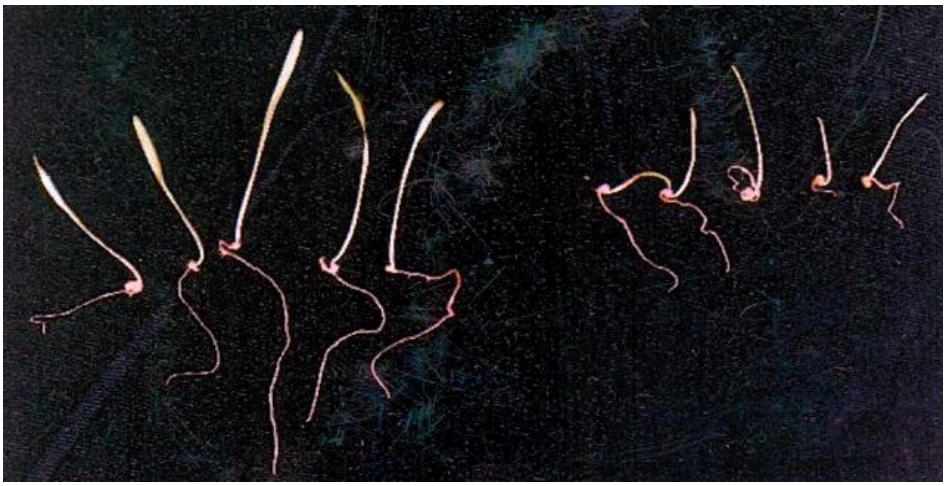

Fig. 2: Pearl Millet seedlings showing increased seedling vigor on seed treatment with Cucurbita extract in acetone at 40% w/v: Unprotected seedlings treated with distilled water is also shown (right)

Table 1: Details of induces tested for systemic acquired resistance

Name of the Medicinal Plant selected	Part of plant used	Solvent	Concentration (wt/v)	Downy mildew incidence	Disease protection over control (untreated) %
Alternaris sessilus	Leaves	Acetone	40	75	24d
		Methanol	40	72	26e
		Chloroform	40	58	41h
		Dist. Water	40	68	31f
Curcuma longa	Rhizome	Acetone	40	98	0
		Methanol	40	97	01a
		Chloroform	40	80	18c
		Dist. Water	40	57	42h
Cuminum cyminum	Seeds	Acetone	40	61	38g
		Methanol	40	85	13b
		Chloroform	40	61	38g
		Dist. Water	40	64	35g
Cinnamon camphora	Fruit	Acetone	40	72	26e
		Methanol	40	68	30f
		Chloroform	40	72	26e
		Dist. Water	40	75	24d
Cucurbita maxima	Fruit	Acetone	40	43	56i
		Methanol	40	76	22d
		Chloroform	40	70	29f
		Dist. Water	40	67	32f
Dist. water control*	-	-	-	98	-

*Unprotected Control. Results are an average of two independent experiments of four replicates of 25 seedlings each. Figures followed by the same letter in the same column are not significantly different at 0.05 level when subjected to DMRT.

Table 2: Effect of Seed treatment with medicinal plant extract on seedling vigor

Treated	MRL (cm)	MSL (cm)	Germination (%)	VI
Untreated Susceptible*	2.25	2.72	99	492.03a
Resistant*	3.2	3.62	98	668.36b
Inducer Treated Susceptible	5.3	6.54	99	1172.16c

MRL - Mean Root Length; MSL - Mean Shoot Length; VI - Vigor Index

*Control - Unprotected susceptible 7042 S Seeds treated with distilled water.

- Untreated resistant IP18294 Seeds treated with distilled water.

Inducer treated Susceptible (7042 S) treated with 40% of acetone extract of cucumber leaves.

Results are an average of two independent experiments of four replicates of 25 seedlings each. Figures followed by the same letter in the same column are not significantly different at 0.05 level when subjected to DMRT.

The results of biochemical analysis of defense related enzymes *viz*., peroxidase, esterase and superoxide dismutase are presented in Figures 3, 4, 5. Inoculation of pearl millet seedlings with the virulent pathotype of *S. graminicola* resulted in further increased activity of the enzymes both in control and induced resistant seedlings. However, the enzyme activities of induced resistant seedlings were much higher than that of control. Upon challenge inoculation, the results of qualitative analysis of the peroxidase during induction of resistance showed varied reaction with respect to presence or absence and also variation in intensity of isoforms expressed in all the test samples. Initially at 0 hour of pathogen inoculation in susceptible cultivar low intensity of the isoforms of peroxidases was observed when compared to resistant cultivar. But at 24 hours of pathogen inoculation increased expression of enzyme was observed with prominent bands (as indicated by arrows) in resistant which was absent in susceptible counterpart. Inducer treated samples showed banding pattern similar to that in resistant cultivar. But a few isoforms which were unique in inducer treated samples were observed (indicated by arrows - Fig. 3). The qualitative analysis of super oxide dismutase activity levels exhibited decreased intensity of the bands in susceptible seedlings. However, increased intensity was observed in resistant cultivar as well as inducer treated sets. Unique bands of interest are indicated by arrows. In inducer treated samples two extra isoforms were observed at 24 h of inoculation. These extra bands were absent in susceptible and resistant controls (Fig. 4). An increased expression of esterase was observed with prominent bands (as indicated by arrows) in Induced resistant sets, which was absent in susceptible counterpart. These bands were also present in resistant plants but to less intensity when compared to induced resistant plants. Inducer treated samples showed banding pattern similar to that in resistant cultivar. But a few isoforms which were unique in inducer treated samples were observed (indicated by arrows - Fig. 5).

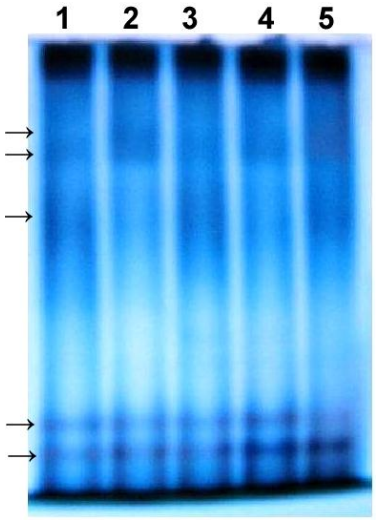

Fig. 3: Native page analysis of Peroxidase

Lanes: 1. Inducer treated seedlings with pathogen inoculation
2. Inducer treated seedling without pathogen inoculation
3. Distilled water treated seedlings with pathogen inoculation
4. Distilled water treated seedlings without pathogen inoculation
5. Resistant seedlings with pathogen inoculation

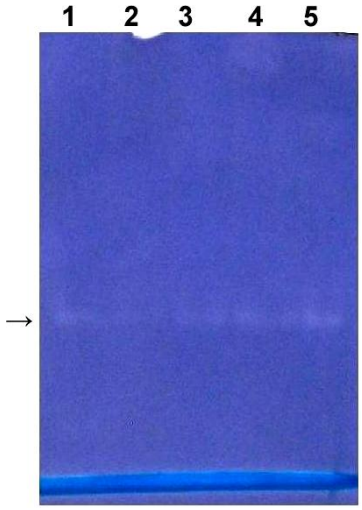

Fig. 4: Native page analysis of Superoxide dismutase

Lanes: 1. Inducer treated seedlings with pathogen inoculation
2. Inducer treated seedling without pathogen inoculation
3. Distilled water treated seedlings with pathogen inoculation
4. Distilled water treated seedlings without pathogen inoculation
5. Resistant seedlings with pathogen inoculation

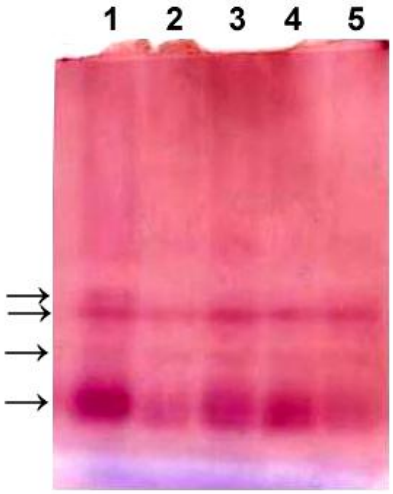

Fig. 5: Native page analysis of Esterase

Lanes: 1. Inducer treated seedlings with pathogen inoculation
2. Inducer treated seedling without pathogen inoculation
3. Distilled water treated seedlings with pathogen inoculation
4. Distilled water treated seedlings without pathogen inoculation
5. Resistant seedlings with pathogen inoculation

Resistance in plants is highly versatile and elastic. Induction of resistance being a technique in controlling the disease manifestation caused by wide range of pathogens, has been applied in many monocotyledonous plants, and has proved to be promising in control of downy mildew in pearl millet. Earlier studies have convincingly established the operation of SAR on prior inoculation of the seedlings with sub optimal dose of the virulent pathotype of *S. graminicola.* Since this method was not ideal, time consuming and possibility of outbreak of disease was more, an approach of seed treatment was developed. Reports on induction of SAR by seed treatment with SAR inducers from plant extracts are available. However, treatment of the plant extracts to the seed has not been yet attempted in pearl millet.

The elicitor moiety present in plant extracts may be protein, low/high molecular carbohydrate or lipid moieties which may be polar or non polar in nature. Hence different extracts were tested. Generally in water extracts protein components and a few high molecular weight carbohydrates can be brought into solution. Similarly in acetone and methanol polar and non polar high and low molecular weight carbohydrate moieties are obtained. Lipid moieties can be obtained by extracting the samples in chloroform. Medicinal plants from different families were tested. Water, acetone, chloroform and methanol extracts of these plants were obtained and the seeds of pearl millet were tested for the effect of these extracts on seed germination.

Effect of different extracts on seed germination shows that irrespective of the solvents used there was a mixed response of seed germination to different extracts tested. Those extracts which showed germination to a higher extant similar to that of control were selected for testing for their ability to induce resistance in pearl millet seeds. Similar observations on effect of seed germination in different extracts of plant have been studied by Cao., *et al*., [8] in Potato.

The concentrations selected for testing for induction of resistance were preliminarily subjected to study their effect on the test fungi *S. graminicola*. The zoospores in the test solution were released to the same extent as in distilled water. Hence it is evident that the chemicals being tested had no antifungal activity.

Prior treatment of pearl millet seeds susceptible to downy mildew with aqueous solution of test extracts as inducers can be used for inducing SAR in pearl millet. On evaluation of the seedlings for expression of downy mildew disease, it was observed that the seedlings raised from seeds that were treated with 40% of *Cucurbita* in acetone showed significantly reduced downy mildew disease, hence more disease protection (Table 1) in comparison with the other treatments. Components in extracts of the family *Solanaceae* was first shown to be a biotic inducer of resistance in tomato by Pearce and Ryan[18]. Reports are also available for induction of resistance by extracts from Solanaceous crops. Later studies have identified the active component responsible for induction of resistance was a polypeptide named Systemin. However in an exceptional case, the active component found in tobacco was not homologous with the systemin found in all other solanaceous crops. It is found to be a carbohydrate modified polypepetide. The finding that the tobacco systemins are not homologous with tomato, potato, pepper, or nightshade systemins raises questions concerning the possible universality of systemins and their structural variability among species. Despite structural differences among the polypeptide defense signals, plant-derived polypeptides that signal defense genes, locally or systemically, are called systemins. Systemins homologous to tomato or tobacco systemins have not been found in species outside the Solanaceae family, but searches for their presence in other species continue. The data indicate that systemins and their receptors may be a common feature of plants, but that structurally different systemin polypeptides may serve the same functions in different plant species[3].

Pearl millet seeds treated with a solution of acetone extract of Cucurbita exhibited significant increase in root and shoot length along with increase in germination compared to seeds soaked only in distilled water. The difference in percentage germination between the inducer treated samples and other treatments was not significantly high as recorded on the 7th day of incubation. However, marked difference in percentage germination with reference to increased seedling vigor in inducer treated plants was observed during the first 3 days of incubation. Similar observations of increase in seedling vigour were observed on inducing resistance in many plants[13, 17]. Thejaswini *et al*. [24], also reported the possibility of inducing systemic acquired resistance (SAR) in host plant against pathogens by seed treatment with

SAR inducers. Cohen[9] has explained this observation as likely to be due to change in hormonal balance during induction of resistance and / or could be due to interaction of different hormones and their quantities which results in expression of phenotype. Changes in histochemical activities in the plant on induction of resistance with respect to the hypersensitive reaction of the host plant and also changes in the activities of defense enzymes were observed on induction of resistance[15]. Early appearance of necrotic spots in induced resistance plants than that of susceptible plants indicates the involvement of hypersensitive reaction in inducing resistant in susceptible plants.

Present study revealed a correlation between activities of peroxidase, superoxide dismutase and esterases which were induced in pearl millet following treatment with the inducing agent. This suggests a role for these enzymes in the immunization of pearl millet. There was increase in activities of all the enzymes quantified within 24 h of inducer treatment. This increase in activity is necessary to restrict the pathogen attack at initial hours of seedling growth. The rapid increase of defense related enzymes immediately after challenge inoculation in inducer treated plants than in the control plants indicates that on treatment, the plants are 'sensitized' to react more rapidly after challenge than control plants. Increase in a number of isoforms of test enzymes during resistance induction as well as on challenging with the pathogen indicates involvement of several isoforms of enzymes in the resistance reaction.

In the present study, induced resistance was found to be associated with enhanced peroxidase activity. The quantitative changes in peroxidase activity in response to inducer treatment, is involved in induction of resistance in pearl millet downy mildew host-pathogen system. Peroxidase is involved in the oxidation of phenolic compounds to toxic quinones, which apparently contributes towards resistance. Peroxidase is also reported to have an important function in secondary cell wall biosynthesis by polymerizing hydroxy and methoxy cinnamic alcohols into lignin forming rigid cross links between cellulose, pectin, hydroxyproline - rich glycoproteins and lignin[25]. Enhanced peroxidase activity may contribute to induced resistance by helping to generate hydrogen peroxide as well as by increasing the concentration of free radicals and their polymerization products.

Establishment of acquired resistance to pathogens may be induced by physiological and/or developmental changes taking place in host growing plants. Indeed, the occurrence of a transition from susceptibility to resistance during development is a widely reported phenomenon in monocotyledons in the case of fungi[16, 2,] and oomycetes[19, 27, 14]. Development related resistance, is well documented from a pathological point of view, few studies have dealt with the genetic and molecular bases of disease control during plant development.

On the contrary, numerous studies have investigated defense mechanisms activated in response to pathogen infection and associated to plant disease resistance[12]. These studies have underlined the key role of the host enzymes which are primarily involved in normal plant metabolism. At the early step of infection, the host plant recognizes some pathogen-secreted molecules which elicit the coordinate activation of defense reactions. At late stages, a number of host-secreted molecules accumulate in the extracellular space and contribute to the control of invasion pathogens. Synthesis and secretion of defense-related proteins are a critical part of the establishment of resistance. A defensive role of some proteins encoded by constitutively expressed genes involved mainly in metabolism but related PR genes by function is strongly suggested by their antimicrobial activity. Another such enzyme which was found to be involved quantitatively and qualitatively in induction of resistance is alanine aminotransferase. Reports have confirmed that this enzyme which is mainly involved in amino acid metabolism generates

amino acid derivative important in activating defense signaling and thereby contributes in bringing about a defense status in otherwise susceptible plants Song *et al*., [23]. Our results are also in concordant with it as we also found an elevated expression of SOD and esterases from seed germination itself in comparison with that of the resistant and susceptible plants of the same age. On inoculation with the pathogen the level of this enzyme further increased several fold clearly indicating its triggering effect as a response to the pathogen.

Thus the results of this study contribute to development of a promising method to effectively inoculate the seeds with required inducer of resistance. The technology employed utilizes activation or enhancement of plants defense mechanism. This system of induced resistance against downy mildew is effective and suggests the possibility of returning the use of some cultivars of pearl millet with all desired qualities but has been withdrawn from cultivation due to their susceptibility to downy mildew. Also, the biochemical aspects of this study may be used as markers during selection of resistant cultivars.

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REFERENCES

1. Abdul Baki, A. A. and Anderson, J. P., Vigor determination in soybean seed by multiple criteria. *Crop Sci*. **13**: 630-633 (1973).
2. Abedon, B.G. and Tracy, W.F., *Corngrass1* of maize (*Zea mays* L.) delays developmental of adult plant resistance to common rust (*Puccinia sorghi* Schw.) and European corn borer (*Ostrinia nubilalis* Hubner). *J. Hered.* **87**: 219-223 (1996).
3. Adermann, K., John, H. and Standker, L., Exploiting natural peptide diversity: novel research tools and drug leads. *Curr. Opin. Biotech.* **15**: 599-606 (2004).
4. Anon., International rules for seed testing. *Seed Sci. Technol.* **13**: 329-332 (1995).
5. Babitha, M.P., Bhat, S.G., Prakash, H.S. and Shetty, H.S., Differential induction of superoxide dismutase in downy mildew resistant and susceptible genotypes of pearl millet. *Plant Pathol.* **51(4)**:480-486 (2002a).
6. Babitha, M.P., Bhat, S.G., Prakash, H.S. and Shetty, H.S., Differential induction of superoxide dismutase in downy mildew resistant and susceptible genotypes of pearl millet. *Plant Pathol.* **51(4)**: 480-486 (2002).
7. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **72**:248-254 (1976).
8. Cao, H., Li, X. and Dong, X., Generation of broad spectrum disease resistance by over expression of an essential regulatory gene in systemic acquired resistance. *Proceedings of National Academy of Sciences*, USA **95**:6531-6536 (1998).
9. Cohen, Y.R., Beta amino butyric acid induced resistance against plant pathology. *Plant Dis.* **86**:448-457 (2002).
10. Farshid. G. K. and Mahesha, H. B., Studies on the esterase and its relationship with commercial characters of silkworm *Bombyx mori* L., *Ann. Bio. Res.* **3**(11):5273-5292 (2012).
11. Halliwell, B. and Gutteridge, J. M. C., *Free radicals in biology and medicine*. 3rd ed. Oxford University Press, UK, pp. 936 (2000).
12. Hammond-Kosack, K. and Parker, J., Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. *Curr. Opin. Biotech.* **14**:177-193 (2003).
13. He, C.Y., Hsaing, T. and D.J. Wolyn., Induction of systemic disease resistance and pathogen defense responses in *Asparagus officianalis* inoculated with nonpathogenic strains of *Fusarium oxysporum*. *Plant pathol.* **51**:225-230 (2002).

14. Hugot, K., Aime, S., Conrod, S., Poupet, A. and Galiana, E., Developmental regulated mechanisms affect the ability of a fungal pathogen to infect and colonize tobacco leaves. *Plant J.* **20**:163-170 (1999).
15. Prathima. D. S., Histological basis of disease resistance in pearl millet against *Sclerospora graminicola*. M.Phil. Thesis. Periyar University. (2008).
16. Moose, S.P. and Sisco, P.H., *Glossy15* Controls the epidermal juvenile-to-adult phase transition in maize. *Plant Cell.* **6**:1343-1355 (1994).
17. Ongena, M., Duby, F., Rossignol, F., Fauconnier, M., Dommes, J. and Thonart., Stimulation of the lipoxigenase pathway is associated with systemic resistance induced in bean by a non pathogenic Pseudomonas strain. *Mol. Plant Microbe Interact.* **17**: 1009-1018 (2004).
18. Pearce, G. and Ryan, C.A., Systemic signaling in tomato plants for defense against herbivores. Isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *J. Biol. Chem.* **278**: 30044-30050 (2003).
19. Reuveni, M., Tuzun, S., Cole, J.S., Siegel, M.R. and Kuc, J., The effects of plant age and leaf position on the susceptibility of tobacco to blue mould caused by *Peronospora tabacina*. *Phytopathol.* **76**: 455-458 (1986).
20. Safeeulla, K. M., *Biology and control of the downy mildews of pearl millet, sorghum and finger millet*. Wesley Press, Mysore, India, (1976).
21. Shetty, S. A., Prakash, H. S. and Shetty H. S., Efficacy of certain plant extracts against seed borne infection of *Trichoconiella padwickii* in paddy (*Oryza sativa*).*Canadian J. Bot.* **67**: 1956-1958 (1989).
22. Singh, S. D. and Gopinath, R., A seedling inoculation technique for detecting downy mildew resistance in pearl millet. *Plant Dis.* 69:582-584 (1985).
23. Song, J.T., Lu, H., McDowell, J.M. and Greenberg, J.T., A key role for ALDI in activation of local and systemic defenses in Arabidopsis. *Plant J.* 40: 200-121 (2004).
24. Thejaswini, P. H., Shetty, H. S. and Mahesha, H. B., Elicitation of induced resistance in pearl millet against *Sclerospora graminicola* through seed treatment with medicinal plant extracts. *Int. J. Pure App. Biosci. 3***(2)**:54-62 (2015).
25. Trujillo, M., Kogel, K. and Huckelhoven, R., Superoxide and hydrogen peroxide play different roles in the non host interaction of barley and wheat with inappropriate formae speciales of *Blumeria graminis. Mol. Plant Microbe Interact.* **17:**304-312 (2004).
26. Van Loon, L.C. and Van Strien, E.A., The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* **55**: 85–97 (1999).
27. Wyatt, S.E., Pan, S.Q. and Kuc, J., beeta-3-Glucanase, chitinase, and peroxidase activities in tobacco tissues resistant and susceptible to blue mould as related to flowering, age and sucker development. *Physiol. Mol. Plant Pathol.* **39**: 433–440 (1991).
28. Ye, X. S., Pan, S. Q. and Kuc, J., Association of pathogenesis-related proteins and activities of peroxidase, β-1,3-glucanase and chitinase with systemic induced resistance to blue mould of tobacco but not to systemic tobacco mosaic virus. *Physiol. Mol. Plant Pathol.* **36**:523-531 (1990).
29. Ye, X. S., Pan, S.Q. and Kuc, J., Specificity of induced systemic resistance as elicited by ethephon and tobacco mosaic virus in tobacco. *Plant Sci.* **84**:1-9 (1992).