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RESEARCH ARTICLE

INA MEDIATED INDUCTION OF RESISTANCE IN PEARL MILLET AGAINST DOWNY MILDEW DISEASE IS ASSOCIATED WITH ENHANCED CHITINASE ACTIVITY

Mythrashree, S. R. and *Amruthesh, K. N.

Applied Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore - 570 006, Karnataka, India

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ABSTRACT

2,6-Dichloroisonicotinic acid (INA) seed treatment at different concentrations (0.5, 1, 2.5 and 5 mM) for 3 and 6 h was evaluated during the interaction of pearl millet with *Sclerospora graminicola* in association with chitinase activity. Susceptible pearl millet seeds treated with INA at 1 mM concentration for 3 h showed significant ($P<0.05$) downy mildew disease resistance compared to 3 h treatments. The present study revealed that INA treated @ 1 mM provided the maximum seed germination 95% and seedling vigor 1552 in comparison to control which offered 79% and 923 of seed germination and seedling vigor, respectively. The results on growth parameter studies revealed that INA treated plants showed enhanced growth parameters when compared to control under greenhouse conditions. Application of INA @ 1 mM reduced the severity of disease with a highest protection of 62% under greenhouse conditions at 6 h treatment and highest reduction found on third day of inoculation in time gap studies. The enhanced chitinase activity was observed at all test points in INA @ 1 mM treated seedlings as compared to the control and peak activity of 10.11 and 3.44 respectively, was found at 24 hai.

INTRODUCTION

Plants are constantly threatened by various biotic and abiotic stresses in their natural environment. Their existence under such conditions is dependent on the ability to perceive external signals and respond in a timely manner. The plant signaling pathways are composed of complex networks that allow the plant to activate an appropriate spectrum of responses depending on the type of stimuli (Anderson *et al*., 2004). Plants cover a range of inducible defense mechanisms that operate to limit the pathogen infection, including host cell death at the site of infection (hypersensitive reaction), increased lignifications and cell wall cross-linking, production of small antibiotic molecules (phytoalexins), production of reactive oxygen species (ROS) and production of pathogen related(PR) proteins.Resistance to biotic stress can be induced in a number of plant species by biological and chemical means (Hammerschmidt and Kuc, 1995). Several chemicals have been reported to induce resistance to pathogens when applied to plants including 2,6-dichloroisonicotinic acid (INA) (Kessmann *et al*., 1994). INA, a synthetic chemical capable of inducing broad- spectrum disease resistance in several plant species was first reported by Metraux *et al*. (1991). INA has been shown to induce resistance under field conditions to various fungal and bacterial diseases of pear, pepper, tobacco (Metraux *et al*., 1991) and rust disease of green bean (Dann and Deverall, 1996). Formulations of INA has been shown to be effective in decreasing susceptibility to pathogens and inducing PR proteins in several other plant species (Metraux *et al*., 1991; Kogel *et al*., 1994; Vernooij *et al*., 1995). INA is interpreted to act by moving systemically through plants, as shown by Metraux *et al*. (1991). Extensive greenhouse studies with *Arabidopsis* and tobacco demonstrated that treatment with INA induced resistance to fungal, bacterial and viral pathogens with associated increase in the accumulation of mRNAs for pathogenesis related (PR) proteins (Ward *et al*., 1991; Friedrich *et al*., 1996) and has offered protection. The findings indicate that INA might be a promising priming agent to induce effective resistance against pearl millet disease.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the first cultivated crop that was used for human food in prehistoric times (Azhaguvel *et al*., 2003). It is one of the major staple cereals of semi-arid regions of the nation with low levels of gross domestic output providing food to millions of people. The world area cropped to pearl millet is about 26 million hectares. In India the crop is grown in about 9.8 million hectares with a total grain production of 7 million tones (Khairwal *et al*., 2008). One of the major biotic yield-reducing factors of pearl millet is the downy mildew disease [*Sclerospora graminicola* (Sacc.) Schroet] resulting in 40-60% crop loss (Thakur *et al*., 2003). Since many pathogens have developed resistance against applicable pesticides, the only way to manage the disease is to control plant pathogens

Corresponding author: Amruthesh, K. N.
Applied Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore - 570 006, Karnataka, India.

(Oostendorp *et al*., 2001). The disease management strategies, which are in practice currently, have their own limitations and hence, newer approaches are being explored and one important option is inducing resistance in the host to exploit its innate immunity. The objective of the present study was to determine INA as inducer against *S. graminicola* on pearl millet under greenhouse conditions as there are no reports on the inhibitory effect of INA against *S. graminicola* and its possible mechanism involved in induced resistance in pearl millet against downy mildew disease.

MATERIALS AND METHODS

Materials

Seeds of pearl millet cultivar (cv.) 7042S highly susceptible to downy mildew pathogen were obtained from International Crops Research Institute for the Semi- Arid Tropics (ICRISAT), Patencheru, Hyderabad, Andhra Pradesh, India.

Pathogen and inoculum preparation

The downy mildew pathogen, *S. graminicola* maintained on its susceptible host 7042S under greenhouse conditions served as source of inoculum. Downy mildew infected leaves were collected in the evening hours and washed with sterile distilled water (SDW) to remove the remnants of sporulation and dust particles and blot dried. The leaves were cut into pieces and placed on Petri plates lined with moist blotters and incubated over night in dark at 26±2º C. Fresh sporangia were harvested into SDW. The harvested sporangia were allowed to stand in dark for 30 mins till the zoospores were released. The concentration of the sporangia was adjusted to 4 x 10^4 zoospores/ ml in SDW (Safeeulla, 1976) and used as inoculum.

Chemical inducers

2, 6-dichloroisonicotinic acid (INA) (Sigma Aldrich, Bangalore) was tested as inducer. INA was dissolved in ethanol (0.5 µl/ ml of SDW) and diluted in SDW.

INA treatments

Pearl millet seeds of 7042S cv. were surface sterilized with 0.02% mercuric chloride for 5 min, rinsed in SDW for 2-3 times. Seeds were air dried followed by dipping in 25 ml of different concentrations (0.5, 1, 2.5 and 5 mM) of INA solutions and SDW. The treated seeds were incubated at 25±2º C in an incubator rotary shaker at 100 rpm (rotation per minute) for 3 h and 6 h and air dried aseptically.

Effect of INA on seed germination and seedling vigor

Germination test was carried out by paper towel method (ISTA, 2003). The elicitors treated seeds were placed on moist germination sheets equidistantly and another Pre soaked paper towel was placed on the first one in order to hold the seeds in position, rolled and wrapped with polythene to prevent drying and incubated for seven days at 25±2º C. After seven days of incubation, seed germination and seedling vigor were analyzed (Abdul Baki and Anderson, 1973). The experiment consisted of four replicates of 100 seeds in all the treatments and repeated thrice.

$$\text{Percent germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds plated}} \times 100$$

Vigor Index = (Mean root length + Mean shoot length) × Germination (%)

Effect of INA on pearl millet-downy mildew disease under greenhouse conditions

The pearl millet seeds primed with INA and SDW for 3 h and 6 h time duration were sown in earthen pots filled with autoclaved soil (1:2:1 ratio of sand, soil and manure). Leaf-whorls of two-day old seedlings were inoculated with a suspension of 4 x 10^4 zoospores/ ml of *S. graminicola* by whorl inoculation method (Singh and Gopinath, 1985). Each treatment consisted of four replicates of 10 pots per replication with 10 seedlings per pot of 14 inch diameter. The pots were arranged in a randomized complete block design (RBD) and maintained under greenhouse conditions (90-95% RH, 25±2° C). Plants were observed daily and rated diseased when they showed any one of the typical symptoms of downy mildew, i.e. sporulation, chlorosis, stunted growth or malformation of the earheads. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. The experiment was repeated thrice with four replicates of 100 plants each. Downy mildew disease protection was calculated using the formula:

$$\text{Downy mildew disease protection} = \frac{\text{C-T}}{\text{C}} \times 100$$

where C - percent downy mildew disease incidence in control; T - percent downy mildew disease incidence in treated plants.

Effect of INA on growth parameters of pearl millet under greenhouse conditions

Evaluation of growth promotion under greenhouse conditions was carried out in pearl millet cv. 7042S seeds primed with INA for 3 h. After treatment, the seeds were blot-dried and sown in earthen pots (14 inch diameter) filled with sand, soil and manure in the ratio of 1:2:1. The experiment consisted of four replicates per treatment with 10 pots in each replicate (10 seeds/ pot) and repeated thrice. The pots were maintained at 25±2º C with 95% RH (relative humidity) and watered regularly. Seeds treated with SDW served as control. 30-days after sowing, seedling height, shoot fresh and dry weight, leaf surface area and number of basal tiller per plant were measured and recorded accordingly.

Spatio-temporal time-gap studies

Spatio-temporal time gap studies were carried out in order to understand the nature of disease protection offered by seed treatment and maintaining spatio-temporal separation between inducer treatments and the pathogen inoculation (Amruthesh *et al*., 2005). The susceptible pearl millet seeds (7042S) treated with INA along with control seeds were sown in autoclaved potting medium as mentioned above and was arranged in RBD. The two-day-old seedlings were challenge inoculated with zoospore suspension of *S. graminicola* (4 x 10^4 zoospore/

ml) by whorl inoculation method with a time gap of 1, 2, 3, 4 and 5 days of emergence in different set of plants. Plants were maintained under greenhouse conditions as mentioned above and were observed for typical symptoms of downy mildew, i.e. sporulation, chlorosis, stunted growth or malformation of the earheads at every 15-day time intervals and were rated for disease when they showed any one of the typical downy mildew symptoms. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. The experiment was repeated thrice with four replicates of 100 plants each.

INA treatment and sampling of plant material for chitinase activity

The expression of chitinase activity as markers of resistance was determined in two-day-old seedlings after treatment with INA @ 1 mM concentration and SDW for 3 h. The treated seeds were plated in Petri plates lined with wet blotter Discs. Two-day-old seedlings were root dip inoculated with *S. graminicola* (4 x 10^4 zoospore/ ml). The seedlings were harvested at different time intervals (0, 2, 4, 6, 8, 12, 18, 24, 48 and 72 hai; hours after inoculation), blot dried, weighed and stored at -80º C prior to analysis.

Chitinase activity

To determine enzyme activity, colorimetric assays were conducted as described by Isaac and Gokhale (1982). One gram of each sample was ground in 1 ml of 0.05 M sodium acetate buffer (pH 5.2) at 4° C. The homogenate was centrifuged at 10,000 rpm for 30 min at 4° C and the supernatant was used to determine enzyme activity. For analysis, samples were assayed using N-acetyl glucosamine as standard. Colloidal chitin in 0.05 M sodium acetate buffer (pH 5.2), purified from chitin following the method of Skujins *et al*. (1965) was used as a substrate. Monomers of N-acetyl glucosamine released after incubation were measured spectrophotometrically at 585 nm using dimethyl amino benzaldehyde reagent (Reissig *et al*., 1955). The enzyme activity was expressed in terms of nmol/ min/ mg protein.

Protein estimation

Protein content in the crude extracts was estimated by dye binding method (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

Statistical analysis

Data from four replicates were analyzed 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 magnitude of F values (P<0.05). Treatment means were separated by Tukey's HSD test.

RESULTS

Effect of INA on seed germination and seedling vigor

INA treatments at different concentrations for 3 h and 6 h enhanced seed germination and seedling vigor over the control. The results revealed that INA treatments enhanced significant seed germination and seedling vigor when treated for 3 h when compared to 6 h treatments. Maximum seed germination (95%) and seedling vigor (1552) was obtained in 1 mM INA treated plants for 3 h followed by 2.5 mM which showed 92% germination and 1419 of seedling vigor. The control pearl millet susceptible seeds recorded 79% seed germination and 923 seedling vigor (Table 1).

Table 1. Effect of INA seed treatment on seed germination and seedling vigor of pearl millet

INA Treatment (mM)	Percent germination		Seedling vigor	
	3 h	6 h	3 h	6 h
0.5	89.3±0.47^{c}	82.3±0.47^{c}*	1356.6±7.28^{c}	1124.8±5.17^{c}
1	95.3±0.47^{a}	89.0±0.40^{a}	1552.6±3.87^{a}	1337.2±4.26^{a}
2.5	92.0±0.40^{b}	84.8±0.47^{b}	1419.1±4.40^{b}	1209.8±5.32^{b}
5	88.5±0.64^{c}	81.5±0.28^{c}	1318.7±6.26^{d}	1090.1±2.02^{d}
Control	79.3±0.62^{d}	79.3±0.62^{d}	923.3±7.58^{e}	923.3±7.58^{e}

Mean from four repeated experiments with four replicates of 100 seeds per treatment in each experiment. Means within columns sharing the same letters are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

Effect of INA on pearl millet-downy mildew disease under greenhouse conditions

INA treated pearl millet plants at different concentrations were tested under greenhouse conditions and a varied degree of downy mildew disease protection was observed ranging from 26% to 62%. Among the different concentrations of INA, maximum downy mildew disease protection of 62% was observed in plants treated with 1 mM concentration for 3 h followed by 55% protection with INA treatment for 3 h at same concentration. The lowest protection of 26% was recorded in pearl millet plants treated with 5 mM concentration for 3 h (Table 2). The SDW treated control plants showed 100% disease incidence.

Table 2. Effect of INA treatment on Per cent downy mildew disease protection

INA Treatment (mM)	Per cent Downy mildew disease protection	
	3 h	6 h
0.5	32.3±0.47^{c}	29.5±0.64^{c}
1	62.3±0.47^{a}	55.8±0.62^{a}
2.5	47.8±0.75^{b}	42.8±0.62^{b}
5	28.3±0.75^{d}	26.3±0.47^{d}
Control	0	0

Mean from four repeated experiments with four replicates of 100 plants per treatment in each experiment. Means within columns sharing the same letters are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

Effect of INA on growth parameters of pearl millet under greenhouse conditions

INA treatments at all the tested concentrations for 3 h showed enhancement in growth parameters under greenhouse conditions and the degree of growth promotion was found to be concentration dependent. Among the different concentrations treated, the pearl millet plants treated with 1 mM concentration recorded maximum increase in vegetative growth parameters like plant height (35.6 cm), shoot fresh (13.6 gm) and dry (4.2 gm) weight, leaf surface area (38.5 cm^2) and number of tillers (4.2) followed by 2.5 mM concentration when compared to SDW treated control plants (Table 3).

Table 3. Effect of INA treatment for 3 h on plant height, shoot fresh weight, shoot dry weight, leaf area and number of basal tillers under green house conditions after 30 DAS

INA Treatment (mM)	Height (cm)*	Shoot fresh weight/ plant (g)*	Shoot dry weight/ plant (g)*	Leaf surface area (cm2)*	No. of basal tillers/plant*
0.5	37.8±0.11^{c}	14.2±0.07^{c}	4.7±0.09^{b}	39.9±0.13^{c}	4.2±0.05^{b}
1	42.4±0.09^{a}	15.6±0.04^{a}	5.3±0.06^{a}	41.4±0.07^{a}	4.5±0.02^{a}
2.5	40.5±0.07^{b}	14.9±0.07^{b}	5.1±0.09^{a}	40.6±0.05^{b}	4.4±0.09^{ab}
5	33.4±0.09^{d}	13.8±0.09^{d}	4.3±0.09^{c}	39.0±0.09^{d}	3.9±0.07^{c}
Control	26.5±0.08^{e}	8.1±0.07^{e}	2.8±0.09^{d}	30.5±0.07^{e}	3.1±0.09^{d}

*Mean from three repeated experiments with four replicates of 100 plants per treatment in each experiment. Means within columns sharing the same letters are not significantly different according to Tukey's HSD test at $P \leq 0.05$

Spatio-temporal time-gap studies

The spatio-temporal time gap studies with INA treatment at 1 mM concentration confirmed the systemic nature of resistance and showed varied degrees of downy mildew disease protection. Maximum disease protection of 59% was observed on third day of consecutive inoculation. The resistance was observed as early as 24 h and reached maximum after three days of pathogen inoculation. Further, resistance so developed was retained throughout the cropping period (Fig. 1).

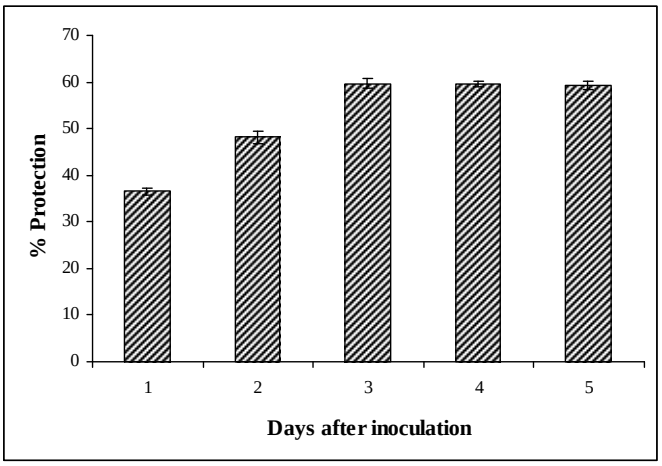

Fig. 1. Demonstration of nature of resistance by INA (1 mM) treatment for 3 h by spatio-temporal separation of the inducer and pathogen inoculation

Effect of INA treatments on chitinase activity

A strong induction of chitinase activity in pearl millet seedlings was observed in INA treated plants at 1 mM concentration. The results revealed that the chitinase activity increased with increase in time with a peak at 24 hai (Fig. 2). However, the increase in chitinase activity was significantly different from that of the control seedlings. Two-day-old seedlings on challenge inoculation showed a steady increase of chitinase activity up to 24 hai and decreased at all other time points tested. The INA treated seedlings on pathogen inoculation showed significantly higher chitinase activity of 10.11 nmol/ min/ mg protein in inoculated seedlings than in susceptible inoculated seedlings 2.46 nmol/ min/ mg protein.

DISCUSSION

The study assessed the effects of INA on *S. graminicola* infection development in pearl millet. The eliciting property of INA to control diseases in different crops has been demonstrated previously (Vernooij *et al*., 1995). Earlier studies by Amruthesh *et al*. (2005) and Pushpalatha *et al*. (2013) found that growth promotion in the form of improved seed germination and seedling vigor of pearl millet were achieved in seed treatments with abiotic agents, at the seedling stage along with other qualitative and quantitative traits. The present results due to treatment are also correlated with previous work done by Zheng *et al*. (1994) who studied the effect of priming on canola and observed that seed germination and seedling emergence were enhanced. Overall, among the concentrations tested, INA at 1 mM showed maximum growth promotion when compared to control.

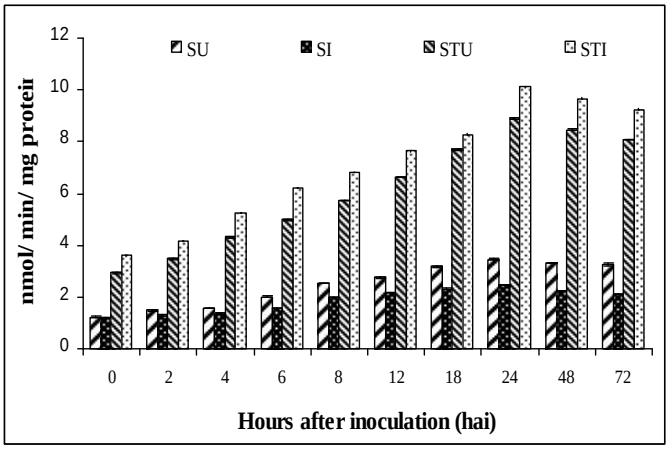

Fig. 2. Temporal pattern of accumulation of the chitinase enzyme in pearl millet seedlings upon seed treatment with INA (1 mM) for 3 h. SU- Susceptible Uninoculated; SI- Susceptible Inoculated; STU- Susceptible Treated Uninoculated; STI- Susceptible Treated Inoculated

The present study revealed enhanced growth parameters in comparison with the control (Table 3). The resistance inducers, namely benzathiadazole, calcium chloride, hydrogen peroxide, Trichoshield and celluloysin were reported to be enhancers of seed quality and vegetative parameters along with significant improvement in yield (Geetha and Shetty, 2002; Niranjan Raj *et al*., 2005; Pushpalatha *et al*., 2013). Seed treatment presents many advantages over other delivery methods and it is reported to alleviate physiological and pathological stresses. Seed treatment resulted in mobilisation, activation and enhancement of various cellular defense responses resulting in induction of resistance (Cornath *et al*., 2002). The elicitors was unique in that, apart from showing the highest capacity of growth promotion, it was also the best inducer of resistance against downy mildew disease, and its performance in terms of growth enhancement and resistance induction was consistent under greenhouse conditions with a protection of 62%. In time gap studies it was evident that a minimum of three days of time interval was required to build up maximum resistance against the pathogen after its exposure to inducer treatment as it has been stated in earlier reports (Niranjan Raj *et al*., 2005;

Murali *et al*., 2012; 2013; Mythrashree *et al*., 2013). Similarly, systemic resistance to the leaf spot pathogen, *Alternaria macrospora*, was induced in cotton leaves by treatment of the cotyledons with a formulation containing INA (Brock *et al*., 1994; Colson-Hanks and Deverall, 2000). Therefore, in pearl millet INA treatments may stimulate inherent defense mechanisms so that the plant can respond more quickly against the invading pathogen as suggested by Dann *et al*. (1998).

In many plants investigated so far, INA treatment is associated with increase in activities of PR proteins (Colson-Hanks and Deverall, 2000). PR proteins include β-1,3-glucanase and chitinase in tobacco, pea, bean and potato (Kombrink *et al*., 1988; Mauch and Staehlin, 1989; Ward *et al*., 1991). Similarly, 1 mM concentration of INA treatment for 3 h to pearl millet seeds acquired the capacity to express faster and stronger defense responses to *S. graminicola* inoculation. Expression of higher levels of hydrolases- chitinases has been shown to provide enhanced resistance to fungal pathogens (Senthilraja *et al*., 2013). Chitinase induction after pathogen infection supplies protection by directly degrading fungal cell wall components and indirectly by releasing some elicitors from the decaying fungal cell wall that might stimulate other plant defense mechanisms like phytoalexin accumulation in the host plant (Edreva *et al*., 2004). The increase in chitinase activity seemed to be a vital aspect of pearl millet defense system, and may play a role in restricting the development of disease symptoms on the pearl millet leaves infected with the pathogen. Moreover, the chitinase activity was maintained at higher levels in INA pretreated and *S. graminicola* challenged pearl millet plants. Furthermore, a subsequent challenge of INA- pretreated plants with the pathogen resulted in higher expression of enzyme activity, compared to control or uninoculated plants. Hence, the function of INA appears to have a direct antifungal effect on the pathogen, consequently increasing their susceptibility to the host chitinase, a lytic enzyme. Therefore, our results suggest that exogenous application of INA enhanced seed quality parameters as well as resistance against downy mildew disease in pearl millet through activation of chitinase enzyme. Exogenous application of INA could enhance disease resistance against downy mildew pathogen infection in pearl millet through the activation of chitinase enzyme.

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REFERENCES

Abdul Baki, A.A. and Anderson, J.D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, 13: 630-633.

Amruthesh, K.N., Geetha, N.P., Lyngs J orgensen, H.J., de Neergaard, E. and Shetty, H.S. 2005. Unsaturated fatty acids from zoospores of *Sclerospora graminicola* induce resistance in pearl millet. *Eur. J. Pl. Pathol.*, 111: 125-137.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R. and Kazan, K. 2004. Antagonistic interaction between abscisic acid and jasmonate- ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *The Pl. Cell*, 16: 3460-3479.

Azhaguvel, P.C., Hash, T., Rangasamy, P. and Sharma, A. 2003. Mapping the d_1 and d_2 dwarfing genes and the purple foliage color locus P in pearl millet. *The American Genetic Association*, 94: 155-159.

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

Brock, P.M., Inwood, J.R.B. and Deverall, B.J. 1994. Systemic induced resistance to *Alternaria macrospora* in cotton *(Gossypium hirsutum). Aust. Pl. Pathol.,* 23: 81-5.

Colson-Hanks, E.S. and Deverall. B.J. 2000. Effect of 2,6-dichloroisonicotinic acid, its formulation materials and benzothiadiazole on systemic resistance to *Alternaria* leaf spot in cotton. *Pl. Pathol.,* 49: 171-178.

Cornath, U., Thulke, O., Katz, V., Schmindling, S. and Kohler, A. 2002. Priming as a mechanism in induced systemic resistance of plants. *Eur. J. Pl. Pathol.,* 107: 113-119.

Dann, E., Diers, B., Byrum, J. and Hammerschmidt,R. 1998. Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *Eur. J. Pl. Pathol.,* 104: 271-278.

Dann, E.K. and Deverall, B.J. 1996. 2, 6-dichloroisonicotinic acid (INA) induces resistance in green beans to the rust pathogen, *Uromyces appendiculatus*, under field conditions. *Aust. Pl. Pathol.,* 25: 199-204.

Edreva, A. 2004. A novel strategy for plant protection: Induced resistance. *J. Cell Mol. Biol.,* 3: 61-69.

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gut Rella, M., Meiers, B., Dincher, S., Staub, T., Uknes, S., Metraux, J.P., Kessmann, H. and Ryals, J. 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Pl. J.,* 10: 61-70.

Geetha, H.M. and Shetty, H.S. 2002. Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzathiadazole, calcium chloride and hydrogen peroxide- a comparative evaluation. *Crop Prot.,* 21: 601-610.

Hammerschmidt, R. and Kuc, J. 1995. Induced Resistance to Disease in Plants. *Kluwer Academic Publishers, Amsterdam.*

Isaac, S. and Gokhale, A.V. 1982. Autolysis: A tool for protoplast production from *Aspergillus nidulans*. *Trans. Br. Mycol. Soc*., 78: 389-394.

ISTA. 2003. International Rules for Seed Testing. *International Seed Testing Association* (Chapter V).

Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S. and Ryals, J. 1994. Induction of systemic acquired resistance in plants by chemicals. *Annu. Rev. Phytopathol*., 32: 439-459.

Khairwal, I.S. 2008. Pearl millet coordinators review report. *Indian Council of Agricultural Research*, Mandor, Jodhpur, pp. 1-23.

Kogel, K.H., Beckhove, U., Dreschers, J., Munch, S. and Romme, Y. 1994. Acquired resistance in barley: the resistance mechanism induced by 2,6-dichloroisonicotinic acid is a phenocopy of a genetically based mechanism governing race-specific powdery mildew resistance. *Pl. Physiol*., 106: 1269-1277.

Kombrink, E., Schroder, M. and Hahlbrock, K. 1988. Several 'pathogenesis-related' proteins in potato are 1, 3-β glucanases and chitinases. Proceedings of the National Academy of Science, United States of America, 85: 782-786.

Mauch, F. and Staehlin, L.A. 1989. Functional implications of the subcellular localization of ethylene-induced chitinase and β-1,3-glucanase in bean leaves. *The Pl. Cell*, 1: 447-457.

Metraux, J.P., Ahl Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J. and Ward, E. 1991. Induced systemic resistance in cucumber in response to 2, 6-dichloroisonicotinic acid and Inducing systemic resistance in cotton pathogens. In: Hennecke H, Verma DPS (eds) Advances in Molecular Genetics of Plant-Microbe Interactions. Proceedings of the 5th International Symposium on the Molecular Genetics of Plant-Microbe Interactions, Interlaken, Switzerland, 1990, Kluwer, Dordrecht, The Netherlands, pp. 432-439.

Murali, M., Amruthesh, K.N., Sudisha, J., Niranjana, S.R. and Shetty, H.S. 2012. Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. *J. Phytol*., 4(5): 30-36.

Murali, M., Sudisha, J., Amruthesh, K.N., Ito, S.I. and Shetty, H.S. 2013. Rhizosphere fungus *Penicillium chrysogenum* promotes growth and induces defence-related genes and downy mildew disease resistance in pearl millet. *Pl. Biol*., 15(1): 111-118.

Mythrashree, S.R., Geetha, N.P., Pushpalatha, H.G., Murali, M., Shetty, H.S. and K.N. Amruthesh. 2013. *Lactuca sativa* mediated chitinase activity and resistance in pearl millet against *Sclerospora graminicola*. *Afr. J. Pl. Sci*., 7(10): 492-503.

Niranjan Raj, S., Shetty, N.P. and Shetty, H.S. 2005. Synergistic effects of Trichoshield on enhancement of growth and resistance to downy mildew in pearl millet. Bio Cont., 50: 493-509.

Ostendorp, M., Kunz, W., Dietrich, B. and Staum, T. 2001. Induced disease resistance in plants by chemicals. *Eur. J. Pl. Pathol*., 107: 19-28.

Pushpalatha, H.G., Sudisha, J. and Shetty, H.S. 2013. Cellulysin induces downy mildew disease resistance in pearl millet driven through defense response. *Eur. J. Pl. Pathol*. DOI 10.1007/s10658-013-0281-9

Reissig, J.L., Strominger, J.L. and Leloir, L.F. 1955. A modified colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem*. 217: 959-966.

Safeeulla, K.M. 1976. Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Wesley Press, Mysore, India.

Senthilraja, G., Anand, T., Kennedy, J.S., Raguchander, T. and Samiyappan, R. 2013. Plant growth promoting rhizobacteria (PGPR) and entomopathogenic fungus bioformulation enhance the expression of defense enzymes and pathogenesis-related proteins in groundnut plants against leafminer insect and collar rot pathogen. *Physiol. Mol. Pl. Pathol.*, 82: 10-19.

Singh, S.D. and Gopinath, R.A. 1985. Seedling inoculation technique for detecting downy mildew resistance in pearl millet. *Pl. Dis*., 72: 425-428.

Skujins, J.J., Potgieter, H.J. and Alexander, M. 1965. Dissolution of fungal cell walls by a streptomycete chitinase and β-(1,3)-glucanase. *Arch. Biochem. Biophys*., 111:358-364.

Thakur, R.P., Rao, V.P., Amruthesh, K.N., Shetty, H.S. and Datar, V.V. 2003. Field surveys of pearl millet downy mildew: Effects of hybrids, fungicides and crop sequence., 33: 387-394.

Vernooij, B., Friedrich, L., Ahl Goy, P., Staub, T., Kessmann, H. and Ryals, J. 1995. 2,6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Mol. Pl. Micro. Int*., 8: 228-234.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl Goy, P., Metraux, J.P. and Ryals, J.A. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Pl. Cell*, 3: 1085-1094.

Zheng, G.H., Wilen, R.W., Slinkard, A.E. and Gusta, L.V. 1994. Enhancement of canola seed germination and seedling emergence at low temperature by priming. *Crop, Sci*., 34:1589 - 1594.

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