RESEARCH ARTICLE

3, 5-Dichloroanthranilic acid (DCA) - an elicitor induces systemic resistance against downy mildew in pearl millet

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ABSTRACT

Seed treatments with synthetic elicitor DCA were evaluated for their potential to induce resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola Schroet*. Disease resistance in plants is associated with the activation of a wide variety of defense responses that serve to prevent pathogen infection. Pearl millet seeds were treated with DCA at different concentrations viz., 10, 20, 50, 100µM for 4h. Among these, 100 µM was found to be optimum, and recorded significant disease protection compare to other concentrations, which revealed maximum germination of 93.7% and seedling vigor 1480 compare to control seedlings. DCA treatment also exhibited anti-sporulation activity under laboratory conditions. Under In-vivo conditions DCA primed seeds exhibited intense decline in downy mildew incidence with significant protection of 64.1% in 100 µM. Vegetative growth parameter studies revealed the enhanced growth of pearl millet when compare to control. Restorative activity of Seedlings sprayed with DCA solution reduced the severity of disease incidence of 33.9% with significant increase in disease protection. Time-gap studies revealed, seed treatment increased disease resistance four days after inoculation. Additionally, Pearl millet seedlings raised after seed treatment with 100µM DCA showed an increased level of the defense-related enzymes: Phenylalanine ammonia-lyase activity and Lipoxygenase, compared with the untreated control. The significant PAL activity was observed at 12h with 4.05U hai and enhanced LOX activity of 5.7U hai was found at 48h.

Keywords: DCA, Pearl Millet, *S. graminicola,* PAL, LOX.

INTRODUCTION

Plants can activate a resistance response upon recognition of a potential pathogen or its products both locally and systemically. The defense response can be accelerated or enhanced by the application of specific compounds, which act as an elicitor or inducer (Lyon *et al*., 1995; Kuc, 2001; Gozzo, 2003). Plant or seed treatment with elicitors often results in a resistance against different pathogens simultaneously (Oostendorp *et al*., 2001). The plant's immunity can be prompted by initial localized infection with pathogens that cause lesions including host cell death (Ross, 1966). The resistance induction in plants results in the

development broad spectrum of immunity in non-infected tissues against a comprehensive range of plant pathogens (viruses, bacteria and fungi) (Vallad and Goodman, 2004; Walters *et al*., 2005; Kuc, 2006). Induced resistance by chemicals is also a promising approach to prevent diseases caused by soil-borne pathogens (Okubara *et al*., 2005). Benzo [1,2,3] thiadiazole derivatives have been shown to mimic the biological activation of systemic acquired resistance by necrogenic pathogens (Kunz *et al.,*1997), synthetic analogue of salicylic acid like Acibenzolar-S-methyl (BTH, ASM), protected cantaloupe against *Colletotrichum lagenarium* and cucumber mosaic virus (CMV) (Smith-Becker *et al*., 2003). Extensive range of chemical agents have been shown to trigger innate defense mechanisms via host-pathogen interactions at physiological, biochemical and molecular levels (Vallad and Goodman, 2004; Kogel and Langen, 2005).

Pearl millet [Pennisetum glaucum (L.) R. Br.] Is one of the most important staple food in the semiarid regions of the world. In India, pearl millet is the third most important cereal crop and is grown over 10 million hectares mainly as a rain fed crop with an annual production of 9.5 million tonnes. The major constraint for pearl millet production is downy mildew disease caused by the obligate, biotrophic, oomyceteus pathogen *Sclerospora graminicola* (Sacc.) Schroet. Disease causes systemic infection in pearl millet that manifests itself as foliar symptoms and malformation of the panicles resulting in severe grain loss. Production resulting upto 80% yield loss (Howarth and yadav, 2002). One of the newer and ecofriendly approaches to manage this disease is by induction of resistance by using biotic and abiotic elicitors. The effect of abiotic and biotic elicitors involves biochemical changes in the host metabolism that may play a role in limiting plant infection.

Abiotic inducers include chemicals which act at various points in the signaling pathways involved in disease resistance. Induced defenses are often triggered by the recognition of conserved pathogen-associated molecular patterns (PAMPs), resulting in PAMP-triggered immunity (PTI) (Gomez-Gomez and Boller, 2002). 3, 5-dichloroanthranilic acid (DCA) a synthetic elicitors, competently induces defense responses to two the phytopathogens (*Hyaloperonospora parasitica* and *Pseudomonas syringe)* by concurrently activating two distinct branches of the plant defense signaling network. (Knoth *et al.,* 2009) DCA and the SA analog INA to be functionally distinct with regard to their dependency on NPR1 and the kinetics of their activities. SA signaling is partially dependent on NPR1 (Non expresser of Pathogenesis- Related genes1), a transcriptional cofactor that is required for the activation of multiple defense genes (Dong, 2004). DCA acts transiently and is only partially dependent on (NPR1) Microarray analyses revealed a cluster of 142 DCA- and INA-responsive genes that show a pattern of differential expression coinciding with the kinetics of DCA-mediated disease resistance (Knoth *et al.,* 2009). *Arabidopsis thaliana* Immune responses against *Hyaloperonospora parasitica* are somewhat facilitated by transcriptional upregulation in response to (LURP) cluster of plant defense genes. Resistance in plants is influenced by Elicitation of Induced defense to recognition of pathogen effectors by Leu-rich repeat-containing plant resistance (R) proteins by making the pathogen avirulent and the plant resistant (Jones and Dangl, 2006). The current study was aimed to investigate the synthetic elicitor 3, 5-dichloroanthranilic acid (DCA) could able to trigger disease resistance and defense enzymes in pearl millet for imparting systemic resistance against downy mildew pathogen. Multiple mechanisms are responsible for induced or acquired resistance, and different types of interactions between pathways were studied.

MATERIALS AND METHODS

Host

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

Pathogen source and inoculum preparation

Downy mildew pathogen *S. graminicola* was isolated from susceptible cv.7042S. The susceptible pathogen was maintained on the same cultivar prior to use under greenhouse conditions (temperature of 22 ± 2 ºC and relative humidity of 80 %). Pearl millet leaves showing prolific sporulation of *S. graminicola* on the abaxial side were collected in the evening hours. The collected leaves were thoroughly washed under running tap water to remove dust and old sporangia. Then the collected leaves were blot dried and cut into

smaller pieces, and placed in moist chamber for sporulation. The next morning, Fresh sporangia were collected and zoospores were released into sterile distilled water. Zoospore concentration was adjusted to 4 X 10^4/ml using hemocytometer and used as inoculum for all inoculation experiments (Safeeulla, 1976).

***In-vitro* studies**

Preparation of inducer and seed treatment

DCA obtained from Sigma DCA solutions were prepared by dissolving 10µM, 20 µM, 50 µM, 100 µM in DMSO (1µl/ml) of 100mL of sterile distilled water and kept for constant agitation for 2h for complete dissolution. For seed treatment, pearl millet seeds were surface sterilized with 0.02% mercuric chloride solution for 4-5 min and then thoroughly rinsed in sterile distilled water. Seeds were submerged in 10ml of inducer solution with different concentrations viz., 10µM, 20 µM, 50 µM, 100 µM. Treated seeds were incubated at 25^0C in a rotary shaker for 4h to facilitate the seed treatment. For the same time interval, Seeds treated with sterile distilled water served as control.

Influence of DCA on seed germination and seedling vigor of pearl millet

Seed germination and seedling vigor were evaluated by treating Seeds with different DCA concentrations of 10µM, 20 µM, 50 µM, 100 µM for 4 h. Germination tests were carried out by the paper towel method (ISTA, 2003). Seeds treated with sterile distilled water for the same duration served as controls. Treated seeds and controls were planted onto paper towels. Treated seeds of pearl millet were placed equidistantly on the paper (100 seeds/ paper towel). The towels were then rolled and wrapped with polythene to prevent drying and incubated for 7 days. The number of seeds germinated were counted and represented in percentage of germination. At the end of 7 days, Seedling vigor was analyzed (Abdul Baki and Anderson, 1973) by measuring the length of the shoot and root of individual seedlings. The experiment was carried out three times with four replicates of 100 seeds each. The vigor index was calculated using the formula:

$$\text{Vigor Index} = (\text{Mean root length} + \text{Mean shoot length}) \times (\% \text{ Germination})$$

Anti-oomycete activity of DCA

Downy mildew infected disease leaves were collected from infected plants grown in greenhouse. Leaves were washed in sterile distilled water, excess of water was removed and blot dried. Leaves were cut in to small discs of 10 mm diameter size by using sterilized cork borers and consequently Immersed in different concentration of DCA solution for 5-10 mins (Wafaa and Haggag, 2002; Musetti *et al*., 2006 and Deepak, 2005). One set of leaf disc dipped in distilled water for the same interval served as control. The treated leaf discs were incubated overnight in a moist chamber for sporulation. After incubation, leaf disc were analyzed under stereo binocular microscope for sporulation and sorted as full inhibition (++), moderate inhibition (+) and no inhibition (-) compare to control set.

***In-vivo* studies**

Effect of seed treatment on pearl millet downy mildew disease

The treated seeds were sown in earthen pots filled with autoclaved soil, sand and manure (in the ratio of 2:1:1). The experiment consisted of four replicates, 10 pots in each replication with 10 seedlings per pot (12-14in diameter) and experiment was repeated thrice. Treated pots were arranged in a randomized complete block design. Seeds treated with sterile distilled water served as control (non-treated).

Seeds treated with the systemic fungicide metalaxyl formulation (Apron 35 SD at 6 g/kg concentration) served as a chemical control. Zoospore suspension of *S. graminicola* (4x10^4zoospores/ml) was prepared as described before. Emerging seedlings at coleoptile stage were challenge-inoculated by the whorl inoculation (Singh and Gopinath, 1985). In the whorl inoculation method, droplets of *S. graminicola* zoospores were dropped onto the leaf whorl of the emerging seedlings and allowed to flow down to the base. Inoculated plants were maintained under greenhouse conditions (20–26 ^{0}C temperature with 90–95% RH) and observed regularly for the development of disease symptoms. The plants were assessed disease when they showed any one of the typical symptoms of downy mildew like sporulation on the abaxial leaf surface, stunted growth, chlorosis, or malformation of the ear heads. Percentage Downy mildew disease incidence was recorded at 30 DAS and final counts were made at 60 DAS as percentage of plants showing symptoms of downy mildew disease.

$$\text{Disease Protection} = \frac{\text{Downy mildew disease incidence in control} - \text{Downy mildew disease incidence in treated plants}}{\text{Downy mildew disease incidence}} \times 100$$

Downy mildew disease protection was calculated using above formula,

Influence of DCA 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 treated DCA for 6 h time duration sown in earthen pots filled with autoclaved soil, sand and manure (in the ratio of 2:1:1) The experiment consisted of four replication with 100 seedlings each and was repeated three times. Plants were maintained under greenhouse conditions (20–26 ^{0}C temperature with 90–95% RH) and observed regularly for the development of disease symptoms. Seeds treated with SDW served as control. After 30 days of sowing, seedling height, shoot fresh and dry weight, leaf surface area and number of basal tiller per plant were measured and recorded accordingly.

Restorative activity of DCA against downy mildew

Susceptible cultivar 7042S seeds were sown to earthen pots and maintained under greenhouse condition as explained before The different concentration of DCA solution were applied to15 day old sporulating downy mildew infected plants. DCA solutions were smeared on to abaxial leaf surface with soft brush constantly for 3 days. One set of infected plants applied with distilled water served as control. The experiment was carried out three times with four replicates of 100 plants each. After 5 days, Inhibition of sporulation were recorded and tabulated.

Effect of DCA on induced resistance by Time gap studies in pearl millet

Spatio-temporal time gap studies were carried out between inducer treatments and the pathogen inoculation (Amruthesh *et al.,* 2005) in order to understand the systemic nature of disease protection offered by seed treatment. The pearl millet seeds (7042S) treated with DCA for 4h were sown in earthen pots filled with autoclaved soil and maintained as explained earlier along with control seeds (seeds treated with sterile distilled water). Pots were arranged in a randomized complete block design. After Two days, seedlings at coleoptile stage were challenge inoculated with zoospore suspension of *S. graminicola* (4 x 10^4 zoospore/ml) following the whorl inoculation method and with a time gap of 1, 2, 3, 4, 5 and 6 days in different set of plants. Plants were maintained under greenhouse conditions and observed regularly for the expression of downy mildew disease. The experiment consisted of four replicates with 100 seedlings each and was repeated three times. At the end of 60 days, Disease incidence and protection of downy mildew disease was calculated as mentioned above.

Biochemical studies

Sampling of seedlings and enzyme extraction

Two-day-old seedlings of susceptible (7042S) and 100µl of DCA treated seeds along with control seedlings (sterile distilled water treated) were root dip inoculated with the zoospore suspension of *S. graminicola* (4 X 10^4 zoospores/ml). The seedlings were harvested at different time intervals 0, 3, 6,9,12, 24, 48 and 72 h post inoculation (hpi) were immediately stored at -80º C prior to analysis and used for biochemical studies. One gram fresh weight of Seedlings were weighted from harvested seedlings and were ground to a fine powder in liquid nitrogen and by homogenizing in different buffers (1ml/g seedlings) for extraction of different enzymes at 4^{0}C. These were centrifuged at 12,000 g for 10 min at 4^{0}C and the supernatants were used for further enzyme assays. Protein content in the crude extracts was estimated by Dye binding method (Bradford, 1976) using BSA (Bovine serum albumin) (Sigma) as a standard.

Phenylalanine ammonia-lyase (PAL, E.C. 4.1.3.5)

PAL enzyme was extracted with 25mM Tris HCL buffer (P^H 8.8). PAL activity was assayed according to the procedure of Beaudoin-Eagan and Thorpe (1985). One hundred µl of extracts were mixed with reaction mixture of 900µl of 50mM L-phenylalanine and 100mM Tris HCl buffer solution. The mixture was placed in a water bath for 120min at 40^{0}C. The reaction was stopped by adding 60µl of 5N HCL. PAL enzyme activity was measured spectrophotometrically at a wavelength of 290nm and expressed as the amount of t-cinnamic acid formed from L-phenylalanine per mg of protein per min.

Lipoxygenase (LOX) (Linoleate: oxygen oxidoreductase, EC 1.13.11.12)

Enzyme activity was measured by following the procedure of Borthakur *et al*. (1987). The substrate for assay was prepared according to the method described by Axelrod *et al*. (1981). 28 mg of Linoleic acid was mixed with an equal volume of Tween-20 in 2 mL of distilled water.

50 µL of 2N NaOH was added to obtain a clear solution and volume of the solution was made up to 10 mL with distilled water. Each time the substrate was prepared fresh and used for the enzyme assay. The reaction mixture contained 0.3 mL of substrate with 3 mL of 0.2 M Sodium phosphate buffer (pH 6.5). The reaction was initiated by adding 10 µl the enzyme extract. The activity was determined spectrophotometrically by monitoring the appearance of the conjugated diene hydroperoxide at 234 nm. The enzyme activity was expressed as a change in the absorbance (δ 234) /mg protein/min. The change in absorbance at 234 nm was recorded for three minute.

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 F values (P = 0.05). Treatment means were separated by Tukey's honestly significant differences (HSD) test.

RESULTS

Influence of DCA on seed germination and seedling vigor

Compared to untreated control, Seeds treated with DCA with different concentration showed significant enhancement of seed germination and vigor index to varying degrees. Highest percentage of seed germination of 93% and 90% was recorded in 100µM and50µM respectively (Fig.1). Similar tendency was also noticed for vigor index in all DCA treated seeds. Maximum seedling vigor of 1480 was recorded in 100µM followed by 1398 in 50µM treated seeds. The untreated control recorded 85% germination and 983 seedling vigor.

Anti-oomycete activity of DCA

Pearl Millet infected leaf disc when treated with different concentrations of DCA exhibited anti-sporulant activity compare to distilled water treated leaf disc (control). Leaf disc when treated with 100µM showed complete inhibition of sporulation followed by 50µM of DCA which showed partial inhibition when compare to control (data not shown). There was a gradual decline in sporulation on the treated leaf discs.

Effect of different concentrations of DCA on potential to activate resistance against downy mildew under greenhouse conditions

Effect of seed treatment on pearl millet downy mildew disease under greenhouse conditions

Seed treatment with DCA at different concentration protected pearl millet plants against downy mildew disease when compared to the untreated control. Overall pictorial assessment of treatments indicated obvious difference in the disease incidence when compared with the control. Highest downy mildew incidence was observed in control with 89.5% followed by 55.2% incidence in 10µM treatment. Among treated plants, least disease incidence was recorded in Metalaxyl with 8.6% and 32.1% in 100µM treated seeds compare to control (Fig. 3). However highest disease protection was recorded in Metalaxyl treated seeds with 90.3% protection. Among treated seeds, disease protection of 64.1, 59.4% was recorded in 100µM and 50µM compare to control sets respectively.

Table.1. Effect of seed treatment on growth parameters of pearl millet plants under greenhouse conditions.

Concentration (µM)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf surface area (cm^2)	No. of tillers/plant
10	29.1±0.2^{d}	12.8±0.1^{b}	2.9±0.1^{c}	31.9±0.3^{d}	2.6±0.3^{b}
20	33.4±0.2^{c}	13.2±0.3^{b}	3.4±0.05^{b}	36.2±0.2^{c}	3.3±0.3^{ab}
50	37.2±0.4^{b}	14.7±0.2^{a}	3.7±0.05^{b}	38.9±0.2^{b}	3.6±0.3^{ab}
100	39.5±0.2^{a}	15.6±0.1^{a}	4.1±0.05^{a}	40.6±0.05^{a}	4.2±0.3^{a}
Control	23.6±0.4^{e}	8.5±0.1^{c}	2.2±0.05^{d}	26.3±0.3^{e}	3.0±0^{b}
Values are means of four independent replicates. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD test (P = 0.05).					

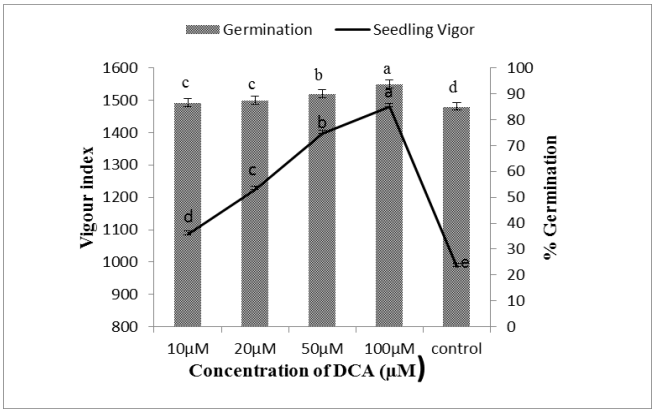

Fig.1. Influence of DCA seed treatment on Germination and Seedling vigor. Values are means of four independent replicates. Bars represent standard errors. Means followed by different superscripts in the columns are significantly different according to Tukey's HSD test (P = 0.05).

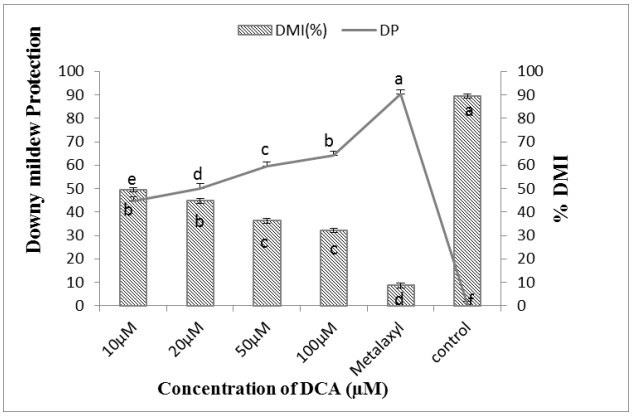

Fig.2. Efficacy of seed treatment with different concentration of DCA on Percentage of downy mildew incidence (DMI) and downy mildew disease protection (DMP) under greenhouse conditions. Values are means of four independent replicates. Bars represent standard errors. Means followed by different superscripts in the columns are significantly different according to Tukey's HSD test (P = 0.05).

Influence DCA on growth parameters of pearl millet under greenhouse conditions

Seed treatment with DCA not only resulted in increased disease resistance against *S. graminicola*, but also considerably enhanced the vegetative growth parameters upon inducer treatment under greenhouse conditions. Among all the concentrations, the maximum increase was recorded in 100µM treatment with all growth parameter like plant height was increased by mean up to 39.5 cm, shoot fresh weight and dry weight were also increased with 15.6 and 4.1gm, leaf surface area of 40.6cm^2 and the number of tillers in control was three whereas 100µM DCA treated plants increased the mean to 4.6 (Table.1). On overall assessment of growth parameter remained found to be reliant on concentration with increase in concentration of the treatment.

Restorative activity of DCA against pearl millet downy mildew

Inducer (with different concentrations) when applied to abaxial surface of leaves showed significant decline in disease reaction when compare to distilled water smeared plants (Fig.4). 100µM DCA applied plants exhibited disease incidence of 33.9% followed by 50µM with 45.4%. Metalaxyl smeared plants showed disease frequency of 6.8% and highest disease incidence of 92.7% was noticed in distilled water treated plants respectively.

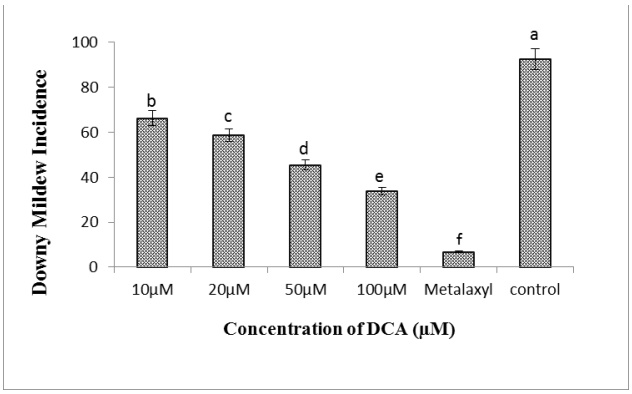

Fig.3. Translaminar activity of DCA in infected pearl millet leaves as indicated by Downy mildew disease Incidence (DMI). Values are means of four independent replications. Bars represent standard errors. Means with different superscripts are significantly different according to Tukey's HSD test (P = 0.05).

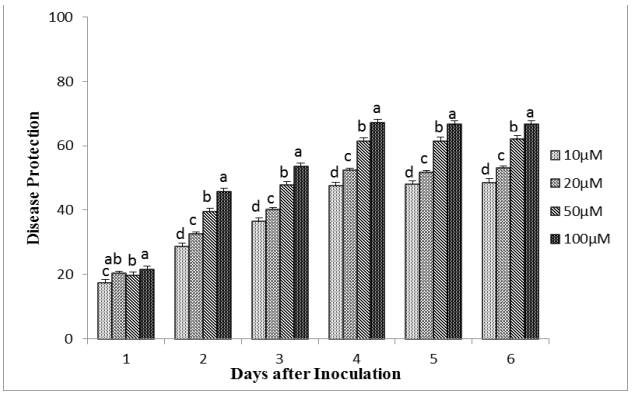

Fig.4. Influence of Seed treatments on Spatio-temporal effect during induction of resistance. Values are means of four replicates. Bars represent standard errors. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD test (P = 0.05).

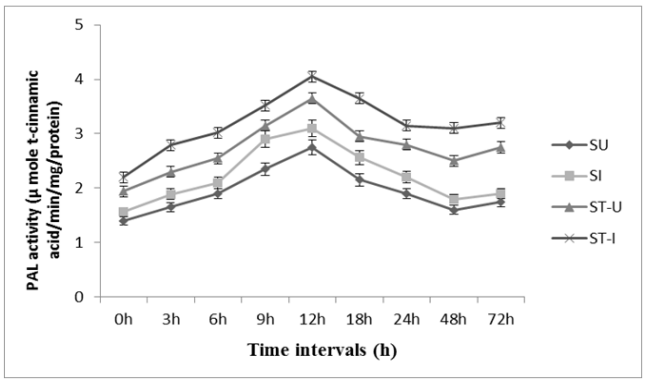

Fig.5. Induction of Phenylalanine ammonia-lyase activity in pearl millet seedlings treated with different concentrations of DCA on inoculation with *Sclerospora graminicola*. STU-susceptible treated uninoculated, STI – susceptible treated inoculated, SU - susceptible uninoculated, SI – susceptible inoculated. Bars indicate standard errors [Tukey's HSD test (P = 0.05)].

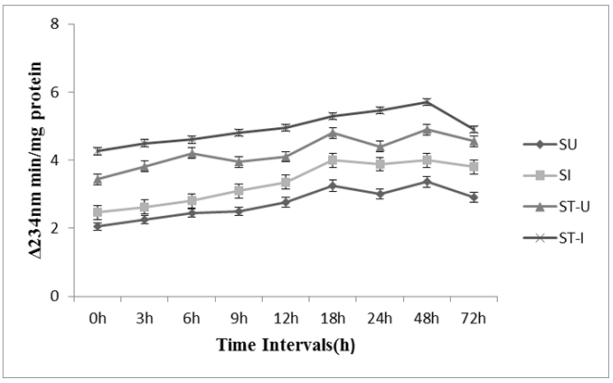

Fig.6. Estimation of Lipoxygenase activity at different time intervals after inoculation. STU – susceptible treated uninoculated, STI – susceptible treated inoculated, SU - susceptible uninoculated, SI – susceptible inoculated. Bars indicates standard errors [Tukey's HSD test (P = 0.05)].

Effect of DCA on induced resistance by Time gap studies in pearl millet

Spatio-temporal studies were assessed for demonstrating the nature of resistance induction by using different concentrations of DCA. Seed treatment with 100µM showed the maximum increase in disease resistance from 21 to 67% on four days after inoculation (Fig.5). Seedlings inoculated on the fourth day established maximum disease protection against downy mildew pathogen. The disease protection was found to be stable at fifth and sixth day and induction of resistance was maintained at the same levels. Seed treatment and seedling inoculation with the downy mildew pathogen were studied for system nature of disease resistance at different time intervals (time gas of 1-6days) respectively.

Influence of DCA seed treatment on enzyme activity

Time course study of PAL activity

Estimation of PAL activity in DCA treated and untreated control seedlings at different time intervals recorded consecutive increase in activity. PAL activity was determined by measuring the conversion of L-phenylalanine to t-cinnamic acid in enzyme assays. The PAL activity increased gradually from 2 hpi and reached up to 72 hpi. In treated seedlings, the maximum activity was observed at 12 hpi and there was a substantial decrease in activity at 24 hpi. The maximum activity of 4.05 U was observed at 12 hpi in induced treated which was 1.9-fold higher than untreated control followed by 3.65 U at 18 hpi. The results revealed that, there is an increase in enzyme activity in DCA treated seedlings upon inoculation showed significantly increase when compared to control inoculated seedlings.

Time course study of LOX activity

LOX activity was more in DCA treated pearl millet seedlings than in untreated control. Seedlings upon challenge inoculation showed a steady increase in enzyme activity from early hours and reached a maximum at 48 hpi compared to the un-inoculated control. In induced treated, 5.7 U of maximum activity were recorded at 48 hpi followed by 5.4 U at 24 hpi. The enzyme activity increased by 2.1-fold at 48 h after inoculation compare to control. In uninoculated seedlings the enzyme activity declined at all the intervals when compared to inoculated seedlings.

DISCUSSION

The sever susceptibility of pearl millet to downy mildew oveing tough challenge to the scientific community aiming towards designing rapid and cheaper management strategy to control disease and yield loss. Number of synthetic and natural chemicals had been reported to enhance host resistance against pathogen infection systemically (Hong *et al*., 1999). Our findings suggest the possible use of DCA a synthetic abiotic inducer to protect pearl millet against the downy mildew disease. In pearl millet downy mildew system certain abiotic elicitors have already been reported like Benzothiadiazole, $CaCl_2$ and H_2O_2

(Geetha and Shetty, 2002), β-aminobutyric acid (Shailashree *et al.,* 2001), Chitosan (Manjunath *et al.,* 2008), Proline (Raj *et al.,* 2004), Cerebroside (Deepak *et al.,* 2003), Elexa (Sharathchandra *et al.,* 2004) and L-methionine (Sarosh *et al.,* 2005).

Seed treatments with DCA enhanced seed germination and seedling vigor of pearl millet under laboratory conditions. None of the DCA concentration caused any phytotoxicity in pearl millet. The gradual decrease in sporulation was observed with application of the different concentrations of DCA solution as compared to control by leaf disc assay (Deepak *et al.,* 2005). Sporulation decreases as the DCA concentration increases. During greenhouse experiments it was observed that, the seed treatment with different concentrations of DCA reduced the disease incidence and offered highest level of disease protection. The systemic and durable nature of resistance was demonstrated by the spatio- temporal separation of the inducer against pathogen inoculation. Studies concluded that a minimum of 4-day time gap prior to pathogen inoculation was necessary for treatment to elicit maximum protection against downy mildew disease. The seeds treated with DCA also enhanced vegetative growth of pearl millet. Seedlings sprayed with DCA also exhibited high level of restorative activity with decline in disease incidence which revealed significant disease protection. The effectiveness of Cyazofamid was reported by earlier workers for their preventive and sporulation inhibitory activity and also for their curative activity against downy mildew in pearl millet (Sudisha *et al.,* 2007).

Induction of resistance has been accessed through signaling pathways using biochemical indicators in the form of induction of defense related enzymes that are activated upon pathogen infection. With the evidence of biochemical studies, we account the involvement of PAL and LOX during the pearl millet and DM disease interaction. Phenylalanine is a critical precursor of a cascade of defense reactions leading to ISR (Dempsey *et al.,* 1999) and is also reported as molecular signals during recognition of pathogen by the host (Lynn and Chang, 1990) the main role of PAL activity in phenylpropanoid metabolism is by converting L-phenylalanine to trans-cinnamic acid (Hahlbrock and sched, 1989). Increased expression of PAL was observed in all time intervals and significant increase was observed after the inoculation. Comparatively highest PAL as recorded in induced resistant seedling at 12 hai. The maximum activity of 4.05 U was observed in treated inoculated seedlings, which was 2-fold higher than control. Lipoxygenase are dioxygenases that catalyze the hydroperoxidation of polyunsaturated fatty acids or their esters that contain a cis, cis-1, 4-pentadiene moiety. In higher plants, the natural substrates for these enzymes are linolenic and linoleic acids (Siedow, 1991; Conconi *et al.,* 1996). Many studies have shown increased LOX activity in plant tissues and cells in response to plant pathogens (Ohta *et al*., 1991). In corresponds to our study, the Lox activity increased with increase in time with a peak at 48 hours after inoculation. However, the increase in Lox activity was significantly higher in induced resistant seedlings with 5.7 U, which was 2.1 fold higher from that of the untreated control seedlings. Lipoxygenase activity has been reported in tomato leaves (*Lycopersicon esculentum* Mill.) for the induction of resistance in response to plant pathogen *Pseudomonas* (Koch *et al*., 1992). Chemically induced LOX (WCI- 2) gene expression correlated with the onset of resistance against *Erysiphe graminis* f.sp. *tritici* in wheat (Gorlach *et al*., 1996). Induction of LOX activity and resistance of the plant has been shown in tobacco infected with *Erysiphe cichoracearum* (Lupu *et al*., 1980) and also induction of resistance against *Magnaporthe grisea* in rice (Ohta *et al*., 1991).

DCA, which acts as plant defense activator that triggers a defined aspect of the plant defense network. Screens for proteins directly targeted by DCA or operating downstream from DCA perception to reveal new components of the plant immune response was reported by earlier workers (Knoth *et al.,* 2009). The defining features of DCA-type elicitors are the presence of the 3- and 5-position chlorines and an amino group at position 2. There are no much reports on application of DCA for defense induction against. DCA induced resistance to two phytopathogens *H. parasitica and Pseudomonas syringae* in *Arabidopsis thaliana.* In microarray analyses, the induction of resistance by DCA and role of NPR1 in were estimated after 48 h of DCA treatment with npr1 mutant. Of the 137 DCA-inducible ACID genes, 20% exhibited NPR1-independent transcriptional up-regulation (Knoth *et al.,* 2009). DCA and INA turned out to be more complex, because their efficiencies in inducing defense activation. Both INA and DCA can formally be considered as two representatives of a variety of related defense-inducing molecules. The defense

activation is completely obstructed in npr1 which discriminates DCA from SA, INA, and BTH (Lawton *et al.,* 1996; Lipinski *et al.,* 1997; Knoth *et al.,* 2007). GO and microarray analyses reveals the genes which are upregulated by DCA treatment and also genes associated with defense response. ACID cluster as a set of genes strictly associated with defense activation. Cluster contains many known defense-related genes and is highly enriched for genes associated with calmodulin binding and kinase activity. Among the upregulated ACID genes are six genes encoding WRKY transcription factors, which have been associated with plant immune responses (Eulgem and Somssich, 2007).

CONCLUSION

Chemicals that transiently activate plant immunity may be beneficial in combating virulent pathogens that threaten crops only during a limited period of time. A transiently active compound like DCA may allow fine-tuned control of defense induction coordinated with the plant's needs, thereby decreasing unwanted side effects caused by long-term defense activation. The present study revealed the efficacy of synthetic elicitor DCA was tested against *S. graminicola* in pearl millet and the evaluation of potentials to imply this compound into the disease management programs.

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