



Original Research Article

DOI: 10.26479/2018.0405.51

ABIOTIC ELICITORS MEDIATED INDUCTION OF RESISTANCE IN PEARL MILLET AGAINST BLAST DISEASE

G L Basavaraj¹, S N Lavanya², K N Amruthesh^{1*}

1. Applied plant pathology laboratory, Department of Studies in Botany,
University of Mysore, Karnataka, India.

2. Department of Studies in Biotechnology, University of Mysore, Karnataka, India.

ABSTRACT: The initiation of defensive responses in plants is a promising retort for controlling pests in conventional agriculture. Some abiotic elicitors like Salicylic acid(SA), β -aminobutyric acid (BABA), and Methyl Jasmonate (MeJ) have been assessed for their ability to advance development and induce resistance against blast disease of Pearl millet caused by *Magnaporthe grisea*. Seeds treated with 50mM BABA exhibited germination of 90.67% and seedling vigor of 1690 followed by seeds treated with 0.50mM SA with germination of 83.61% and seedling vigor of 1470 compared to control with germination of 68.3% and seedling vigor of 843.7 respectively. Seeds treated with BABA showed improved vegetative growth parameters when contrasted with other elicitors. Under greenhouse conditions, the BABA revealed the utmost disease protection of 61.3% against blast disease followed by SA with 52.4% of protection when compared to untreated seedlings. It was also found that the activity of the defense- related enzymes like Phenylalanine ammonia lyase (PAL), Peroxidase (POX) and β -1,3-glucanase were increased significantly in resistant and inoculated seedlings after challenged with the pathogen.

KEYWORDS: BABA, Elicitors, Induced Resistance, Pearl millet, Blast disease.

Corresponding Author: Dr. K N Amruthesh* Ph.D.

Applied plant pathology laboratory, Department of Studies in Botany,
University of Mysore, Karnataka, India. Email Address: dr.knamruthesh@botany.uni-mysore.ac.in

1. INTRODUCTION

Currently, disease control is to a great extent based on the use of fungicides, bactericides, insecticides and chemical compounds detrimental to plant invaders, causative agents or vectors of

plant diseases. In any case, the precarious impact of these chemicals or their degradation products on the environment and human wellbeing emphatically requires the scan for new, innocuous methods for disease control. Since the late expanding assortment of evidence on the natural phenomenon of induced resistance has been accumulated, culminating in its successful practical application in the recent decade[1]. A large number of components are accounted for to incite obstruction in plants pathogens causing easily affected necrotic response (HR), avirulent and constricted pathogenic strains, pests elicitors of biotic origin, abiotic elicitors, and chemical products for example, benzothiadiazole (BTH), β -aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), salicylic acid, and some inorganic salts [2][3]. The cell wall is the actual site where plants and pathogens attempt to prevail by contrasting wall-reinforcing and wall-degrading strategies. When pathogens start weakening the plant cell wall components, plants are capable of sensing the loss of wall integrity and subsequently activate the defense signaling pathways. Pathogens try to escape the plant defenses and sometimes take advantage of the host cell wall metabolism to facilitate their entry into the tissue [4]. When a plant acknowledges an infective agent, the structural defense mechanism is instantly activated and a progression of cell wall modifications like formation of papillae, callose deposition, hydrogen peroxide generation (H_2O_2), within the middle of the cell membrane and also the cellwall in order that pathogens faces a stiff physical obstruction that is tough to attack. These changes occur rapidly and in high intensity in resistant cultivars compared to the susceptible cultivars. These structures prevents the haustoria formation and availability of nourishment to the pathogen [5]. Different elicitors are known to act by reinforcing the cell wall by upgrading the synthesis and accumulation of compounds like phenolics, flavonoids, hydrogen peroxide, callose and other cell wall cross-linking polymers [6]. Elicitor applications lead to resistance reactions including; the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), production of phytoalexins, induction of hypersensitive reaction (HR), callose deposition, lignin accumulation and expression of resistance related genes [7][8]. PAL is the first important enzyme in the phenyl propanoid metabolism and plays a major role in the regulation of biosynthesis of phenols in plants and catalyzes the conversion of phenylalanine to trans-cinnamic acid, which provides the precursors for flavonoid pigments, lignin and phytoalexins associated with the disease resistance, which can measure the ability of plant disease resistance and play an important role in plant disease resistance responses. POX is involved in the formation of cell wall strengthening agents like lignin and suberin, they are required for cell wall cross-linking of HRGPs, they regulate the metabolism of active oxygen species and active nitrogen species, and more importantly they are essential for HR [9]. β -1,3-glucanases are hydrolyzing enzymes which act on β -1,3-glucans of pathogen cell wall. Glucanases can degrade cell walls of pathogens and thereby control their spread; these degraded fragments can further act as elicitors which trigger defense responses in the host [10]. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the earliest

sustenance known to people and has been staple nourishment from many years in numerous parts of the world. Globally, a greater part of the pearl millet production is devoured as a staple food (95%) and, the rest of the pearl millet production (5%) is utilized as animal feed or fodder [11]. Pearl millet is affluent in essential nutrients and generally is being advanced as a health crop with nutraceutical benefits. In recent years Pearl millet is assaulted by blast disease caused by *M.grisea* (Herbert) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc.) reported from all the pearl millet growing regions of the world attacking distinctive aerial parts of the plant at all phases of its development beginning from the seedling stage (causing lesions and untimely drying of young leaves) to the panicle causing huge economic loss to the pearl millet growing farmers [12]. The blast disease of pearl millet is currently dealing with essentially through chemical fungicides which have their own particular restrictions, so present examination was planned to assess the viability of some abiotic elicitors to induce disease resistance against *M.grisea*, the causal agent of blast disease of pearl millet.

2. MATERIALS AND METHODS

2.1 Seed sample

Seeds of pearl millet susceptible cultivars (ICMB95444) and resistant cultivars (ICMR06222) to blast disease were obtained from ICRISAT, Hyderabad, India and AICPMIP Mysore centre, Mysuru, Karnataka, India.

2.2. Screening, isolation and identification of *M.grisea*

The collected pearl millet leaf samples were surface sterilized with 0.02% sodium hypochlorite followed by subsequent rinsing with sterile distilled water (SDW). The leaves with disease symptoms (lesions) were cut into small pieces of 1-2 sq.cm size followed by standard blotter method [13] and were kept for incubation at 25±2°C for 48 hours. After incubation, the leaf bits were examined under a stereo microscope and the fungal specks of *M.grisea* were chosen carefully with the help of sterile inoculation needle and relocated on to oat meal agar (OMA) media under aseptic conditions and incubated at 25±2°C for 7 days. At the end of the incubation phase, the fungi were further known based on the morphological, conidial, and cultural characteristics [14].

2.3 Preparation of inoculum

M.grisea was grown on OMA medium and the conidia from freshly sporulating cultures of *M.grisea* were scrapped from the surface with the help of a sterile spatula and inoculum was prepared by mixing required amount of SDW so as to set the concentration of suspension at 1×10⁵conidia mL⁻¹ using Haemocytometer and were used as a standard inoculum throughout the study.

2.4 Preparation of elicitors for seed treatment

Chemicals like BABA, SA, and MeJ are procured from Sigma-Aldrich(USA) and elicitors of different concentrations like BABA-25 mM, 50 mM, 75 mM, 100 mM, SA-0.25 mM, 0.50 mM, 0.75mM,1.0 mM and MeJ-0.02 µM,0.04 µM,0.06 µM,0.08 µM were prepared by using SDW.

2.5 Effect of seed treatment with abiotic elicitors on seed germination and seedling vigor of Pearl millet

The seeds of pearl millet after sterilization treated with abiotic elicitors by placing 400 seeds in 50 mL of elicitor in a rotary shaker at 25°C for 3h and 6h, respectively. The seeds treated with SDW were kept as control. After treatment the seeds were air-dried aseptically and were placed equidistantly on three layers of moistened blotter discs placed on Petri plates to evaluate percent seed germination [15] and another set of treated seeds were subjected to paper towel method to record seedling vigor [16]. The experiment consisted of four replicates of 100 seeds each and repeated thrice. After 7 days of incubation, percent germination, root length and shoot length were recorded and vigor index was calculated.

2.6 Effect of seed treatment with abiotic elicitors on blast disease protection of pearl millet under greenhouse conditions

The pearl millet treated and control seeds were sown to earthen pots containing soil, sand and manure (2:1:1). Seeds treated with the Tricyclazole (6g/kg) served as a positive control. Fourteen day old seedlings were challenged with the pathogen as described earlier. Inoculated and uninoculated plants were maintained under greenhouse conditions (20-26°C temperature with 90-95% RH) and observed regularly for the development of blast symptoms like specks on leaves and gradually enlarge to form a spindle-shaped spots or lesions with greyish necrotic centres. Percentage of blast disease incidence was recorded at 30 DAS and final counts were made at 60 DAS. Each treatment consisted of 10 pots with 10 seedlings per pot and the experiment was repeated four times.

2.7 Evaluation of seed treatment with abiotic elicitors on vegetative growth parameters of pearl millet under greenhouse conditions

Pearl millet seeds treated with different abiotic elicitors for 6 h of time duration sown to earthen pots filled with autoclaved soil, sand and manure (in the ratio of 2:1:1). The experiment consisted of 10 pots, with 10 seedlings per pot and the experiment was repeated four times. Seeds treated with SDW served as control. Plants were maintained under greenhouse conditions (20-26°C temperature with 90-95% RH) and observed regularly. After 60 days of sowing, plant height, number of productive tillers, length of earheads, girth of earhead and weight of 1000 seeds were recorded accordingly.

Biochemical studies

2.8 Sampling of pearl millet seedlings for biochemical studies

Susceptible treated, untreated and resistant seeds were subjected to paper blotter method and incubated at 26°C for 14 days. After incubation seedlings were carefully removed and inoculated with the conidial suspension of *M.grisea* (1×10^5 conidia/ml). The seedlings were harvested at different time intervals of 0, 3, 6, 9, 12, 24, 48 and 72 h post inoculation (hpi) were immediately stored at -80°C for further biochemical studies. Protein content in the crude extracts was estimated

by Dye binding method [17] using BSA (Bovine serum albumin) (Sigma) as a standard.

2.9 Phenylalanine ammonia-lyase assay (PAL, EC. 4.1.3.5)

PAL activity was assayed according to the procedure Beaudoin-Eagan *et al* [18]. One gram fresh weight of seedlings was weighed from harvested seedlings and was ground to a fine powder in liquid nitrogen and by homogenizing with 25mM Tris-HCl buffer pH 8.8 (1ml/g seedlings). One hundred μ l of extracts were mixed with the reaction mixture of 900 μ l of 50mM L-phenylalanine and 100mM Tris HCl buffer solution. The mixture was placed in a water bath for 120min at 40°C. The reaction was stopped by adding 60 μ l of 5N HCl. PAL enzyme activity was measured spectrophotometrically at 290nm and expressed as the amount of t-cinnamic acid formed from L-phenylalanine per mg of protein per min.

2.10 Peroxidase assay (POX, EC.1.11.1.7)

POX activity was assayed according to the procedure Hammerschmidt *et al* [19]. Enzyme was extracted by homogenizing 1g of seedlings in 10 mM potassium phosphate buffer (pH 6.9) in pre-chilled mortar and pestle. The reaction mixture (3 ml) consisted of 0.25% v/v guaiacol in 10 mM potassium phosphate buffer (pH 6.0) containing 100 mM hydrogen peroxidase. The crude enzyme (10 μ l) was added to initiate the reaction, which was measured spectrophotometrically at 470 nm. POX activity is expressed in terms of the change in absorbance at 470 $\text{min}^{-1} \text{mg}^{-1}$ protein.

2.11 β -1,3-glucanase assay (GLU, EC. 3.2.1.6)

β -1,3-glucanase activity was assayed according to the method of Pan *et al* [20] with glucose as standard. β -1,3-glucanase enzyme was extracted by homogenizing 1g of seedlings in 50 mM sodium acetate buffer (pH 5.2). Laminarin 0.1% (Sigma) in 0.05 M sodium acetate buffer (pH 5.2) was used as the substrate and 50 μ l of enzyme extract and incubated for 15 min at 37° C. The reaction was stopped by adding 0.5 ml of DNS reagent, incubated in boiling water bath for 10 min and cooled. Finally 1ml of distilled water was added and measured spectrophotometrically at 540 nm. Enzyme activity was expressed in terms of μ moles per mg per min.

2.12 Statistical analysis

The data from all the above experiments were analyzed separately for each experiment and were subjected to arcsine transformation and analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by F values ($P \leq 0.05$). Treatment means were separated by Tukey's Honestly Significant Differences (HSD) test.

3. RESULTS AND DISCUSSION

3.1 Effect of abiotic elicitors on seed germination and seedling vigor of pearl millet

Pearl millet seeds which are susceptible to blast disease were treated with abiotic elicitors at different time intervals were analyzed for their effect on seed germination and vigor index. Among all the treatments, BABA at 50mM concentration for 6h of treatment offered maximum seed germination of 90.67% and seedling vigor of 1690 followed by SA (0.50mM) offered 83.61% of seed

germination and 1470.7 seedling vigor (Table 1) at the same time interval, whereas MeJ (0.04 μ M) showed seed germination of 80.33% and seedling vigor of 1261.6. The control seedlings offered 68.3% and 843.7 of seed germination and seedling vigor respectively. It was also evident that, abiotic elicitor treatments offered a similar trend of increase in per cent seed germination and seedling vigor. Elicitors at 6h of treatment with ideal concentration which exhibit a maximum percentage of seed germination and seedling vigor were taken for further studies.

Table 1: Effect of abiotic elicitor treatments for 3h and 6h on seed germination and seedling vigor of Pearl millet.

Sl.no.	Elicitor	Concentration	3h		6h	
			Germination	Vigor	Germination	Vigor
1	SA	0.25mM	79.0 \pm 1.73 ^{bcd}	1123.0 \pm 7.8 ^c	81.00 \pm 1.15 ^b	1230.0 \pm 7.6 ^d
2		0.50mM	81.0 \pm 1.73 ^{bcd}	1236.7 \pm 11.6 ^d	83.61 \pm 1.20 ^{bc}	1470.7 \pm 15.7 ^f
3		0.75mM	78.7 \pm 1.20 ^{bcd}	1124.0 \pm 6.2 ^c	80.00 \pm 1.58 ^b	1347.3 \pm 4.6 ^e
4		1.0mM	77.3 \pm 1.45 ^{bcd}	1136.3 \pm 26.9 ^c	78.33 \pm 0.88 ^b	1350.3 \pm 18.6 ^e
5	BABA	25mM	82.3 \pm 0.88 ^{cde}	1303.3 \pm 31.7 ^{dc}	84.33 \pm 2.02 ^{bc}	1666.3 \pm 12.0 ^g
6		50mM	86.7 \pm 1.45 ^e	1658.3 \pm 20.1 ^f	90.67 \pm 0.88 ^c	1690.0 \pm 5.7 ^g
7		75mM	83.7 \pm 0.88 ^{de}	1342.0 \pm 16.2 ^e	83.33 \pm 1.85 ^{bc}	1380.3 \pm 7.5 ^e
8		100mM	81.67 \pm 1.76 ^{cd}	1259.0 \pm 14 ^d	82.7 \pm 1.20 ^{bc}	1277.0 \pm 25.9 ^{dc}
9	MeJ	0.02 μ M	73.3 \pm 1.85 ^{ab}	1005.0 \pm 14.0 ^b	78.67 \pm 0.88 ^b	1103.3 \pm 3.5 ^b
10		0.04 μ M	76.7 \pm 1.76 ^{bcd}	1127.3 \pm 13.3 ^c	80.33 \pm 1.76 ^b	1261.6 \pm 18.0 ^{dc}
11		0.06 μ M	74.7 \pm 1.76 ^{abc}	1028.6 \pm 14.4 ^b	78.67 \pm 0.88 ^b	1129.3 \pm 10.3 ^{bc}
12		0.08 μ M	69.33 \pm 2.02 ^a	802.3 \pm 11.0 ^a	74.0 \pm 2.08 ^a	1100.3 \pm 13.0 ^b
13	Control		67.3 \pm 1.45 ^a	807.3 \pm 7.4 ^a	68.33 \pm 1.45 ^a	843.7 \pm 15.6 ^a

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 ($P \leq 0.05$).

3.2 Evaluation of abiotic elicitors for blast disease protection under greenhouse condition

Seeds treated with different abiotic elicitors induced blast disease resistance in Pearl millet when compared to untreated sets (Fig.1). Overall possible estimation at 60 DAS indicated the noticeable differences in disease protection among the treatments. Seeds treated with BABA offered maximum of 61.3% protection followed by SA which offered 52.4% protection, whereas MeJ recorded least protection with 41.8% while 99.2% disease incidence was observed in control plants.

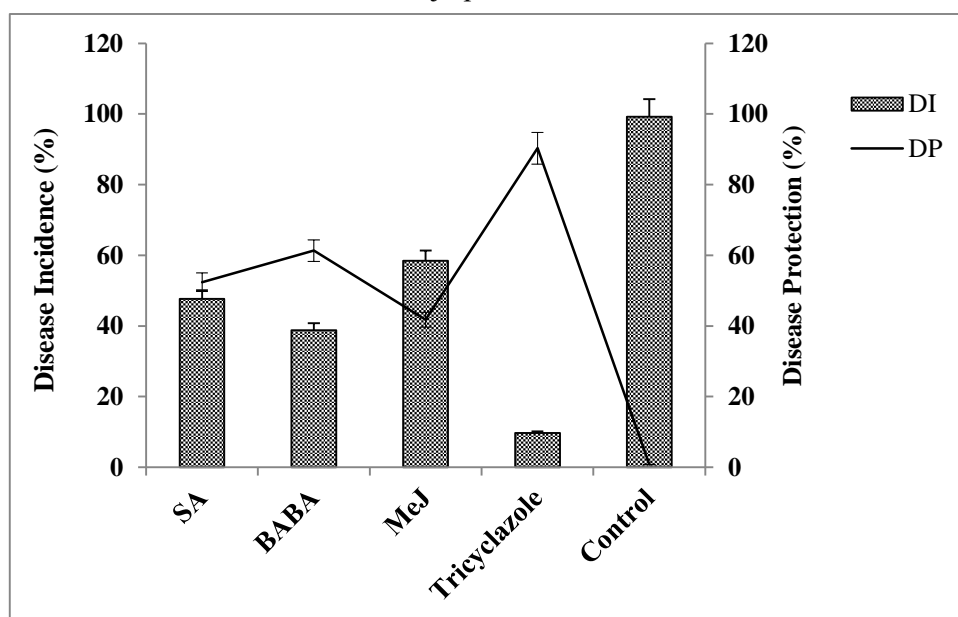


Fig 1: Effect of abiotic elicitors on disease protection and disease incidence under greenhouse conditions.

3.3 Evaluation of abiotic elicitors for vegetative growth parameters under greenhouse conditions

Seeds treated with different abiotic elicitors were also resulted in increased vegetative growth parameters along with disease protection. The effect of abiotic elicitor treatments on vegetative growth parameters was evaluated at the end of 60 DAS. The results offered significant difference among the abiotic elicitor treatments (Table 2). Among all the elicitors tested, BABA treatments showed maximum plant height with 117.5cm, days required for 50% flowering with 40.7days, number of reproductive tillers per plant was 3.1, length of earhead (11.9cm), girth of earhead (4.4cm) and weight of thousand seeds (11.4gm) followed by SA and MeJ. About 1.16 to 1.55 fold increase in plant growth parameters were observed in BABA at treated plants compared to control. Based on the above, BABA at 50mM concentration 6h of treatments which recorded ideal vegetative growth and offered best disease protection was taken for further biochemical studies.

Table 2: Effect of abiotic elicitor on vegetative growth parameters

Abiotic elicitor	Height of plants (cm)	Days required for 50% Flowering	No. of reproductive tillers	Length of earhead(cm)	Girth of earhead (cm)	1000 seed weight (g)
SA (0.50mM)	97.1 ±1.1 ^c	42.0 ±0.58 ^{ab}	2.4 ±0.21 ^a	10.8 ±0.15 ^b	4.1 ±0.04 ^{bc}	10.4 ±0.21 ^a
BABA (50mM)	117.5 ±2.3 ^d	40.7 ±0.33 ^a	3.1 ±0.09 ^b	11.9 ±0.26 ^c	4.4 ±0.11 ^c	11.4 ±0.27 ^b
MeJ (0.04µM)	85.7 ±1.6 ^b	42.7 ±0.33 ^b	2.1 ±0.15 ^a	9.9 ±0.09 ^a	3.9 ±0.04 ^{ab}	10.2 ±0.09 ^a
Control	75.8 ±1.5 ^a	43.7 ±0.33 ^b	1.7 ±0.21 ^a	9.3 ±0.25 ^a	3.5 ±0.09 ^a	9.8 ±0.06 ^a

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 ($P \leq 0.05$).

3.4 Biochemical studies

3.4.1 Estimation of Phenylalanine ammonia lyase (PAL) activity

The activity of PAL was significantly increased in resistant and susceptible BABA treated seedlings along with control seedlings after inoculation, compared to uninoculated sets. Resistant seedlings offered maximum PAL activity at 6 hai with 35.3U (Fig 2a) followed by BABA treated seedlings at same time interval with 33.2U when compared to control with 4.8U. There was a threefold increase in PAL activity was noticed in resistant and BABA treated inoculated seedlings over the inoculated control seedlings.

3.4.2 Estimation of Peroxidase (POX) activity

Peroxidase activity was estimated in both inoculated and uninoculated seedlings raised from resistant, BABA treated and susceptible seeds (Fig 2b). The maximum POX activity of 49U was observed in resistant inoculated pearl millet seedlings at 9 hai followed by BABA treated inoculated seedlings with 47.1U. In control inoculated seedlings the maximum activity recorded was 17.6U at 48 hai and at 72 hai uninoculated control seedlings recorded maximum activity of 5.2U.

3.4.3 Estimation of β -1,3-glucanase activity

The maximum activity of 4.8U has observed in BABA treated inoculated seedlings at 24 hai followed by resistant inoculated seedlings with 4.6U at 24 hai. The control inoculated and uninoculated seedlings recorded with 1.9U and 1.2U at 24 hai respectively. There was 2.4 and 2.5 fold increase in β -1,3-glucanase activity in resistant inoculated and BABA-treated inoculated seedlings over the control respectively.

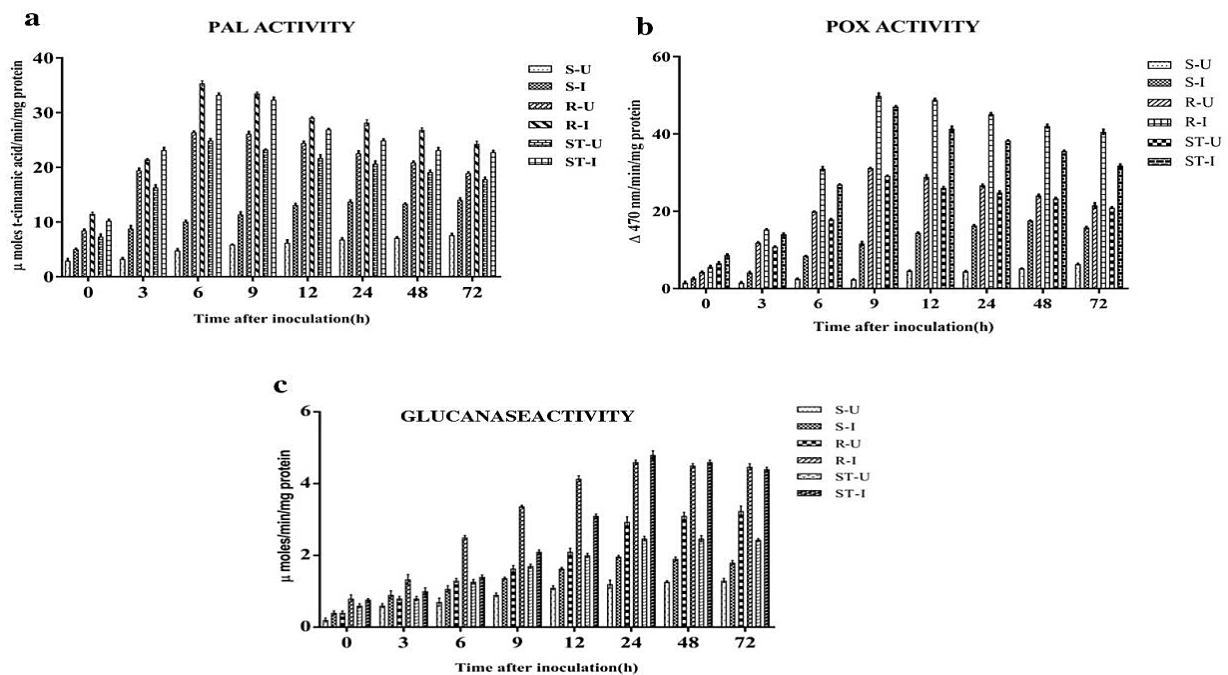


Fig 2: Time course study of (a) PAL (b) POX and (c) β -1, 3-glucanase enzyme activity in pearl millet seedlings upon treatment with BABA. SU Susceptible uninoculated: SI Susceptible inoculated: RU Resistant

uninoculated: RI Resistant inoculated: STU Susceptible treated uninoculated: STI Susceptible treated inoculated. Each value is the mean for three replicates (n=3) and vertical bars indicates the standard error.

Management of plant diseases has become multidimensional in the last few decades. Many approaches have been roped into this area of crop protection owing to undesired effects of chemical pesticides. The present study was aimed at evaluating the efficacy of selected abiotic elicitors in promotion of plant growth and disease resistance against blast disease of pearl millet. Growth promotion and induction of systemic resistance in various crops using different abiotic elicitors like SA, BABA, MeJ, BTH, INA etc., have been reported by various workers [21][22][23]. These abiotic elicitors are known to activate the signaling pathway that depends on SA or through the activation of novel signaling cascade not dependent on SA but on jasmonic acid or ethylene [24]. The seeds of pearl millet were treated with different concentrations of abiotic elicitors for 3h and 6h to study their effect on seed germination and seedling vigor. In the present study, the 6h treatments were more effective than the 3h treatments with maximum germination of 90.67% and seedling vigor of 1690 was observed in pearl millet seeds treated with BABA (50mM) for followed by SA treatment. Similar observations of enhancement of seed germination and seedling vigor were noticed in pearl millet seeds treated with BABA, BTH and SA from previous reports [25]. These abiotic elicitors also offered protection to pearl millet against blast disease and maximum protection of 61.3% was observed in BABA (50mM) treated seeds under greenhouse conditions. Our results correlated with previous reports where in Pearl millet seeds treated with BABA, BTH, SA, thiamine and trehalose were evaluated for their efficacy under field and greenhouse conditions [26][27]. The Experiments on vegetative parameters like plant height, days required for 50% flowering, number of reproductive tillers per plant, length of earhead, girth of earhead and weight of 1000 seeds offered better results as compared to the untreated control. These results are corroborated with that of earlier reports [28] in pearl millet. The present study indicated up to 1.5 fold increase in vegetative growth parameters when compared to control seedlings. Our results are also corroborated with similar studies [29] in pearl millet. When a pathogen or an elicitor permeates into the plant system several defense responses will be activated to thwart the infection from the pathogen. Upon pathogen infection several pre-existing physical barriers and inducible defense responses in the form of induction of defense related enzymes will be activated. Many defense related enzymes are the result of interaction between the host and the pathogen, which in turn alters the cell metabolism. A similar increase in the activity of defense related enzymes like PAL, POX and β -1,3-glucanase were noticed when 3,5-Dichloroanthranilic acid were used as elicitors in pearl millet against downy mildew disease [30]. In the present study, the PAL activity was increased up to 6 hai in BABA treated inoculated seedlings and resistant inoculated seedlings. The maximum activity of PAL was noted in resistant inoculated seedlings with 35.3U followed by BABA treated seedlings with 33.2U which was 4.65 fold increase than the control. The results are similar to the earlier reports [31][32]. Induced

resistance in hydroponic tomato against *Ralstonia solanacearum* wilt triggered by different elicitors like chitosan, salicylic acid and jasmonic acid was associated with increased phenolic content, enhanced activities of defense enzymes including PAL and the increased lignification in comparison to the control plants [33]. Chitosan treated grapevine showed elicitation of PAL activities [34]. The peroxidase (POX) activity was maximum in resistant seedlings with 49.9U whereas in BABA treated seedlings show activity of 47.1U. It was 2.7 fold increases than the control. Significantly increased POX, PAL and catalase were recorded in rice plants which were treated with the elicitor algino-oligosaccharides which led to the development of resistance to *M.grisea* [35]. Methyl jasmonate (MeJ) treatment significantly enhanced the resistance against *M. oryzae* in both cultivars but the treated resistant cultivar maintained a higher level of resistance than the same treated susceptible [36]. The activity of β -1,3-glucanase was maximum in BABA treated inoculated seedlings with 4.8U after 24 hai which was 3.7 fold increase than the control, whereas in susceptible inoculated seedlings maximum activity was 1.96U. Induction of resistance in cotton against *Verticillium dahliae* toxins was achieved by treating the callus cells with SA which showed increased activities of the enzymes chitinase and β -1,3-glucanase [37]. Acibenzolar-S-methyl (ASM), ethylene (ET) and jasmonic acid (JA) were evaluated for their efficiency to induce resistance against *Pyricularia oryzae* in wheat and it was found that these elicitor treatments elevated the levels of POX, PPO, chitinase and β -1,3-glucanase activities which led to the increased resistance against *Pyricularia oryzae* [38].

4.CONCLUSION

The present study has clearly demonstrated the efficacy of some abiotic elicitors in growth promotion and protection against blast disease of pearl millet, and the results support the application of BABA as an elicitor of systemic resistance in pearl millet against blast disease caused by *M. grisea*.

ACKNOWLEDGEMENT

The first author is grateful to University Grants Commission (UGC), New Delhi, for providing FDP-Teacher fellowship under XII plan and authors are also acknowledge the support provided by ICAR-AICPMIP, Mysore Centre and University of Mysore, Mysuru, for providing necessary facilities for this research work.

CONFLICT OF INTEREST

The authors declare no conflict of interests

REFERENCES

1. Kuc, J. Concepts and Direction of Induced Systemic Resistance in Plants and its Application. Eur J of Plant Patho. 2001: 107: 7-12.
2. Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., et al. Induction of systemic acquired resistance in plants by chemicals. Annu Rev Phytopathol.1994: 32: 439-459.

3. Cohen, Y., Vaknin, M., and Mauch-Mani, B. BABA-induced resistance: milestones along a 55-year journey. *Phytoparasitica*. 2016:44: 513-538
4. Bellincampi, D., Cervone, F., and Lionetti, V. Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. *Front Plant sci*. 2014: 5: 228
5. Underwood, W. The plant cell wall: a dynamic barrier against pathogen invasion. *Front Plant Sci*. 2012: 3: 85
6. Nicholson, R. L., and Hammerschmidt, R. Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol*. 1992:30: 369-389.
7. Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S. E., and Metraux, J. P. Reactive oxygen species and plant resistance to fungal pathogens. *Phytochem*. 2015: 112: 54-62.
8. Matsukawa, M., Shibata, Y., Mizutani, M. A., Mori, H., Wang, P., Ojika, M., et al *Nicotiana benthamiana* Calreticulin 3a is required for the Ethylene-Mediated Production of Phytoalexins and Disease Resistance Against Oomycete Pathogen *Phytophthora infestans*. *Mol plant microbe interact*. 2013: 26: 880-892.
9. Mayer, A. M. Polyphenol oxidases in plants and fungi: going places? A review. *Phytochem*. 2006:67: 2318-2331.
10. Ranieri, A., Nali, G. D., and Urso, G. Peroxidase activity in *Cucurbita pepo* L. leaves exposed to ozone. *Agricoltura Mediterranea, Special Volume*, 1995: 47-54.
11. ICRISAT (International Crops Research Institute for Semi-Arid Tropics). Research for impact: Annual report. Patancheru, Andhra Pradesh, India. 1997
12. Wilson, J. P., and Gates, R. N. Forage yield losses in hybrid pearl millet due to leaf blight caused primarily by *Pyricularia grisea*. *Phytopathology*. 1993:83: 739-743.
13. ISTA. Proceedings of the international Seed Testing Association, International Rules for Seed Testing. *Seed Science and Technology*. 2005:21: 25-30.
14. Mathur, S. B., and Kongsdal, O. Common laboratory seed health testing methods for detecting fungi. *ISTA*. 2003: 89-96.
15. Singh, S. D., and Gopinath, R. A seedling inoculation technique for detecting downy mildew resistance in pearl millet. *Plant Dis*. 1985: 72: 42-58.
16. Abdulbaki, A. A., and Anderson, J. D. Vigor analysis in Soya bean seeds by multiple criteria. *Crop Sci*. 1973:13: 630-633.
17. 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*. 1976:72: 248-254.
18. Beaudoin-Eagan, L. D., and Thorpe, T. A. Tyrosine and Phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiol*. 1985: 78: 438-441.

19. Hammerschmidt, R., Nuckles, E., and Kuc, J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol Plant Path.* 1982;20: 73-82.
20. Pan, S.Q., Ye, X.S., and Kuc, J. A technique for detection of chitinase, β -1,3-glucanase and protein patterns after a single separation using polyacrylamide gel electrophoresis or isoelectro focusing. *Phytopathology* 1991;81: 970-974.
21. Justyna, P. G., and Ewa, K. Induction of resistance against pathogens by β -aminobutyric acid. *Acta Physiol Plant.* 2013: 35: 1735-1748.
22. Thakur, M., and Sohal, B. S. Role of elicitors in inducing resistance in plants against pathogen infection: a review. *ISRN Biochemistry.* 2013;20: 1155-1165.
23. Manikandan, A., and Muthukrishnan., S. Foliar Application of Salicylic Acid Stimulates Flowering and Induce Defense Related Proteins in Finger Millet Plants. *Univ Jnl of Plant Sci.* 2014: 2: 14-18.
24. Zimmerli, L., Jakab, G., Metraux, J. P., and Mauch-Mani, B. Potentiation of pathogen specific defence mechanisms in *Arabidopsis* by beta-aminobutyric acid. *Proceedings of the National Academy of Sciences U.S.A.* 2000: 97: 12920-12925.
25. Geetha, H. M., and Shetty, H. S. Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide. A comparative evaluation. *Crop Prot.* 2002: 21(8): 601-610.
26. Shailasree, S., Sarosh, B. R., Vasanthi, N. S., and Shetty, H. S. Seed treatment with beta aminobutyric acid protects *Pennisetum glaucum* systemically from *Sclerospora graminicola*. *Pest Manage Sci.* 2001;57(8): 721-728.
27. Murali, M., Sudisha, J., Amruthesh, K. N., and Shetty, H. S. Rhizosphere fungus *Penicillium chrysogenum* promotes growth and induces defense-related genes and downy mildew disease resistance in pearl millet. *Plant Biol.* 2013;15, 111–118
28. Pushpalatha, H. G., Mythrashree, S. R., Shetty, R., Geetha, N.P., Sharathchandra, R.G., Amruthesh, K.N., and Shetty, H.S. Ability of vitamins to induce downy mildew disease resistance and growth promotion in pearl millet. *Crop Prote.* 2007: 26: 1674-1681.
29. Sharathchandra, R. G., Niranjan Raj, S., Shetty, N. P., Amruthesh, K. N., and Shetty, H. S. ElexaTM - A chitosan formulation induces growth promotion and downy mildew disease resistance in pearl millet. *Crop Prot.* 2004;23: 881-888.
30. Lavanya, S. N., and Amruthesh, K. N. 3, 5-Dichloroanthranilic acid (DCA) - an elicitor induces systemic resistance against downy mildew in pearl millet. *Int J Life Sci.* 2016;4: 97-106.
31. Sundravadana, S., Alice, D., Kuttalam, S., and Samiyappan, R. Azoxystrobin induces lignification-related enzymes and phenolics in rice (*Oryza sativa* L.) against blast pathogen (*Pyricularia grisea*). *J Plant Interact.* 2007;2(4): 219-224.

32. Shailasree, S., and Melvin, P. β - Amino Butyric Acid - Resistance Inducing Agent in Pearl Millet. *J Plant Biochem and Physiol.* 2015:2: 144.
33. Niranjan-Raj, S., Shetty, N. P., and Shetty, H. S. Proline - an inducer of resistance against pearl millet downy mildew disease caused by *Sclerospora graminicola*. *Phytoparasitica.* 2004: 32(5): 523-527.
34. Trotel-Aziz, P., Couderchet, M., Vernet, G., and Aziz, A. Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. *Eur J of Plant Patho.* 2006: 114: 405-413.
35. Zhanga, S., Tanga, W., Jiangb, L., Houa, Y., Yanga, F., Chenb, W., and Lia, X. Elicitor activity of algino-oligosaccharide and its potential application in protection of rice plant (*Oryza saliva* L.) against *Magnaporthe grisea*. *Biotechnol and Biotech Equip.* 2015:29: 646-652.
36. Li, Y., Nie, Y., Zhang, Z., Ye, Z., Zou, X., Zhang, L., and Wang, Z. Comparative proteomic analysis of methyl jasmonate induced defense responses in different rice cultivars. *Proteomics.* 2014:14: 1088-1101.
37. Li, Y. Z., Zheng, X. H., Tang, H. L., Zhu, J. W., and Yang, J. M. Increase of β -1,3-glucanase and chitinase activities in cotton callus cells treated by salicylic acid and toxin of *Verticillium dahlia*. *Acta Bot Sin.* 2003:45: 802-808.
38. Rios, J. A., Rodrigues, F. A., Debona, D., Resende, R. S., Moreira, W. R., and Andrade, C. C. L. Induction of resistance to *Pyricularia oryzae* in wheat by acibenzolar-S-methyl, ethylene and jasmonic acid. *Trop Plant Pathol.* 2014:39: 224-233.