



RESEARCH ARTICLE

Biochemical basis of chemically induced disease resistance against sugary disease of sorghum caused by *Sphacelia sorghi*

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Abstract

Sorghum, a multipurpose food crop and one of the top five grain crops globally, is threatened by sugary disease (ergot), which causes significant yield losses in hybrid seed production. This study aimed to investigate the biochemical basis of disease resistance in sorghum induced by various chemical compounds against sugary disease caused by *Sphacelia sorghi*. The research evaluated the effects of chitosan, salicylic acid, acibenzolar-s-methyl, beta-aminobutyric acid, ethylene, jasmonic acid, benzoic acid, indole-3-butyric acid, benzothiadiazole and zinc oxide on the activity of phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) at different concentrations and across various time intervals (3, 6 and 9 days) following post-pathogen inoculation. All treatments significantly increased PAL and PPO activities compared to the control. Chitosan at 1000 ppm (part per million) exhibited the highest PAL activity, while salicylic acid at 1000 ppm recorded the highest PPO activity. The lowest PAL and PPO activities were observed in the control group inoculated with the pathogen alone. The enhanced activity of PAL and PPO in treated plants suggests a strong defensive response in sorghum against *S. sorghi*. Based on their ability to significantly enhance PAL and PPO activities, the use of disease-tolerant genotypes in combination with prophylactic sprays of chitosan or salicylic acid at 1000 ppm is recommended as a cost-effective strategy for managing sugary disease (ergot) in sorghum.

Keywords: chitosan; ergot; phenylalanine ammonia lyase (PAL); plant defence; polyphenol oxidase (PPO); *Sphacelia sorghi*

Introduction

Sorghum (*Sorghum bicolor* (L.) Pers.) has been a crucial staple crop for human consumption since ancient times, particularly in the semi-arid regions of the world. It is a multipurpose cereal ranked among the top five globally and is used as a source of food, fodder, livestock feed and biofuel feedstock (1). Globally, the crop is cultivated on over 40.07 million ha, producing approximately 57.89 million t of grains annually (2). In India, sorghum production stands at 8.71 million t, with major cultivation regions including Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, Uttar Pradesh, Gujarat and Rajasthan.

However, sorghum production is severely constrained by various fungal diseases (3). Major fungal diseases affecting global sorghum production include downy mildew, leaf blight, smuts, rust, anthracnose, charcoal rot, grain mold, ergot or sweet sickness or sugary disease and stripe disease (4-6). The occurrence and intensity of these diseases fluctuate with the seasons, with higher rates observed during the wet season compared to the dry season.

Ergot, also known as sugary disease, represents a significant challenge to sorghum cultivation, particularly

when susceptible varieties are planted under favourable environmental conditions. It poses a particular threat to hybrids and hybrid seed production, particularly in male sterile lines that exhibit poor flowering synchrony or delayed seed formation (7). Honeydew-contaminated grain has reduced feed intake and causes toxicity when fed to livestock. Ergot-infected seeds exhibited lower germination and seedling emergence and the infection may exacerbate the incidence and severity of grain molds such as *Curvularia* species, *Fusarium* species, *Alternaria* species and *Cladosporium* species (8).

To reduce losses caused by sugary disease, cultivating resistant cultivars presents a more cost-effective and viable alternative compared to chemical treatments. Consequently, it is crucial to thoroughly investigate both the sources of resistance and the factors that contribute to it. Comparative analyses of biochemical constituents in sorghum during the diseases' progression have frequently facilitated a deeper understanding of the resistance mechanisms and aid in identifying resistant genotypes (9).

The primary aim of this study was to examine the metabolic alterations resulting from treatment with various inducers and to elucidate the role of key defense enzymes

such as PAL and PPO. These enzymes are involved in the oxidation of phenolic compounds, which are commonly found in plants and play a vital role in their defense mechanisms (10). Following physical injury or pathogen infection, plants often exhibit increased PAL and PPO activity, which can contribute to the production of quinones from phenols. This enzymatic process not only strengthens plant cell walls but also generates antimicrobial compounds, thereby enhancing the plants' defense system against biotic stressors.

Such mechanisms are crucial in the induction and development of resistance against *S. sorghi*. In this context, the present study aims to investigate the biochemical basis of disease resistance in sorghum as induced by various chemical compounds against sugary disease caused by *S. sorghi*.

Materials and Methods

The present research investigations were conducted in the Sorghum Pathology Laboratory and Glasshouse of the Department of Plant Pathology, College of Agriculture. Field experiments were conducted during the *kharif* season in 2022-23 and 2023-24 at the Livestock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand.

Sorghum seeds of cytoplasmic male sterility line (CMS A 104) were surface sterilization using 70 % ethanol for 30 s, followed by two washes with sterile distilled water. Then 3 to 5 seeds were sown in 25 cm diameter pots containing a sterilized mixture of field perlite, soil and farm yard manure in a 1:1:1 ratio. The seedlings were grown in a glasshouse under natural condition. The pots were irrigated every two days.

The *S. sorghi* was cultured on T₂ agar media for 5-7 days. The conidial concentration obtained from the media was adjusted to 1×10^6 conidia/mL and supplemented with 0.2 % Tween 20. During the flowering stage, when the spikelets exhibited newly emerged stigmas, the panicles were treated with chemical inducers exogenously. All the treatments were applied as a foliar spray using a handheld sprayer at 20 mL per plant, at different concentrations (1, 10, 100 and 1000 ppm).

On the next day, each treatment was sprayed uniformly with conidial suspension at 1×10^6 conidia/mL is applied to submerge panicles by using hand sprayer. To ensure optimal conditions for pathogen infection and development, plants were enclosed in plastic bags for a period ranging from 24 to 72 hr, maintaining a consistently high level of humidity (90-92 %) and temperature (19 °C) throughout the period. This method created an environment conducive to the progression of the pathogen by simulating conditions favourable for infection and subsequent disease establishment.

Enzyme activities

To assay PAL and PPO enzyme activities, fresh leaf samples were collected at specific time intervals; before the application of chemical inducers and on the 3rd, 6th and 9th days after the application of inducers and inoculation with

the test pathogen *S. sorghi*. In the control treatment, plants were inoculated only with the test pathogen. Detailed descriptions of the enzyme analyses are provided under separate headings (11).

Analysis of phenylalanine ammonia-lyase (PAL)

One g of leaf sample was homogenized in 3 mL of ice-cold 0.1 M sodium borate buffer with a pH of 7.0. The buffer solution also included 1.4 mM 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidone. The resulting homogenate was filtered through muslin cloth and the filtrate then centrifuged at 16000 g for 15 min at a temperature of 4 °C. The supernatant obtained after centrifugation was used as the enzyme extract.

For the assay, the reaction mixture consisted of 0.4 mL of enzyme extract, 0.5 mL of 0.1 M borate buffer at pH 8.8 and 0.5 mL of 12 mM L-phenylalanine. The mixture was then incubated for 30 min at a temperature of 30 °C. After incubation, the optical density (OD) of the solution was measured at 290 nm. PAL activity was assessed by quantifying trans-cinnamic acid, following the method outlined by (12). Enzyme activity was expressed as micromoles of trans-cinnamic acid per min per g of fresh weight ($\text{min}^{-1} \text{g}^{-1}$ fresh weight).

PAL Activity =

$$\frac{\text{OD Value} \times \text{Standard Value} \times \text{Volume during Assay}}{\text{Weight of the Sample}} \times 100 \quad (\text{Eqn. 1})$$

Analysis of polyphenol oxidase (PPO)

PPO activity was determined following the method described by (13). Leaves samples weighing one g was homogenized in 2 mL of 0.1 M sodium phosphate buffer with a pH of 6.5 in mortar and pestle. Subsequently, the homogenates were centrifuged at 16000 g for 15 min at a temperature of 4 °C and supernatant was used as enzyme extract.

The assay mixture was prepared by combining 0.2 mL of enzyme extract with 1.5 mL of 0.1 M sodium phosphate buffer at pH 6.5 and 0.2 mL of 0.01 M catechol. The change in absorbance was recorded at 495 nm at 30 s intervals for up to 3 min. The enzyme activity was quantified as the rate of change in absorbance per min per mg of fresh tissue weight.

PPO Activity =

$$\frac{\text{Change in OD Value} \times \text{Reaction Volume}}{2.5 \times \text{Volume of Enzyme Extract}} \times 100 \quad (\text{Eqn. 2})$$

Statistical analysis

The experimental data collected in this study were statistically analysed to determine the significance of differences using the standard procedures for a three-factorial complete randomized design (14). Analysis and interpretation were conducted following Fisher's analysis of variance method (15). The level of significance for 'F' and 'T' tests was set at $p = 0.01$ was used. Critical differences were calculated wherever 'F' test was significant.

Results and Discussion

Effect of chemical inducers on defense related enzymes against *S. sorghi*

The chemical inducers are capable of increasing PAL and PPO activity in sorghum when applied against *S. sorghi*. The chemical inducers treated sorghum plants at different concentrations (1, 10, 100 and 1000 ppm) synthesized higher amount of PAL (Table 1) and PPO (Table 2) compared to the control before the application of chemical inducers and on the 3rd, 6th and 9th days after the application of inducers and inoculation with the test pathogen *S. sorghi*

Effect of phenylalanine ammonia lyase (PAL) activity against inoculation with *S. sorghi*

Under glasshouse conditions, all chemical inducers evaluated at different concentrations (1, 10, 100 and 1000 ppm) showed increased activity of PAL enzyme, which persisted until the 9th

day post inoculation with the pathogen. Baseline PAL activity, measured prior to pathogen inoculation, ranged from 0.010 to 0.019 $\mu\text{mol}/\text{min}/\text{g}$ fresh weight (FW) as trans-cinnamic acid (TCA) and was statistically non-significant across treatments.

Chemical inducers applied at 1000 ppm, in all the treatments activity of PAL was increased till day 6th and started declining thereafter. On 3rd day the enzyme activity ranged between 0.031 to 0.072 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA), with the maximum activity of 0.072 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) in chitosan and minimum activity of 0.031 $\mu\text{mol}/\text{min}/\text{g}/\text{FW}$ (TCA) in beta-amino butyric acid treatment. On 6th day, the enzyme activity increased and ranged between 0.068 to 0.114 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA). The maximum activity on 6th day was for salicylic acid with 0.114 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) and minimum was for treatment acibenzolar-s methyl with 0.068 $\mu\text{mol}/\text{min}/\text{g}/\text{FW}$ (TCA). On 9th day the activity was ranged between 0.066 to 0.110 $\mu\text{mol}/\text{min}/\text{g}/\text{FW}$ (trans-

Table 1. Biochemical changes of phenylalanine ammonia-lyase (PAL) activity due to *S. sorghi* in $\mu\text{mol}^{-1} \text{min}^{-1} \text{g}^{-1} \text{FW}$

Sl. No.	Treatment	Concentration (ppm)	Before application	3 rd day	6 th day	9 th day
1	Chitosan	1000	0.013	0.072	0.112	0.110
		100	0.016	0.065	0.094	0.091
		10	0.010	0.044	0.051	0.050
		1	0.013	0.018	0.022	0.021
2	Salicylic acid	1000	0.013	0.070	0.114	0.108
		100	0.017	0.036	0.068	0.064
		10	0.011	0.022	0.028	0.025
		1	0.013	0.019	0.025	0.022
3	Acibenzolar-s methyl	1000	0.012	0.032	0.068	0.066
		100	0.014	0.026	0.057	0.056
		10	0.012	0.021	0.032	0.029
		1	0.014	0.017	0.023	0.022
4	Beta-amino butyric acid	1000	0.012	0.031	0.069	0.067
		100	0.015	0.026	0.063	0.056
		10	0.014	0.019	0.026	0.025
		1	0.011	0.012	0.021	0.020
5	Ethylene	1000	0.01	0.035	0.075	0.073
		100	0.012	0.029	0.059	0.058
		10	0.011	0.021	0.035	0.034
		1	0.015	0.018	0.025	0.024
6	Jasmonic acid	1000	0.013	0.062	0.106	0.104
		100	0.014	0.032	0.071	0.065
		10	0.012	0.028	0.030	0.029
		1	0.014	0.020	0.027	0.026
7	Benzoic acid	1000	0.015	0.047	0.076	0.075
		100	0.014	0.027	0.049	0.040
		10	0.018	0.025	0.028	0.026
		1	0.015	0.019	0.026	0.025
8	Indole 3 butyric acid	1000	0.015	0.055	0.094	0.091
		100	0.013	0.023	0.061	0.053
		10	0.012	0.022	0.025	0.023
		1	0.014	0.017	0.021	0.020
9	Benzothiadiazole	1000	0.019	0.064	0.079	0.077
		100	0.015	0.028	0.046	0.044
		10	0.015	0.018	0.024	0.020
		1	0.012	0.016	0.020	0.019
10	Zinc oxide	1000	0.010	0.046	0.083	0.082
		100	0.012	0.024	0.043	0.042
		10	0.013	0.02	0.027	0.021
		1	0.016	0.018	0.024	0.019
11	Control	1000	0.016	0.017	0.019	0.018
		100	0.016	0.017	0.019	0.018
		10	0.016	0.017	0.019	0.018
		1	0.016	0.017	0.019	0.018
			±S.Em.	C.D.		
Chemical inducers (A)			0.001	0.003		
Concentration (B)			0.001	0.002		
A × B (Chemical inducers × Concentration)			0.002	0.006		
Interval (C)			0.001	0.002		
A × C (Chemical inducers × Interval)			0.002	0.006		
B × C (Concentration × Interval)			0.001	0.003		
A × B × C (Chemical inducers × Concentration × Interval)			0.004	0.011		

Table 2. Biochemical changes of polyphenol oxidase (PPO) activity due to *S. sorghi* in $\text{min}^{-1}\text{mg}^{-1}$ FW

Sl. No.	Treatment	Concentration (ppm)	Before application	3 rd day	6 th day	9 th day
1	Chitosan	1000	0.086	0.131	0.167	0.165
		100	0.081	0.114	0.156	0.150
		10	0.068	0.091	0.124	0.105
		1	0.073	0.074	0.085	0.082
2	Salicylic acid	1000	0.065	0.126	0.176	0.174
		100	0.067	0.120	0.143	0.136
		10	0.080	0.116	0.125	0.121
		1	0.068	0.071	0.078	0.074
3	Acibenzolar-s methyl	1000	0.067	0.102	0.142	0.141
		100	0.076	0.105	0.135	0.133
		10	0.073	0.110	0.121	0.116
		1	0.067	0.071	0.092	0.086
4	Beta-amino butyric acid	1000	0.054	0.110	0.158	0.143
		100	0.057	0.105	0.138	0.135
		10	0.049	0.084	0.120	0.096
		1	0.057	0.060	0.089	0.067
5	Ethylene	1000	0.081	0.128	0.159	0.152
		100	0.078	0.121	0.150	0.149
		10	0.076	0.114	0.124	0.115
		1	0.074	0.078	0.086	0.082
6	Jasmonic acid	1000	0.073	0.123	0.150	0.145
		100	0.070	0.095	0.135	0.128
		10	0.066	0.107	0.118	0.112
		1	0.074	0.082	0.092	0.088
7	Benzoic acid	1000	0.084	0.124	0.154	0.147
		100	0.073	0.110	0.135	0.125
		10	0.081	0.114	0.117	0.110
		1	0.063	0.070	0.083	0.080
8	Indole 3 butyric acid	1000	0.070	0.107	0.147	0.144
		100	0.076	0.104	0.144	0.139
		10	0.083	0.097	0.122	0.120
		1	0.076	0.080	0.083	0.078
9	Benzothiadiazole	1000	0.057	0.100	0.122	0.117
		100	0.068	0.107	0.113	0.111
		10	0.076	0.102	0.108	0.105
		1	0.070	0.073	0.080	0.076
10	Zinc oxide	1000	0.076	0.121	0.150	0.143
		100	0.080	0.114	0.131	0.127
		10	0.056	0.093	0.103	0.101
		1	0.050	0.070	0.076	0.073
11	Control	1000	0.056	0.059	0.063	0.062
		100	0.056	0.059	0.063	0.062
		10	0.056	0.059	0.063	0.062
		1	0.056	0.059	0.063	0.062
Chemical inducers (A)			±S.Em.	C.D.		
Concentration (B)			0.003	0.009		
A × B (Chemical inducers × Concentration)			0.002	0.005		
Interval (C)			0.006	0.017		
A × C (Chemical inducers × Interval)			0.002	0.005		
B × C (Concentration × Interval)			0.006	0.017		
A × B × C (Chemical inducers × Concentration × Interval)			0.004	0.010		
			0.012	0.031		

cinnamic acid). The maximum activity of 0.110 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) was in chitosan and minimum activity of 0.066 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) was in acibenzolar-s methyl treatment.

Chemical inducers applied at 100 ppm, in all the treatments activity of PAL was increased till day 6th and started declining thereafter. On 3rd day the enzyme activity ranged between 0.023 to 0.065 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) with maximum activity of 0.065 $\mu\text{mol}/\text{min}/\text{g}/\text{fresh weight}$ (TCA) in chitosan and minimum activity of 0.023 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) in indole 3 butyric acid treatment. On 6th day the enzyme activity increased and ranged between 0.043 to 0.094 $\mu\text{mol}/\text{min}/\text{g}/\text{FW}$ (TCA). The maximum activity on 6th day was for chitosan with 0.094 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) and minimum for zinc oxide treatment with 0.043 $\mu\text{mol}/\text{min}/\text{g}/\text{fresh weight}$ (TCA). On 9th day the activity ranged between 0.040 to 0.091 $\mu\text{mol}/\text{min}/\text{g}/\text{fresh weight}$ (TCA). The maximum activity of 0.091 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) was observed in

chitosan and minimum activity of 0.040 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) in benzoic acid treatment.

Chemical inducers applied at 10 ppm, in all the treatments, activity of PAL was increased till day 6th and started declining thereafter. On 3rd, 6th and 9th day the maximum activity of 0.044, 0.051 and 0.050 $\mu\text{mol}/\text{min}/\text{g}/\text{FW}$ (TCA) was recorded in chitosan treatment respectively and minimum activity of 0.018, 0.024 and 0.020 $\mu\text{mol}/\text{min}/\text{gram}/\text{FW}$ (TCA) were observed in benzothiadiazole treatment.

Pathogen inoculated only with pathogen showed non-significant increase in PAL activity before the application and at different intervals of time.

The effective prevention of pathogen invasion largely depends on the host cells' ability to promptly and accurately detect pathogens, which subsequently triggers a range of defense mechanisms (16, 17). These mechanisms include the production of secondary metabolites, reactive oxygen

species (ROS), defense enzymes and pathogenesis-related proteins (PRs), all of which work collectively to counteract pathogen infection (18). Elicitor signalling plays a crucial role by enhancing host defense responses through processes such as cell wall reinforcement, antimicrobial metabolite synthesis and the activation of defense enzymes and PR proteins, as well as by inducing a hypersensitive response, thereby successfully managing pathogen manifestation (19).

The findings of this study demonstrate that during chemical elicitor-induced resistance to sugary disease, PAL and PPO play a vital role and the speed and intensity with which these defensive enzymes are triggered and accumulated is strongly connected with the degree of resistance elicited by that particular elicitor (20). In general, all the tested chemical elicitors, i.e. chitosan, salicylic acid, acibenzolar-s methyl, beta-amino butyric acid, ethylene, jasmonic acid, benzoic acid, indole 3 butyric acid, benzothiadiazole and zinc oxide showed higher activities of PAL and PPO compared to control (21).

Effect of polyphenol oxidase (PPO) activity against inoculation with *Sphacelia sorghi*

Under glass house conditions, all chemical inducers evaluated at various concentrations (1, 10, 100 and 1000 ppm) showed increased PPO activity and the effect was observed till 9th day of inoculation with the pathogen. In all the treatments, an amount of activity of PPO was observed before the pathogen inoculation. The reading of PPO activity varied between 0.049 to 0.086 min⁻¹mg⁻¹FW (fresh weight) and found non-significant between the treatments.

Chemical inducers applied at 1000 ppm, in all the treatments activity of PPO was increased till day 6th and started declining thereafter. On the 3rd day, enzyme activity ranged between 0.100 to 0.131 min⁻¹mg⁻¹FW with maximum activity of 0.131 min⁻¹mg⁻¹FW observed in chitosan and minimum activity of 0.100 min⁻¹mg⁻¹FW observed in benzothiadiazole treatment. On the 6th day, enzyme activity further increased, ranging between 0.122 to 0.176 min⁻¹mg⁻¹FW. The maximum activity on 6th day was for salicylic acid with 0.176 min⁻¹mg⁻¹FW and minimum was for treatment benzothiadiazole with 0.122 min⁻¹mg⁻¹FW. On 9th day the activity was ranged between 0.117 to 0.174 min⁻¹mg⁻¹FW. The maximum activity of 0.174 min⁻¹mg⁻¹FW in salicylic acid and minimum activity of 0.117 min⁻¹mg⁻¹FW in benzothiadiazole treatment were found on 9th day.

In all the treatments of chemical inducers applied at 100 ppm, activity of PPO increased till day 6th and started declining thereafter. On the 3rd day, enzyme activity ranged from 0.095 to 0.121 min⁻¹mg⁻¹FW with maximum activity of 0.121 min⁻¹mg⁻¹FW in ethylene and minimum activity of 0.095 min⁻¹mg⁻¹FW in jasmonic acid treatment. On 6th day the enzyme activity increased and ranged between 0.113 to 0.156 min⁻¹mg⁻¹FW. The maximum activity on 6th day was for chitosan with 0.156 min⁻¹mg⁻¹FW and minimum for treatment benzothiadiazole with 0.113 min⁻¹mg⁻¹FW. On 9th day the activity ranged between 0.118 to 0.150 min⁻¹mg⁻¹FW. The maximum activity of 0.150 min⁻¹mg⁻¹FW in chitosan and minimum activity of 0.118 min⁻¹mg⁻¹FW in benzothiadiazole treatment on 9th day.

While, chemical inducers applied at 10 ppm, in all the treatments activity of PPO was increased till day 6th and

started declining thereafter. On 3rd, 6th and 9th day the maximum activity of 0.116, 0.125 and 0.121 min⁻¹mg⁻¹FW was recorded in salicylic acid treatment respectively and minimum activity of 0.084, 0.120 and 0.096 min⁻¹mg⁻¹FW were observed in beta-aminobutyric acid treatment on 3rd, 6th and 9th day after inoculation respectively.

Only pathogen inoculated treatment showed non-significant increase in PPO activity before the application and at different intervals of time.

It shows that treatment with chitosan and salicylic acid at 100 ppm concentration was found to be most cost effective (Table 2). These treatments provided maximum disease control with favorable economic returns, making them suitable for commercial use in hybrid seed production.

A study reported elevated activities of polyphenol oxidase and peroxidase in sorghum leaves infected with *Drechslera sorghicola*, correlating with disease development (22). The activity of PAL in the leaves of barley genotypes with resistance to the spot blotch pathogen *B. sorokiniana* was found to be elevated (23). Another study, examined *Sorghum bicolor* cultivars against *Macrophomina phaseolina* for peroxidase activity and salicylic acid and found positive relationship between biochemical parameters and disease resistance of cultivars PJ-1430 and SU-1080 (24).

The chemical inducers viz., salicylic acid and chitosan evaluated at different concentrations (1, 10, 100 and 1000 ppm) showed much higher activity of PAL and PPO enzymes than other tested chemical inducers. The effect was peaked at 6th day after inoculation and lasted till 9th day of pathogen inoculation. Salicylic acid is known to specifically interact with proteins that are involved in the degradation of intracellular hydrogen peroxide (H₂O₂) (25). This interaction can lead to increased intracellular levels of H₂O₂ and result in the inactivation of *Aspergillus brasiliensis* conidia (26). Additionally, a study demonstrated that salicylic acid exhibits direct antimicrobial effects against *Penicillium expansum* conidia (27). This effect is likely due to its ability to penetrate the cell wall and interact with the plasma membrane, which can disrupt the lipid bilayer and/or damage proteins, which is essential for maintaining cell permeability. This disruption can lead to an elevated concentration of reactive oxygen species. Chitosan treatment induces systemic acquired resistance responses and regulates several defense genes, including the activation of PAL and PPO enzymes, phytoalexins and PR proteins, which results in induced resistance (28-30). Chitosan treatment also has direct effect as antifungal compound by inhibiting mycelial growth, sporulation (31-32).

Conclusion

Constitutive PAL and PPO activities were recorded in all treatments before the application of chemical inducers, as well as on the 3rd, 6th and 9th days after the application of inducers and inoculation with the test pathogen *S. sorghi*. In all treatments, there was a progressive increase in PAL and PPO activities starting from the 3rd day post-inoculation, with peak activities observed on the 6th day. However, by the 9th day, a decline in both these enzyme activities was noted.

Notably, sorghum plants treated with chitosan and salicylic acid exhibited significantly higher PAL and PPO activities compared to untreated seedlings, across various concentrations and time intervals. The enhanced activity of PAL and PPO in treated plants suggests a potential mechanism for the identification of resistant genotypes, as these enzymes are involved in the plant defense response.

Furthermore, integrating the use of disease-tolerant genotypes with prophylactic sprays of chitosan or salicylic acid has proven to be effective for managing sugary disease in sorghum. The combination of genetic resistance and chemical inducers can synergistically enhance the plants' defense mechanisms, thereby reducing the impact of pathogen infection and contributing to improved crop health and yield.

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Authors' contributions

SD led the conceptualization, design, material preparation and data collection as well as data analysis for this research as part of his Ph.D. thesis. YS served as the major advisor, providing invaluable guidance and support, including help with securing funding. SM and SS contributed to the study through critical review and editing of the manuscript. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest: The author(s) declare that they have no competing interests.

Ethical issues: None

References

- Ananda GKS, Myrans H, Norton SL, Gleadow R, Furtado A, Henry RJ. Wild sorghum as a promising resource for crop improvement. *Front Plant Sci.* 2022;11:1108. <https://doi.org/10.3389/fpls.2020.01108>
- Khalifa M, Eltahir EAB. Assessment of global sorghum production, tolerance and climate risk. *Front Sustain Food Syst.* 2023;7:1184373. <https://doi.org/10.3389/fsufs.2023.1184373>
- Abreha KB, Ortiz R, Carlsson AS, Geleta M. Understanding the sorghum-*Colletotrichum sublineola* interactions for enhanced host resistance. *Front Plant Sci.* 2021;12:641969. <https://doi.org/10.3389/fpls.2021.641969>
- Wang ML, Dean R, Erpelding J, Pederson G. Molecular genetic evaluation of sorghum germplasm differing in response to fungal diseases: rust (*Puccinia purpurea*) and anthracnose (*Colletotrichum graminicola*) Euphytica. 2006;148(3):319–30. <https://doi.org/10.1007/s10681-005-9040-0>
- Das IK, Rajendrakumar P. Disease resistance in sorghum. In: Das IK, Padmaja PG, (eds). Biotic stress resistance in millets. Academic Press, Cambridge, Massachusetts; 2017. pp 23–67. <https://doi.org/10.1016/B978-0-12-804549-7.00002-0>
- Mengistu G, Shimelis H, Laing M, Lule D. Breeding for anthracnose (*Colletotrichum sublineolum* Henn.) resistance in sorghum: Challenges and opportunities. *Aust J Crop Sci.* 2018;12(12):1911–20. <https://doi.org/10.21475/ajcs.18.12.12.p1230>
- Pazoutova S, Frederickson DE. Genetic diversity of *Claviceps africana* on sorghum and Hyparrhenia. *Plant Pathol.* 2005;54:749–63. <https://doi.org/10.1111/j.1365-3059.2005.01255.x>
- Kebede D, Dramadri IO, Rubaihayo P, Odong T, Edema R. Resistance of sorghum genotypes to Ergot (*Claviceps species*). *Agric.* 2023;13:1100. <https://doi.org/10.3390/agriculture13051100>
- Zanjare SR, Suryawanshi AV, Zanjare SS, Shelar VR, Balgude YS. Screening of groundnut (*Arachis hypogaea* L.) genotypes for identification of sources of resistance against leaf spot disease. *Legume Res.* 2023;46(3):288–94. <https://doi.org/10.18805/LR-4370>
- Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules.* 2019;24:2452. <https://doi.org/10.3390/molecules24132452>
- Worth HDL, Sutton BS, Ainsworth GC. Ainsworth and Bisby's dictionary of fungi. 7th Ed. Commonwealth Mycological Institute, Kew; Surrey, England; 1983. 445. <https://doi.org/10.1017/S0024282984000360>
- Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol Plant Pathol.* 1984;25(2):111–23. [https://doi.org/10.1016/0048-4059\(84\)90050-X](https://doi.org/10.1016/0048-4059(84)90050-X)
- Mayer AM. Polyphenol oxidases in plants and fungi: going places? A review. *Phytochem.* 2006;67(21):2318–31. <https://doi.org/10.1016/j.phytochem.2006.08.006>
- Walter SD, Belo IJ. Design and analysis of factorial clinical trials: The impact of one treatment's effectiveness on the statistical power and required sample size of the other. *Stat Methods Med Res.* 2023;32(6):1124–44. <https://doi.org/10.1177/09622802231163332>
- Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley and Sons; 1984. <https://doi.org/10.1017/S0014479700014496>
- Prsic J, Ongena M. Elicitors of plant immunity triggered by beneficial bacteria. *Front Plant Sci.* 2020;11:594530. <https://doi.org/10.3389/fpls.2020.594530>
- Jones JDG, Staskawicz BJ, Dangl JL. The plant immune system: From discovery to deployment. *Cell.* 2024 Apr 25;187(9):2095–116. <https://doi.org/10.1016/j.cell.2024.03.045>
- Bolwell PP, Page A, Pislewska M, Wojtaszek P. Pathogenic infection and the oxidative defences in plant apoplast. *Protoplasma.* 2021;217(1-3):20–32. <https://doi.org/10.1007/BF01289409>
- Lorrain S, Vailleau F, Balague C, Roby D. Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.* 2003;8(6):263–71. [https://doi.org/10.1016/S1360-1385\(03\)00108-0](https://doi.org/10.1016/S1360-1385(03)00108-0)
- Lavanya SN, Niranjana-Raj S, Jadimurthy R, Sudarsan S, Srivastava R, Tarasatyavati C, et al. Immunity elicitors for induced resistance against the downy mildew pathogen in pearl millet. *Sci Rep.* 2022;12(1):4078. <https://doi.org/10.1038/s41598-022-07839-4>

21. Park CH, Yeo HJ, Park YE, Chun SW, Chung YS, Lee SY, et al. Influence of chitosan, salicylic acid and jasmonic acid on phenylpropanoid accumulation in germinated buckwheat (*Fagopyrum esculentum* Moench). *Foods*. 2019;8(5):153. <https://doi.org/10.3390/foods8050153>
22. Khan AJ, Deadman ML, Srikandakumar A, Al-Maqbali YM, Rizvi SG, Al-Sabahi J. Biochemical changes in sorghum leaves infected with leaf spot pathogen, *Drechslera sorghicola*. *The Plant Pathol J*. 2001;17(6):342–46.
23. Kaur S, Bhardwaj RD, Kaur J, Kaur S. Induction of defense-related enzymes and pathogenesis-related proteins imparts resistance to barley genotypes against spot blotch disease. *J Plant Growth Regul*. 2021;41:1–15. <https://doi.org/10.1007/s00344-021-10333-2>
24. Kumari N, Sharma I, Alam A, Sharma V. Screening of sorghum genotypes and biochemical changes for resistance to damage caused by *Macrophomina phaseolina*. *Arch Phytopathol Plant Protect*. 2016;48(9–12):760–75. <https://doi.org/10.1080/03235408.2016.1140569>
25. Chen Z, Silva H, Klessig DF. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Sci*. 1993;262(5141):1883–86. <https://doi.org/10.1126/science.8266079>
26. Scaramuzza N, Cigarini M, Mutti P, Berni E. Sanitization of packaging and machineries in the food industry: Effect of hydrogen peroxide on ascospores and conidia of filamentous fungi. *Int J Food Microbiol*. 2020;316:108421. <https://doi.org/10.1016/j.ijfoodmicro.2019.108421>
27. Neto RAC, Maraschin M, Di Piero RM. Antifungal activity of salicylic acid against *Penicillium expansum* and its possible mechanisms of action. *Int J Food Microbiol*. 2015;215:64–70. <https://doi.org/10.1016/j.ijfoodmicro.2015.08.018>
28. Xing K, Zhu X, Peng X, Qin S. Chitosan antimicrobial and eliciting properties for pest control in agriculture: A review. *Agron Sustain Dev*. 2015;35:569–88. <https://doi.org/10.1007/s13593-014-0252-3>
29. Sharif R, Xie C, Zhang H, Arnao MB, Ali M, Ali Q, et al. Melatonin and its effects on plant systems. *Molecules*. 2018;23(9):2352. <https://doi.org/10.3390/molecules23092352>
30. Carmona SL, Villarreal-Navarrete A, Burbano-David D, Gómez-Marroquín M, Torres-Rojas E, Soto-Suárez M. Protection of tomato plants against *Fusarium oxysporum* f. sp. lycopersici induced by chitosan. *Rev Colomb Cienc Hortic*. 2021;15(3):e12822. <https://doi.org/10.17584/rcch.2021v15i3.12822>
31. Kumaraswamy RV, Kumari S, Choudhary RC, Pal A, Raliya R, Biswas P, et al. Engineered chitosan-based nanomaterials: Bioactivities, mechanisms and perspectives in plant protection and growth. *Int J Biol Macromol*. 2018;113:494–506. <https://doi.org/10.1016/j.ijbiomac.2018.02.130>
32. El-araby A, Janati W, Ullah R, Uddin N, Bari A. Antifungal efficacy of chitosan extracted from shrimp shell on strawberry (*Fragaria × ananassa*) postharvest spoilage fungi. *Heliyon*. 2024;10(7):e29286. <https://doi.org/10.1016/j.heliyon.2024.e29286>

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