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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 T_2 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 ^{0}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 (min^{-1} g^{-1} fresh weight).

PAL Activity =

$$\frac{\text{OD Value x Standard Value x Volume during Assay}}{\text{Weight of the Sample}} \text{ x } 100$$

(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 =

$$\frac{\text{Change in OD Value x Reaction Volume}}{\text{2.5 x Volume of Enzyme Extract}} \text{ x } 100$$

(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 µmol/min/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 µmol/min/gram/FW (TCA), with the maximum activity of 0.072 µmol/min/gram/FW (TCA) in chitosan and minimum activity of 0.031 µmol/min/g/FW (TCA) in beta-amino butyric acid treatment. On 6th day, the enzyme activity increased and ranged between 0.068 to 0.114 µmol/min/gram/FW (TCA). The maximum activity on 6th day was for salicylic acid with 0.114 µmol/min/gram/FW (TCA) and minimum was for treatment acibenzolar-s methyl with 0.068 µmol/min/g/FW (TCA). On 9th day the activity was ranged between 0.066 to 0.110 µmol/min/g/ FW (trans-

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

Sl. No.	Treatment	Concentration (ppm)	Before application	3rd day	6th day	9th 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 $min^{-1}mg^{-1}$ FW

Sl. No.	Treatment	Concentration (ppm)	Before application	3rd day	6th day	9th 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
			±S.Em.	C.D.		
	Chemical inducers (A)		0.003	0.009		
	Concentration (B)		0.002	0.005		
	A × B (Chemical inducers × Concentration)		0.006	0.017		
	Interval (C)		0.002	0.005		
	A × C (Chemical inducers × Interval)		0.006	0.017		
	B × C (Concentration × Interval)		0.004	0.010		
	A × B × C (Chemical inducers × Concentration × Interval)		0.012	0.031		

cinnamic acid). The maximum activity of 0.110 µmol/min/gram/FW (TCA) was in chitosan and minimum activity of 0.066 µmol/min/gram/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 µmol/min/gram/FW (TCA) with maximum activity of 0.065 µmol/min/g/fresh weight (TCA) in chitosan and minimum activity of 0.023 µmol/min/gram/FW (TCA) in indole 3 butyric acid treatment. On 6th day the enzyme activity increased and ranged between 0.043 to 0.094 µmol/min/g/FW (TCA). The maximum activity on 6th day was for chitosan with 0.094 µmol/min/gram/FW (TCA) and minimum for zinc oxide treatment with 0.043 µmol/min/g/fresh weight (TCA). On 9th day the activity ranged between 0.040 to 0.091 µmol/min/g/fresh weight (TCA). The maximum activity of 0.091 µmol/min/gram/FW (TCA) was observed in chitosan and minimum activity of 0.040 µmol/min/gram/FW (TCA) in benzoic acid treatment.

Chemical inducers applied at 10 ppm, in all the treatments, activity of PAL was increased till day 6thand started declining thereafter. On 3rd, 6th and 9th day the maximum activity of 0.044, 0.051 and 0.050 µmol/min/g/FW (TCA) was recorded in chitosan treatment respectively and minimum activity of 0.018, 0.024 and 0.020 µmol/min/gram/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 collecetively 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^{-1}mg^{-1}FW (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^{-1}mg^{-1}FW with maximum activity of 0.131 min^{-1}mg^{-1} FW observed in chitosan and minimum activity of 0.100 min^{-1}mg^{-1}FW observed in benzothiadiazole treatment. On the 6th day, enzyme activity further increased, ranging between 0.122 to 0.176 min^{-1}mg^{-1}FW. The maximum activity on 6th day was for salicylic acid with 0.176 min^{-1}mg^{-1}FW and minimum was for treatment benzothiadiazole with 0.122 min^{-1}mg^{-1}FW. On 9th day the activity was ranged between 0.117 to 0.174 min^{-1}mg^{-1}FW. The maximum activity of 0.174 min^{-1}mg^{-1} FW in salicylic acid and minimum activity of 0.117 min^{-1}mg^{-1}FW 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^{-1}mg^{-1}FW with maximum activity of 0.121min^{-1}mg^{-1}FW in ethylene and minimum activity of 0.095 min^{-1}mg^{-1}FW in jasmonic acid treatment. On 6th day the enzyme activity increased and ranged between 0.113 to 0.156 min^{-1}mg^{-1}FW. The maximum activity on 6th day was for chitosan with 0.156 min^{-1}mg^{-1}FW and minimum for treatment benzothiadiazole with 0.113 min^{-1}mg^{-1}FW. On 9th day the activity ranged between 0.118 to 0.150 min^{-1}mg^{-1}FW. The maximum activity of 0.150 min^{-1}mg^{-1}FW in chitosan and minimum activity of 0.118 min^{-1}mg^{-1} FW 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^{-1}mg^{-1}FW was recorded in salicylic acid treatment respectively and minimum activity of 0.084, 0.120 and 0.096 min^{-1}mg^{-1}FW 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 (H_2O_2) (25). This interaction can lead to increased intracellular levels of H_2O_2 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 ccontributed 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

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