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ESTIMATION OF BIOCHEMICAL PARAMETERS IN SORGHUM CULTIVARS TO CHARCOAL ROT [*MACROPHOMINA PHASEOLINA* (TASSI) GOID.]

R. SUKANYA*[1], S. K. JAYALAKSHMI[2], K. SREERAMULU[3] AND G. GIRISH[4]
[1]Department of Plant Pathology University of Agricultural Sciences, Raichur - 584 104, INDIA
[2]Department of Plant Pathology, College of Agricultural Sciences, Kalaburgi - 585 101, INDIA
[3]Department of Biochemistry, Gulbarga University, Kalaburgi - 585 101, INDIA
[4]Department of Genetics and Plant breeding, College of Agricultural Sciences, Kalaburgi - 585 101, INDIA
e-mail: sukanyachavan888@gmail.com

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***Corresponding author**

ABSTRACT
Estimation of biochemical parameters were carried out in sorghum cultivars DSV-4 (resistant), GS-23 (Moderately resistant), M-35-1 (moderately susceptible) and SPV-86 (susceptible) to charcoal rot caused by *M. phaseolina* (Tassi) Goid. revealed that, SA and *M. phaseolina* treated plants showed high level of hydrogen peroxide, SA, phenolics and polyphenol oxidase in roots and shoots of sorghum genotypes assayed compared to control. Whereas in SA and *M. phaseolina* treated plants showed low level of catalase accumulation compared to control. It was found that accumulation of hydrogen peroxide was 1.0 to 2.0, SA 1 to 1.75, phenolics 0.5 to 1.65 and polyphenol oxidase was 1.1 to 1.68 fold, reveals that accumulation of these parameters are more in resistant cultivars than susceptible cultivars over control, whereas catalase accumulation found to be 0.90 to 2.31 fold more in susceptible cultivars over control.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is is an important crop worldwide, yield and quality of sorghum is affected by wide array of biotic and biotic stresses. Among the biotic factors of many diseases, charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. is causig more yield loss in rabi sorghum growing areas as it has wide host range. As plants possess a wide range of active defense responses that contribute to resistance against a variety of pathogens. Induced resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. Pre-treatment of plants with avirulent pathogens (biotic inducers) or chemical compounds (abiotic inducers) can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial infection sites. Typically, this inducible resistance system known as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria and fungi (Ryals *et al*., 1996). The mechanism of SAR is a general strategy that is used by plants to defend them selves and has been shown for different plant species such as tobacco, cucumber, potato and rice. However, SAR can also be induced by SA. Upon infection, SA levels increases systemically in tobacco and cucumber.

The detection of increased SA levels in systemic leaves and in the phloem led many researchers to believe that SA might be a systemic signal for SAR. The evidence for and against this hypothesis has been the subject of previous reviews (Dempsey *et al*., 1999).

Although the primary signal for SAR in plants is still elusive, it is known that this signal should be mobile. The defense gene products include polyphenol oxidase (PPO), peroxidase (POD) that catalyzes the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis.

The utilization of pathogenic microorganisms as inducing agents has not been successful under field conditions. However, low molecular weight chemicals capable of inducing SAR can have significant implications in crop protection strategies. Here studies undertaken on screening of sorghum genotypes against charcoal rot under field condition and estimation of salicylic acid, H_2O_2, activities of catalases, total phenolics and polyphenol oxidase in resistant and susceptible cultivars of sorghum.

MATERIALS AND METHODS

Plant materials inoculation with *M. phaseolina*

Seeds of sorghum cultivars resistant (DSV-4 and GS-23), moderately susceptible (M35-1) and susceptible (SPV-86) to charcoal rot disease were procured from the Agriculture Research Station, Kalaburgi, India. Seeds were surface sterilized with 0.1 % aqueous $HgCl_2$ for 1-2 min and then thoroughly washed with double distilled water. The culture was maintained on sterilized sandy loam soil mixed with maize powder at 19:1 w/w**.** The pathogen inoculum was prepared by culturing the fungus on potato dextrose agar (PDA) medium for 7 days in Petri-plates. The sclerotial suspension was prepared by pouring 20 ml of sterile distilled water in each Petri-plate. The concentration of sclerotia was adjusted to $1X10^5$ mL^{-1}. Shoots and roots were separated and used for enzyme extraction. All other chemicals and reagents used were of analytical grade.

There were two replications with 100 seeds for each treatment. The first set was supplied with distilled water to serve as a control, while set two was used for pathogen *M. phaseolina* inoculation and set three as used for salicylic acid treatment (0.4mM). The seeds were germinated in Petri plates lined with double layer of filter paper at 26ºC for 10 days (Jayalaxmi, 2009). Then, the ten days old seedlings the shoots and roots of the cultivar were collected for the estimation of SA, hydrogen peroxide, phenols and determination of catalases and polyphenol oxidase.

Estimation of hydrogen peroxide in sorghum cultivars infected with

M. phaseolina

For the estimation of H_2O_2, Noreen and Ashraf (2009) method was followed. Fresh sample of shoot and root were homogenized in 2 mL of 0.1 % (w/v) Tri chloro acetic acid in a pre-chilled pestle and mortar. The homogenate was centrifuged at 12,000× g for 15 min and the supernatant was collected. Absorbance of the reaction mixture consisting of 0.5 ml supernatant, 0.5 mL sodium phosphate buffer (pH 7.0) and 1 ml of 1 M KI was read at 390 nm. The H_2O_2 content was determined by using an extinction coefficient of 0.28 µM/cm and expressed as µM/g FW.

Estimation of total phenolics in sorghum cultivars infected with *M. phaseolina*

Root and shoot samples (1g) were homogenized in 10mL of 80 per cent methanol and agitated for 15 min at 70ºC. 1mL of the methanolic extract was added to 5 ml of distilled water and 250 μL of Folin Ciocalteau reagent (1 N) and the solution was kept at

25ºC. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. Absorbance values were calibrated to a standard graph generated with known concentrations of catechol. The amount of phenolics was expressed as μg catechol mg^{-1} protein (Zieslin and Ben-Zaken, 1993).

Estimation of salicylic acid in sorghum cultivars infected with *M. phaseolina*

Root and shoots of sorghum from control and *M. phaseolina* infected plants of different genotypes were collected from 10 days old plants, weighed, and frozen in liquid nitrogen. For each sample, 1g of the frozen tissue was extracted for free salicylic acid essentially as described previously (Malamy *et al.,* 1992). Briefly, the tissue was homogenized in 3 mL of 90 per cent methanol. After centrifugation, the pellet were re-extracted with 100 per cent methanol. The combined supernatant was dried in a speed vacuum with heat (40ºC). The residue was resuspended in 2.5mL of 5 per cent trichloroacetic acid and sonicated for 10 min. The free SA was then separated from conjugated SA through organic extracts with two volumes of ethyleacetate, -cyclopentane, iso-propanol (50:50:1). The aqueous phase contains the conjugated SA will be acidified with HCl to pH 1 and was boiled for 30 min to release SA from any acid labile conjugated forms. The released free SA was then extracted with the organic mixture and analyzed as above. The organic phase containing the free SA was dried with a heat of 60ºC. The dried extract was suspended in 1mL of 96 per cent methanol and Ferric chloride reagent is prepared by adding 1 gm of $FeCl_3$ to 100 ml of 1 per cent ferric chloride and measure the absorbance of the violet colored complex using UV-Visible spectrophotometer at wavelength of 525 nm against blank sample (without salicylic acid).

Determination of activity of catalases in sorghum cultivars infected with *M. phaseolina*

The activity of catalase was determined bas per the Rao *et al*. (1997) following the consumption of H_2O_2 at 240 nm for 1 min in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 10 mM of 10 per cent substrate. One unit of activity was defined as the amount of enzyme catalyzing the decomposition of H_2O_2 (U/mg protein/ min). Estimation of protein was carried out by Lowry's *et al*. (1951).

Determination of activity of polyphenol oxidase (PPO) in sorghum cultivars infected with *M. phaseolina*

PPO activity was determined according to Mayer *et al*. (1965). The reaction mixture consisted of 200 ìl enzyme extract and 1.5mL of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min/mg/ protein. Estimation of protein was carried out by Lowry's *et al*. (1951).

RESULTS

Inoculation with *M. phaseolina*

The study reveals that there is a marked reduction in infection caused by the pathogen *M. phaseolina* was observed in seed treated with SA and seed treatment followed by *M. phaseolina* compared to the seeds treated with pathogen alone treated seeds exhibited infection by the pathogen. Seeds without SA and pathogen treated seeds kept as control.

Overall, the results revealed that, the seeds of susceptible cultivar of M35-1 and SPV-86 pretreated with SA delayed in expression and there was no infection on seedlings. Whereas, the seeds treated with *M. phaseolina* showed complete collapse of the seedlings. In contrast, the seedlings of DSV-4 and GS-23 pretreated with SA showed highest level of protection and other treatments *viz*., control and *M. phaseolina* treated seedlings remained healthy throughout the course of experiment.

Estimation of H_2O_2 in sorghum cultivars infected with *M. phaseolina*

Table 1: Estimation of hydrogen peroxide in sorghum cultivars infected with *M.phaseolina*

Varieties	Concentration of hydrogen peroxide (μM/g FW)					
	Control		Salicylic acid (0.4 mM)		*M. phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot
DSV-4	0.621 ± 0.11	0.839 ± 0.17	1.196 ± 0.18	1.367 ± 0.19	1.492 ± 0.23	1.505 ± 0.25
GS-23	0.535 ± 0.09	0.759 ± 0.15	1.071 ± 0.13	1.35 ± 0.17	1.439 ± 0.21	1.442 ± 0.26
M-35-1	0.460 ± 0.10	0.575 ± 0.14	0.660 ± 0.11	0.757 ± 0.14	0.668 ± 0.18	0.768 ± 0.21
SPV-86	0.330 ± 0.07	0.450 ± 0.12	0.439 ± 0.09	0.614 ± 0.11	0.464 ± 0.20	0.585 ± 0.16

Mean ± SE of three independent experiments

Table 2: Estimation of total phenolics in sorghum cultivars infected with *M. phaseolina*

Varieties	Concentration of phenols (μg/g FW)					
	Control		Salicylic acid (0.4 mM)		*M. phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot
DSV-4	820 ± 2.1	940 ± 2.4	840 ± 2.2	1200 ± 2.3	1340 ± 2.6	1556 ± 2.4
GS-23	650 ± 2.0	780 ± 2.3	680 ± 2.1	905 ± 2.2	960 ± 2.1	1150 ± 2.2
M-35-1	425 ± 1.8	460 ± 2.0	213 ± 1.9	250 ± 2.2	240 ± 2.5	270 ± 2.3
SPV-86	250 ± 1.7	240 ± 1.8	125 ± 1.8	140 ± 1.9	135 ± 1.9	145 ± 2.0

Mean ± SE of three independent experiment

Table 3: Estimation of salicylic acid in sorghum cultivars infected with *M. phaseolina*

Varieties	Concentration of salicylic acid (μM/g FW)					
	Control		Salicylic acid (0.4 mM)		*M. phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot
DSV-4	0.82 ± 0.17	0.74 ± 0.18	1.44 ± 0.17	1.38 ± 0.19	1.26 ± 0.15	1.16 ± 0.14
GS-23	0.74 ± 0.13	0.66 ± 0.17	1.14 ± 0.15	1.2 ± 0.18	1.20 ± 0.18	1.10 ± 0.16
M-35-1	0.39 ± 0.12	0.38 ± 0.16	0.42 ± 0.14	0.50 ± 0.16	0.42 ± 0.16	0.38 ± 0.15
SPV-86	0.36 ± 0.10	0.30 ± 0.15	0.37 ± 0.12	0.44 ± 0.13	0.38 ± 0.14	0.30 ± 0.12

Mean ± SE of three independent experiments

Table 4: Estimation of activity of catalases in sorghum cultivars infected with *M. phaseolina*

Varieties	Concentration of catalases (U/mg protein/ min)					
	Control		Salicylic acid (0.4 mM)		*M. phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot
DSV-4	2.38 ± 1.10	1.78 ± 0.51	1.27 ± 0.11	0.80 ± 0.11	1.03 ± 1.52	0.90 ± 0.12
GS-23	4.54 ± 1.60	3.28 ± 1.70	2.27 ± 0.25	2.00 ± 0.11	3.03 ± 1.67	2.30 ± 0.81
M-35-1	8.88 ± 0.57	7.23 ± 0.66	8.32 ± 0.88	7.56 ± 0.72	7.80 ± 0.87	7.60 ± 0.76
SPV- 86	8.33 ± 0.77	7.89 ± 0.87	9.20 ± 0.44	8.83 ± 0.78	8.50 ± 0.77	7.94 ± 0.88

Mean ± SE of three independent experiments

Table 5: Determination of activity of PPO in sorghum cultivars infected with *M. phaseolina*

Varieties	Concentration of polyphenol oxidase (PPO) (U/mg protein/ min)					
	Control		Salicylic acid (0.4 mM)		*M. phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot
DSV-4	4.32 ± 3.20	5.11 ± 1.80	6.99 ± 0.52	7.30 ± 2.60	6.90 ± 3.40	8.20 ± 2.50
GS-23	4.22 ± 2.10	4.31 ± 3.10	6.82 ± 3.21	7.00 ± 0.75	6.60 ± 3.22	7.20 ± 0.19
M-35-1	3.50 ± 2.11	3.90 ± 1.90	3.90 ± 2.15	4.50 ± 1.11	4.30 ± 1.67	4.80 ± 2.50
SPV-86	3.30 ± 1.50	3.80 ± 1.20	3.70 ± 0.52	3.88 ± 1.70	3.90 ± 2.20	4.20 ± 2.50

Mean ± SE of three independent experiments

H_2O_2 level in roots and shoots of resistant genotype DSV-4 was recorded 0.621 and 0.839 μM/g FW respectively was recorded in control plants. Where as in case of root and shoots of moderately resistant genotype GS-23, the H_2O_2 levels was 0.535 and 0.759 μM/g FW respectively. Whereas in case of roots and shoots of susceptible cultivars M-35-1 and SPV-86 the level of H_2O_2 was 0.460 and 0.575, 0.330 and 0.450 μM/g FW respectively. This reveals that the level of H_2O_2 was more in root and shoots of resistant and moderately resistant cultivar than in case of susceptible cultivars.

It has been observed that, in case of SA treated resistant genotype (DSV-4) the level of H_2O_2 was increased by 2.0 fold in roots and 1.7 fold in shoots. Where as in case of moderately resistant genotype (GS-23) the level of H_2O_2 was increased by 1.9 and 1.6 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of H_2O_2 was increased by 1.4 and 1.3 fold where as in SPV-86 the level of H_2O_2 was increased by 1.2 and 1.0 fold in root and shoot respectively compared to

the control.

In case of pathogen *M. phaseolina* treated resistant genotypes (DSV-4) the level of H_20_2 was increased by 2.6 fold in roots and 1.8 fold in shoots. In case of moderately resistant genotype (GS-23) the level of H_20_2 was increased by 2.4 and 1.8 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of H_20_2 was increased by 1.30 and 1.35 fold and in another susceptible cultivar SPV-86 the level of H_20_2 was increased by 1.10 and 1.19 fold in root and shoot respectively over the control plants.

This study reveals that SA and *M. phaseolina* treated plants showed high level of H_20_2 in roots and shoots of sorghum genotypes assessed compared to control (Table 1).

Estimation of total phenolics in sorghum cultivars infected with *M. phaseolina*

The phenolics in roots and shoots of resistant cv DSV-4 was 820 and 940 μg/g FW. Where as in case of moderately resistant cv GS-23 the phenolics observed was 650 μg/g and 780 μg/g FW in roots and shoots respectively. Whereas in case of roots and shoots of susceptible cultivars M-35-1 and SPV-86 the level of phenolics recorded were 425 and 460, 250 and 240 μg/g FW respectively. This reveals that the level of phenolics was more in root and shoots of resistant and moderately resistant cultivars than in case of susceptible cultivars.

In case of SA treated plants of resistant genotype (DSV-4) the level of phenolics in roots and shoots were increased by 1.02 fold in roots and 1.27 fold in shoots and in case of moderately resistant genotype (GS-23) the level of phenolics was increased by 1.04 and 1.16 fold in roots and shoots. In susceptible genotype M-35-1, the level of phenolics was increased by 0.58 and 0.65 fold where as in SPV-86 the level of phenolics was increased by 0.50 and 0.56 fold in root and shoot respectively over the control.

In case of pathogen *M. phaseolina* treated resistant genotype (DSV-4) the level of phenolics was increased by 1.63 fold in roots and 1.65 fold in shoots. In case of moderately resistant genotype (GS-23) the level of phenolics was increased by 1.47 and 1.50 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of phenolics was increased by 0.56 and 0.58 fold where as in SPV-86 the level of phenolics was increased by 0.54 and 0.58 fold in root and shoot respectively over the control.

This study reveals that SA and *M. phaseolina* treated plants showed high level of phenolics in roots and shoots of sorghum genotypes assessed compared to control

(Table 2).

Estimation of salicylic acid in sorghum cultivars infected with *M. phaseolina*

In case of roots and shoots of resistant cv DSV-4, SA level was recorded to the extent of 0.82 and 0.74 μM/g FW where as in roots and shoots of moderately resistant cv GS-23 SA levels recorded to the extent of 0.74 in roots and 0.66 μM/g FW in shoots respectively. In susceptible cultivars M-35-1 and SPV-86 the level of SA recorded were 0.39 and 0.38, 0.36 and

0.30 μM/g FW in roots and shoots respectively. This reveals that, the level of SA was more in root and shoots of resistant and moderately resistant cultivars than in case of susceptible cultivars.

In case of 0.4 mM SA treated resistant genotype (DSV-4) it has been observed that SA was increased by 1.75 fold in roots and 1.86 fold in shoots and in case of moderately resistant genotype (GS-23) the level of SA was increased by 1.64 and 1.81 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of SA was increased by 1.1 and 1.3 fold where as in SPV-86 the level of SA was increased by 1.05 and 1.20 fold in root and shoot respectively over the control.

In case of pathogen *M. phaseolina* treated resistant genotype (DSV-4) the level of SA was increased by 1.53 fold in roots and 1.56 fold in shoots and in case of moderately resistant genotype (GS-23) the level of SA was increased by 1.50 and 1.60 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of SA was increased by 1.07 and 1.00 fold where as in SPV-86 susceptible cultivar also the level of SA was increased by 1.05 and 1.00 fold in root and shoot respectively over the control.

This study reveals that SA and *M. phaseolina* treated plants showed high level of SA in roots and shoots of sorghum genotypes assayed compared to the control (Table 3).

Estimation of activity of catalases in sorghum cultivars infected with *M. phaseolina*

In case of roots and shoots of resistant cv DSV-4, catalase activity recorded were 2.38 and 1.78 U/mg protein/ min where as in roots and shoots of moderately resistant cv GS-23 catalase activity recorded were 4.54 and 3.28 U/mg protein/min respectively. Whereas in roots and shoots of susceptible cultivar M-35-1 and SPV-86 the catalase activity was recorded 8.88 and 7.23, 8.33 and 7.89 U/mg protein/ min respectively. This reveals that the catalase activity was more in root and shoots of susceptible cv than in case of resistant and moderately resistant cultivar (Table 4).

In case of 0.4 mM SA treated resistant genotype (DSV-4) the catalase activity was decreased by 1.8 fold in roots and 2.2 fold in shoots. Similarly in case of moderately resistant genotype (GS-23) the catalase activity was decreased by 2.0 and 1.64 fold in roots and shoots. In susceptible genotype M-35-1 the catalase activity was decreased by 1.06 and 0.95 fold and in SPV-86 the catalase activity was decreased by 0.90 and 0.94 fold in roots and shoots respectively over the control.

In case of pathogen *M. phaseolina* treated resistant genotype (DSV-4) the catalase activity was decreased by 2.31 fold in roots and 1.9 fold in shoots and in case of moderately resistant genotype (GS-23) the catalase activity was decreased by 1.49 and 1.40 fold in roots and shoots respectively. In susceptible genotype M-35-1 the catalase activity was decreased by 1.13 and 0.95 fold where as in another susceptible genotype SPV-86 the catalase activity was increased by 0.98 and 1.03 fold in root and shoot respectively over the control.

This study reveals that SA and *M. phaseolina* treated plants showed high level of catalase activity in susceptible genotypes of sorghum assayed compared to control as well as resistant genotypes.

Determination of activity of polyphenol oxidase (PPO) in sorghum cultivarsinfected with *M. phaseolina*

In roots and shoots of resistant cv DSV-4, PPO level was

recorded 4.32 and 5.11 U/mg protein/ min respectively. Where as in case of roots and shoots of moderately resistant cv GS-23 PPO recorded were 4.22 in roots and 4.31 U/mg protein/ min respectively. Whereas in case of roots and shoots of susceptible cultivars M-35-1 and SPV-86 the level of PPO observed was 3.50 and 3.90, 3.30 and 3.80 U/mg protein/ min respectively. This reveals that the level of PPO was more in root and shoots of resistant and moderately resistant cultivar than in case of susceptible cultivars.

In case of 0.4 mM SA treated resistant genotype (DSV-4) the level of PPO was increased by 1.66 fold in roots and 1.68 fold in shoots and in case of moderately resistant genotype (GS-23) the level of PPO was increased by 1.60 and 1.62 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of PPO was increased by 1.10 and 1.15 fold similarly in another susceptible genotype SPV-86 the level of PPO was increased by 1.12 and 1.18 fold in root and shoot respectively as over the control.

In case of pathogen *M. phaseolina* treated resistant genotype (DSV-4) the level of PPO was increased by 1.60 fold in roots and 1.62 fold in shoots and in case of moderately resistant genotype (GS-23) the level of PPO was increased by 1.56 and 1.60 fold in roots and shoots respectively. In susceptible genotype M-35-1 the level of PPO was increased by 1.22 and 1.23 fold where as in SPV-86 the level of PPO was increased by 1.18 and 1.10 fold in root and shoot respectively over the control plants. This study reveals that, SA and *M. phaseolina* treated plants showed high level of PPO in roots and shoots of sorghum genotypes assayed compared to control (Table 5).

DISCUSSION

Biochemical resistance or susceptibility in plants against any disease depends mainly on pre-existing, pre-formed or induced substances by the pathogen in the host. The nutritional status and concentration of biochemical constituents in plants prior to infection may determine the severity of disease. Sometimes, host plant is induced to synthesize these compounds upon infection. In the present study taking one genotypes from each category of resistant, moderately resistant, moderalely susceptible and susceptible groups, an attempt has been made to find out the changes in biochemical parameters present in sorghum plants due to charcoal rot caused by *M. phaseolina*. When the plants are subjected to environmental stresses, the balance between the production of AOS and the scavenging system may be causing oxidative damage (Scandalous, 1993; Alscher *et al*., 1997). In the susceptible cultivar SPV-86 has an efficient scavenging system, which results in low levels of H_2O_2 accumulation. Results correlated with the Madhusudhan *et al*. (2009) showed increased peroxidase, salicylic acid, lipid peroxidation, protein oxidation, hydrogen peroxide and decreased catalase in incompatible host tobamovirus interaction in comparison to compatible hosts. Same way the inoculation of *M. phaseolina* showed increase in H_2O_2 accumulation in resistant cultivars like DSV-4 and GS-23 showed incompatible reaction compared with control and susceptible cultivars showing compatible reaction.

Phenolic compounds are play a major role in plant defence mechanisms. High levels of phenolics were observed in all the cultivars of sorghum upon treatments compared with the control. Phenolic compounds are found in all sorghum cultivars, with variation in the concentration. Present studies have shown that phenolic compounds are associated with plant resistance to pathogenic attack. Phenol content attains a maximum value in resistant cultivars treated with pathogen in DSV-4 and GS-23 whereas, it declined in control ones and susceptible cultivars. In sorghum, a relationship between total sugars and phenols in sorghum roots and the first internodes and *M. phaseolina* infection was recorded in resistant genotypes having 2 - 3 times higher levels than susceptible ones (Anahosur and Naik, 1985; Patil *et al.,* 1985; Vandana *et al*., 2014) these phenol levels could assist in identifying sources of resistance.

Salicylic acid (SA), a plant hormone widely distributed in angiosperms, is known to be a key element in pathogen defense and its simple and accurate quantification in plants is therefore of prominent importance. Hypersensitive response, systemic acquired resistance, pathogenesis-related (PR) gene expression, oxidative burst, and programmed cell death are the various resistance mechanisms involved by SA signaling (Klessig and Malamy 1994; Ryals *et al*., 1996). The quantification of SA revealed that, in incompatible interactions, the accumulation is more than those of the compatible interactions. SA accumulation is more in resistant cultivars (DSV-4 and GS-23) treated with pathogen as compared to control. Whereas SA accumulation was found to be less in susceptible cultivars (M-35-1 and SPV-86). The central role of salicylic acid is mediating defense against a wide variety of pathogens. These results are in coordination with that of observations in barley, where salicylic acid accumulation in infected leaves was pathogen specific (Vallelian-Bindschedler *et al*., 1998).

Catalase (EC 1.11.1.6) scavenges hydrogen peroxide (H_2O_2) and it directly dismutates to H_2O (Chen *et al*., 1993). Salicylic acid involved in plant defense responses binds and inhibits catalase activity and consequently elevates cellular levels of H_2O_2. Study reveals that the results are in accordance with these studies as catalase accumulation was more in susceptible cultivars SPV-86 and M-35-1 as compared to the resistant cultivars such as DSV-4 and SPV-86.

It was observed that higher activities of PPO in the resistant cultivars than that of susceptible cultivars of sorghum, in all the treatments. The induced PPO activities in roots and shoots treated with SA, and with pathogen might have also been implicated in induced defense responses against the pathogen invasion. However, low PPO activities were observed in susceptible cultivars. Therefore, induction of PPO is quite likely to govern some mechanism of biochemical resistance in resistant cultivars (DSV-4 and GS-23) that arose from the interaction between *M. phaseolina* and host.

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