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RESEARCH ARTICLE

Salicylic acid and salicylic acid sensitive and insensitive catalases in different genotypes of chickpea against *Fusarium oxysporum* f. sp. *ciceri*

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Abstract Differential expression of catalase isozymes in different genotypes of chickpea resistant genotypes- A1, JG-315, JG-11, WR-315, R_1-315, Vijaya, ICCV-15017, GBS-964, GBM-10, and susceptible genotypes- JG-62, MNK, ICCV-08321, ICCV-08311, KW-104, ICCV-08123, ICC-4951, ICC-11322, ICC-08116 for wilt disease caused by *Fusarium oxysporum*. f. sp. *ciceri* (Foc) was analyzed. Salicylic acid (SA) and H_2O_2 concentrations were determined in control as well as in plants infected with *F. ciceri* and found that the high and low levels of salicylic acid and H_2O_2 in resistant and susceptible genotypes of chickpea respectively. Catalase isozyme activities were detected in the gel and found that no induction of new catalases was observed in all the resistant genotypes and their some of the native catalase isozymes were inhibited; whereas, induction of multiple catalase isozymes was observed in all the screened susceptible genotypes and their activities were not inhibited upon Foc or SA treatments. The above results support the possible role of these isozymes as a marker to identify which genotype of chickpea is expressing systemic acquired resistance.

Keywords Catalase · Chickpea · *Fusarium oxysporum* f. sp. *ciceri* · H_2O_2 · Salicylic acid

Abbreviations

Foc	*Fusarium oxysporum* f. sp. *ciceri*
SA	Salicylic acid

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Introduction

Plants possess a wide range of active defense responses that contribute to resistance against a variety of pathogens. Pretreatment of plants with biotic or abiotic inducers can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial site of infection. When a virulent pathogen infects plants, it often triggers the synthesis of various defense related enzymes and antimicrobial compounds at the site of infection, which restricts the growth of pathogen and renders it avirulent, resulting a hypersensitive response (HR) (Dixon and Lamb 1990). This is followed by an increase in the levels of salicylic acid (SA) at the site of HR lesions (10 to 50-fold) and throughout the plant (2 to 5-fold) (Klessig and Malamy 1994; Ryals et al. 1996). SA is most important systemic signal molecule, several attempts have been made to induce resistance by increasing SA content of the plant. The role of SA and SA inhibitable catalases in disease resistance was well studied in tobacco (Chen and Klessig 1991; Chen et al. 1993) and chickepea (Raju et al. 2007, 2008).

Catalases are heme containing enzymes involved primarily prevailing the potential damaging effect caused by H_2O_2. H_2O_2 has been identified as a potential signal in plant defense response came with the identification of catalase as a salicylic acid binding protein. Catalase was proposed to be a receptor that becomes inactive after SA binding. Plants unlike animals, have multiple isoforms of catalases that are the main route of H_2O_2 degradation and hence inhibition of catalase activity results in increased levels of H_2O_2 or related active oxygen species (AOS) acts as a signal for the expression of defense-related genes and possibly in the development of enhanced disease resistance (SAR) (Chen et al. 1993)

Elevated levels of H_2O_2 are toxic to the plants, whereas at lower concentration it acts as signal molecule. Catalase from cucumber, tomato, Arabidopsis and tobacco were substantially inhibited by SA whereas those from maize, rice and

some genotypes of chickpea (Raju et al. 2007) were insensitive (Sanchez-casas and Klessing 1994). Thus there is a close correlation between the SA and inhibition of catalases by SA in different genotypes of chickpea. Considerably higher intrinsic levels and increased activities of antioxidant enzymes, like catalase in susceptible genotypes and decreased levels in resistant genotypes were observed under the influence of SA (Raju et al. 2007), which further supports the possible role of these enzymes in establishing the SAR.

Inhibition of catalases by SA plays an important role in establishing SAR in chickpea genotype ICCV10 (Raju et al. 2007, 2008). The induction of systemic acquired resistance (SAR) by SA and Foc, was investigated in two different genotypes of chickpea L550 and ICCV10 which were susceptible and resistant to wilt disease caused by Foc and found that susceptible genotypes could not express SAR whereas, resistant genotypes expressed SAR (Raju et al. 2008). Gayatridevi et al. (2012) have purified the catalase isozymes from the resistant chickpea genotype ICCV-10 and showed that one of the catalase from the shoot was insensitive to SA whereas other two catalases were inhibited by SA at the physiological concentration.

The chickpea is one of the most important crop in arid and semi arid tropics and represent the largest number of genotypes. Each genotype has its own resistant characters. It is very difficult for the breeder to distinguish between resistant and susceptible genotypes unless they are specified. It is not known in other genotypes of chickpea plants, whether catalases are inhibited by SA. In order to confirm the correlation between the levels of SA and SA sensitive catalases in different genotypes of chickpea, the present study was aimed to screen different genotypes of chickpea plants for the inhibition of catalases by SA.

Materials and methods

Treatment

Seeds of Chickpea (*Cicer arietinum* L.) resistant and susceptible genotypes (Table 1) to wilt disease caused by Foc were procured from the Agriculture Research Station, Gulbarga, India. The seeds were surface sterilized with 0.1 % aqueous $HgCl_2$ for 1–2 min and then thoroughly washed with double distilled water. There were three replications with 60 seeds for each treatment. The first set was supplied with distilled water to serve as a control, while set two was inoculated with the pathogen and a third set was treated with 0.8 mM of salicylic acid. All the petri plates lined with double layer of filter paper were kept wet by supplying respective solutions and allowed to germinate at 26 °C up to 10 days. Shoots and roots were separated and used for enzyme extraction. All the chemicals and reagents used were of analytical grade.

Inoculation with *F. oxysporum* f. sp. *ciceri* (race1)

Culture of *Fusarium oxysporum* f. sp. *ciceri* (race 1) causing wilt disease in chickpea was obtained from the Agriculture Research Station Gulbarga, India. 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 a petri-plates. The micro conidial was prepared by pouring 20 ml of sterile distilled water in each Petri-plate. The concentration of micro conidia was adjusted to 3,000 conidial mL^{-1}. Then, the 10 days old seedlings (shoot length: 10 cm) were sprayed with a spore suspension prepared as above. The shoots and roots of the genotypes were collected after 10 days of treatment for the isolation of catalases.

Enzyme extraction

Weighed sample (5 g) (shoots and root) were grounded in a pre chilled pestle and mortar in 5 ml ice cold 0.1 M Tris–HCl buffer, pH 7.5 containing 5 mM β-Mercaptoethanol. The extract was centrifuged at 10,000 g for 25 min at 4 °C. The supernatant thus obtained was used as enzyme source for the determination of catalase activity. The protein concentration of supernatant was determined by Lowry et al. (1951) method using bovine serum albumin as a standard.

Enzyme assay

The activity of catalase was determined by 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 % substrate. One unit of activity was defined as the amount of enzyme catalyzing the decomposition of H_2O_2 μM/min.

Native PAGE and activity staining

Equal amount of protein extracted from the shoot and root samples of control, SA and pathogen treated were subjected to native PAGE using 8 % polyacrylamide under non-denaturing conditions (Laemmli 1970) without SDS. Catalase isoforms were visualized by following the modified method described by (Woodbury et al. 1971). Gels were soaked in 1.3 mM H_2O_2 for 25 min at room temperature and briefly rinsed with distilled water containing 1 % potassium ferricynaide and 1 % ferric chloride and visualized catalase isozymes.

Table 1 Catalase isoforms* of chickpea in different genotypes treated with SA and Foc

Sl. No.	Cultivar	Genotype	Number of catalase isoforms					
			Control		SA treatment		Foc treatment	
			Root	Shoot	Root	Shoot	Root	Shoot
1	A-1	R	1	1	0	1	0	1
2	JG-315	R	1	3	0	2	0	2
3	JG-11	R	1	3	1	1	1	1
4	WR-315	R	1	1	0	1	0	1
5	R_1-315	R	1	1	1	0	1	0
6	Vijaya	R	1	3	1	1	1	1
7	ICCV-15017	R	2	1	1	1	1	1
8	GBS-964	R	1	2	0	2	0	2
9	GBM-10	R	3	1	1	1	1	1
10	ICC-4951	S	1	1	2	2	2	2
11	ICCV-08321	S	2	2	6	5	6	5
12	ICCV-08311	S	1	1	3	4	3	4
13	KW-104	S	1	2	3	4	3	4
14	MNK	S	1	1	2	3	2	3
15	JG-62	S	1	1	2	2	2	2
16	ICC-08116	S	2	2	3	3	3	3
17	ICC-11322	S	ND	ND	ND	ND	ND	ND
18	ICCV-08123	S	ND	ND	ND	ND	ND	ND

*Catalase isoforms were determined by Native-PAGE. *S* Susceptible, *R* Resistant. *ND* Not detected

H_2O_2 estimation

For the estimation of H_2O_2, method of Noreen and Ashraf (2009) was followed. Fresh sample of shoot and root were homogenized in 2 ml of 0.1 % (w/v) TCA 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 μMcm^{-1} and expressed as $\mu M/g^{-1}$ FW.

Estimation of SA

Root and shoot of chickpea from control and Foc infected plants of different genotypes were collected from 10 days old plants, weighed, and frozen in liquid nitrogen. For each sample, 1 g of the frozen tissue was extracted for free salicylic acid essentially as described previously (Malamy et al. 1992; Hennig et al. 1993). Briefly, the tissue was homogenized in 3 mL of 90 % methanol. After centrifugation, the pellet was reextracted with 100 % methanol. The combined supernatant was dried in a speed vacuum with heat (40 °C). The residue was resuspended in 2.5 mL of 5 % 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 was acidified with HCl to pH 1 and 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 under nitrogen. The dried extract was suspended in 96 % methanol. The absolute values were measured at 300 nm for the determination of SA (Kokat and Burda, 1998).

Results

Detection of catalase isozymes in native PAGE

In plant cells, catalase enzymes exhibit multiple isoforms and their composition were analyzed by native PAGE. Native gels stained for catalase activity in the control shoots and roots of resistant genotypes revealed more than one isoforms, and activities of some of these isoforms was inhibited upon treatment with SA or Foc. Whereas, control root and shoot extract of susceptible genotypes revealed

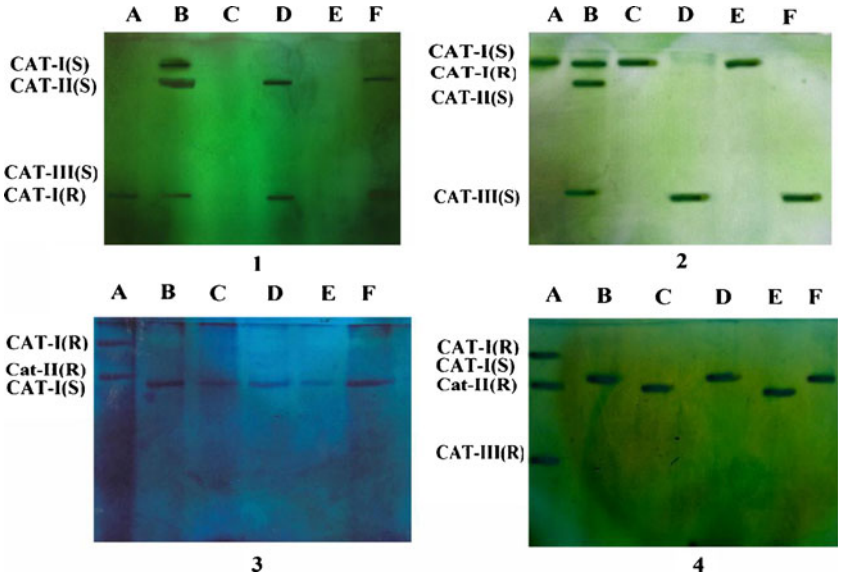

Fig. 1 Native gel stained for catalase activity from root and shoot extract of chickpea resistant genotypes JG-315 (**1**), JG-11(**2**), A-1 (**3**) and WR-315 (**4**), (*A*) control root; (*B*) control shoot: (*C*) SA treated root; (*D*) SA treated shoot; (*E*) pathogen infected root; (*F*) pathogen infected shoot; Equal amount of protein was loaded on gels (20 μg)

induction of new isoforms of catalases in addition to their native isoforms upon treatment with SA and Foc (Table 1). For example, in A1 genotype, root catalase was inhibited upon treatments. Similar inhibition was also observed in all other resistant genotypes. However, in susceptible genotype ICC4951, induction of new catalases was observed in both root and shoot. Similar observations were noted in all other susceptible genotypes. Native gel stained for catalase isoforms in some of the resistant genotypes are shown in Fig. 1. The chickpea genotype JG-315 showed single isoform in control root (1A) and shoot (1B) extract, upon treatments with SA or Foc, only the root catalase was inhibited (1C and E), but not the shoot catalase (1D and F) respectively. However, in JG-11 genotype, single isoform of catalase in root (2A) and three isoforms in shoot (2B) of control extract were observed. Upon treatments with SA or Foc, root catalase was not inhibited whereas, shoot catalases I and II were inhibited. Similar type of inhibition patter was observed in other resistant genotypes viz. A-1 and WR-315 (Fig. 1: 3 and 4). On the other hand, the susceptible genotype JG62 was shown to contain single isoform of catalase in both the control root Cat- I(R) and shoot Cat-I(S) extract (Fig. 2; 1A and B). However, upon treatments with SA or Foc, induction of new isoforms of catalase in shoots as well as in roots (shown in arrows) was observed. Similar pattern of induction of new catalase isoforms was observed in all the susceptible genotypes (Fig. 2: 2, 3 and 4).

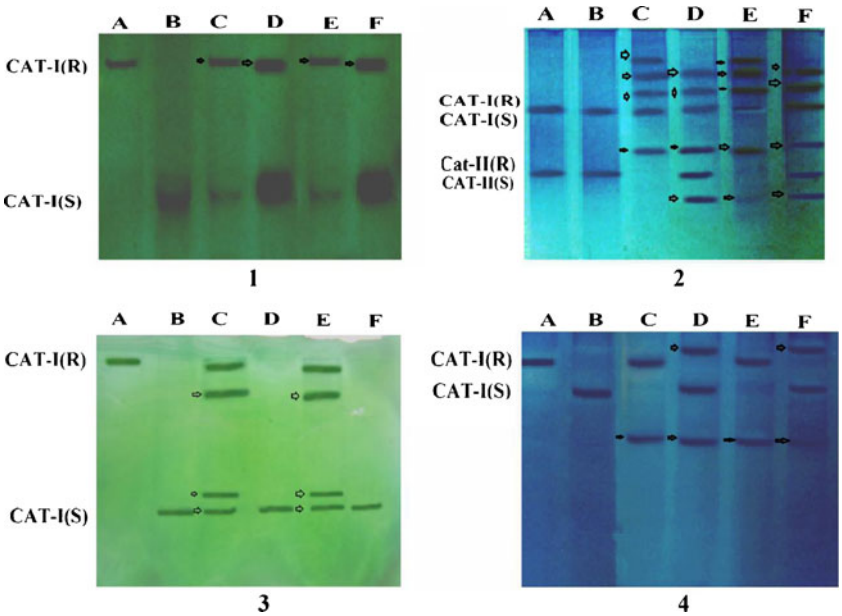

Fig. 2 Native gel stained for catalase activity from root and shoot extract of chickpea susceptible genotypes: JG-62 (**1**), ICCV-08321 (**2**), ICCV-08311 (**3**) and MNK (**4**). (*A*) control root; (*B*) control shoot: (*C*) SA treated root; (*D*) SA treated shoot; (*E*) pathogen infected root; (*F*) pathogen infected shoot; Equal amount of protein was loaded on gels (20 μg). *Arrows* indicate induction of new isofoms of catalases

Table 2 Levels of H_2O_2, catalase activity and SA in control, Foc and SA treated roots and shoots of resistant and susceptible genotypes of chickpea

Sl. No.	Cultivar	H_2O_2 level (μM/g fresh Wt)						Catalase activity (U/mg)	
		Control		SA		FOC		Control	
		R	S	R	S	R	S	R	S
1	A-1	0.20±0.01	0.24±0.05	0.51±0.00	0.56±0.03	0.50±0.04	0.54±0.02	20±2.01	18±1.66
2	JG-315	0.31±0.02	0.36±0.02	1.86±0.00	0.74±0.06	1.07±0/07	0.90±0.02	15±1.11	33±3.22
3	JG-11	0.41±0.01	0.38±0.01	1.9±0.03	1.40±0.09	1.80±0.09	1.50±0.09	12±1.04	41±4.10
4	WR-315	0.36±0.06	0.34±0.00	1.88±0.03	1.58±0.07	1.75±0.06	1.52±1.11	16±1.32	18±1.81
5	R_1-315	0.51±0.00	0.46±0.02	2.10±0.03	0.69±0.06	2.20±1.02	0.70±0.07	10±1.01	13±1.26
6	Vijaya	0.47±0.03	0.24±0.01	1.60±0.05	0.60±0.05	1.90±1.00	0.42±0.05	13±1.29	39±3.89
7	ICCV-15017	0.21±0.06	0.32±9.00	0.72±0.03	0.88±0.08	0.77±0.06	0.80±0.05	23±2.14	15±1.51
8	GBS-964	0.12±0.03	0.36±0.02	0.97±0.06	0.80±0.07	1.01±0.06	0.86±0.06	13±1.29	22±1.99
9	GBM-10	0.21±0.01	0.41±0.02	0.61±0.09	1.09±0.09	0.60±0.04	1.11±0.09	41±4.12	20±2.05
10	ICC-4951	0.37±0.01	0.29±0.04	0.06±0.04	0.05±0.01	0.06±0.05	0.04±0.01	17±1.65	13±1.16
11	ICCV-08321	0.26±0.00	0.38±0.03	0.01±0.01	0.11±0.01	0.01±0.00	0.17±0.01	21±2.01	22±2.08
12	ICCV-08311	0.19±0.01	0.16±0.04	0.05±0.02	0.02±0.01	0.04±0.01	0.01±0.00	13±1.30	09±0.83
13	KW-104	0.23±0.03	0.21±0.02	0.05±0.02	0.05±0.02	0.04±0.01	0.06±0.00	36±3.54	35±3.67
14	MNK	0.19±0.03	0.11±0.03	0.04±0.01	0.05±0.03	0.04±0.02	0.05±0.02	19±1.60	25±2.78
15	JG-62	0.20±0.05	0.18±0.01	0.06±0.00	0.06±0.04	0.06±0.03	0.04±0.01	20±1.32	27±3.08
16	ICC-08116	0.23±0.02	0.21±0.01	0.12±0.01	0.08±0.01	0.10±0.01	0.10±0.03	21±1.08	19±2.11
17	ICC-11322	0.19±0.01	0.20±0.02	0.10±0.01	0.09±0.02	0.10±0.01	0.08±0.01	23±1.34	20±2.98
18	ICCV-08123	0.16±0.01	0.20±0.02	0.09±0.02	0.10±0.00	0.09±0.00	0.07±0.01	26±2.65	18±3.11

Sl. No.	Cultivar	Catalase activity (U/mg)				SA levels (μM)			
		SA		FOC		Control		FOC	
		R	S	R	S	R	S	R	S
1	A-1	02±0.12	16±1.54	02±0.02	17±1.67	0.08±0.001	0.7±0.002	1.20±0.03	1.23±0.03
2	JG-315	00±0.00	23±2.17	00±00	21±1.98	0.08±0.001	0.3±0.001	1.7±0.001	2.3±0.003
3	JG-11	11±1.01	26±1.98	13±1.03	31±2.76	0.07±0.002	0.8±0.001	2.8±0.001	3.6±0.003
4	WR-315	14±1.13	00±00	16±1.54	00±00	0.04±0.002	0.6±0.003	2.1±0.002	3.1±0.021
5	R_1-315	10±0.87	00±00	10±1.12	00±00	0.05±0.001	0.6±0.001	1.9±0.003	2.1±0.040
6	Vijaya	10±1.05	03±0.6	08±0.78	00±00	0.06±0.001	0.6±0.003	4.6±0.021	4.1±0.060
7	ICCV-15017	10±0.67	13±0.8	09±0.90	15±1.10	0.07±0.002	0.8±.0021	2.0±0.023	2.6±0.054
8	GBS-964	09±00	21±1.80	06±00	18±1.31	0.08±0.004	0.08±0.04	1.6±0.027	1.9±0.011
9	GBM-10	05±0.13	18±1.97	03±0.30	17±1.13	0.06±0.002	1.2±0.002	1.7±0.040	2.9±0.05
10	ICC-4951	33±2.98	41±4.13	31±3.07	40±4.01	0.05±0.001	1.0±0.016	2.0±0.032	2.8±0.076
11	ICCV-08321	56±5.31	49±4.67	59±5.64	43±4.00	0.4±0.001	0.3±0.002	0.5±0.001	0.2±0.002
12	ICCV-08311	11±0.93	23±1.87	12±1.11	27±2.12	0.6±0.001	0.5±0.001	0.7±0.041	0.5±0.032

Table 2 (continued)

Sl. No.	Cultivar	Catalase activity (U/mg)				SA levels (μM)			
		SA		FOC		Control		FOC	
		R	S	R	S	R	S	R	S
13	KW-104	47±4.67	51±4.98	43±4.12	57±5.65	1.0±0.014	0.9±0.005	1.0±0.067	1.0±0.035
14	MNK	40±3.19	42±4.13	41±1.40	46±4.43	0.6±0.016	0.4±0.009	0.7±0.087	0.5±0.022
15	JG-62	39±0.08	41±2.09	40±1.09	40±2.88	0.7±0.026	1.3±0.031	07±0.021	1.4±0.017
16	ICC-08116	39±2.90	41±2.71	40±2.98	41±3.88	0.3±0.021	0.7±0.035	0.3±0.04	0.7±0.060
17	ICC-11322	38±3.07	40±3.56	43±1.56	43±3.77	0.4±0.033	0.6±0.036	0.4±0.02	0.7±0.040
18	ICCV-08123	37±2.98	45±4.1	35±3.98	42±4.10	1.5±0.006	1.2±0.004	1.5±0.003	1.3±0.003

Each value represents the Mean ± SE average of three independent experiments. *R* Root, *S* Shoot, *SA* Salicylic acid treated, and *FOC Fusarium oxysporum* f.sp. *ciceri* infected

Hydrogen peroxide content

SA and Foc treated plants showed high levels of H_2O_2 in roots and shoots of all the chickpea genotypes compared with the water treated controls. The level of H_2O_2 increased by 2 folds in roots and shoots of susceptible genotypes upon treatments. In the resistant genotypes H_2O_2 levels were increased by 3–5 folds in roots and shoots as compared with that of water treated controls. For example, in resistant genotype GBM-10, highest level of H_2O_2 was observed in SA treated soot (1.09 μM) and Foc infected shoot (1.11 μM) whereas, in susceptible genotype ICCV-08321, contains lowest level of H_2O_2 in SA and Foc treated root (0.01 μM) respectively. Overall, the accumulation of H_2O_2 was higher in resistant genotypes that of the susceptible genotypes (Table 2).

Catalase activity

Upon treatments with SA and Foc, the catalase activity of susceptible genotypes increased significantly by 1.5 to 2.6 fold in roots and 2 to 3 folds in shoots with respect to their water treated control. On the other hand, catalase activities of resistant genotypes markedly decreased by 37–40 % in roots and 27–30 % in shoots compared with their respective controls (Table 2)

SA estimation

Levels of the SA were found to be substantially higher in Foc treated roots and shoots than those of control root and shoot of resistant genotypes whereas, in susceptible genotypes showed lower levels of SA compared with their corresponding root and shoots of control plant extract. Among the number of genotype screened, the resistant genotype GBM-10 showed the highest levels of SA (2.9 μM) whereas, the susceptible genotype ICCV-08321 showed lowest levels of SA (0.2 μM) in Foc infected shoot (Table 2). Overall, 3–4 fold increase of SA was observed in the resistant genotypes than that of susceptible genotypes (Table 2).

Discussion

Pre-treatment of plants with different biotic (pathogens and insect pests) and abiotic inducers (chemicals) induce plant resistance, that defends the plants against their subsequent attack. The plant phytohormones induce plant defense against many biotic and abiotic stresses (Maffei et al. 2007; Noreen and Ashraf 2009). This induction of plant defense is mediated through various physiological, biochemical and molecular mechanisms (Mohd et al. 2011). Salicylic acid is an important and well-studied endogenous

plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in plant defense in addition to their impact on plant growth and development (Vicent and Plasencia 2011; Lu 2009; Mohd et al. 2011). SA also activates the generation of ROS and other defensive processes such as hypersensitive response and cell death (Hayat et al. 2009). Hydrogen peroxide is an important signaling molecule that mediates the synthesis of many defensive compounds in plants in response to biotic and abiotic stresses. (Maffei et al. 2007; Noreen and Ashraf 2009; Kawano 2003; Barbehenn et al. 2010). Hydrogen peroxide content was elevated in the SA-treated plants.

In the present study, we have shown that the activity of catalase enzymes and its isozyme pattern exhibited differently in relation to genotype upon treatment with SA and Foc (Table 1). The induction of multiple isoforms of catalase indicates that the susceptible genotypes have an efficient scavenging system which results in low levels of H_2O_2 accumulation (Table 2), which would not serve as a second messenger for the induction of defense responses. From this observation, it is clear that the plant recognizes SA through a mechanism identical with those used to detect pathogen infection and therefore, unable to establish SAR. In contrast, resistant genotypes have shown high levels of H_2O_2 through lower activities of catalase enzymes, upon treatments with SA and Foc. Salicylic acid has been reported to inhibit catalase, the H_2O_2 scavenging enzyme, increasing H_2O_2 level in treated tobacco leaves (Wendenhenne et al. 1998). During plant pathogen interactions, the activities and levels of the reactive oxygen species (ROS) detoxifying enzyme, catalase was suppressed by SA and nitric oxide (Klessig et al. 2000). In resistant genotypes the increased levels of H_2O_2 are due to inhibition of catalases that would serve as a second messenger for the induction of defense responses.

Production of ROS at elevated levels is a common feature of defense response in plants, when they are challenged by pathogens and elicitors, H_2O_2 functions as a second messenger, mediating the systemic expression of various defense related genes in tomato plants (Martha et al. 2001). Production of ROS in plants in response to stress is a common phenomenon (War et al. 2011). They play a potent role in plant defense against biotic and abiotic stresses either by direct toxicity or by activating defensive enzymes (Maffei et al. 2007; Noreen and Ashraf 2009). Among the ROS, H_2O_2 is very important, because it is stable and easily diffusible through the cell membranes (Maffei et al. 2007). H_2O_2 triggers several physiological and molecular processes in plants that signal the production of various defensive compounds and enzymes, which in turn modify plant resistance against stress. It has been suggested that SA application leads to the uptake of exogenous SA into the veins that results in H_2O_2 accumulation (Ganesan and Thomas 2001).

In the present study, it was found that the pathogen-inoculated shoots and roots contain high level of free SA compared with controls. Catalases of shoot and root extract also exhibited differences in sensitivity to SA. The difference in SA sensitivity of catalases from these different tissues correlates with the tissue specific expression of these enzymes. In contrast, rice root and cell-suspension cultures had a low level of free SA and contained SA-insensitive catalases. This correlation supports the proposal that plant SA-sensitive catalases play a role in certain aspects of the SA mediated response (Howe and Jander 2008). At the site of infection, SA levels can reach maximum, a concentration sufficient to cause substantial inhibition of catalase and ascorbate peroxidase, the other major H_2O_2 scavanging enzyme (Durner et al. 1997). The increase in SA levels was also observed in cucumber, tobacco and Arabidopsis, infected by *Colletotrichum lagenarium*, tobbacco necrosis virus, turnip crinkle virus respectively followed by SAR development and induction resistance (Dempsey and Klessig 1994).

The susceptible genotypes (for wilt disease) expressed multiple catalases, followed by no induction of SAR, while the resistant genotypes did not express multiple catalases and their native catalase isoforms were inhibited by SA, which followed by expression of SAR (Raju et al. 2007, 2008). In the present study we confirmed the same by screening 16 different genotypes of chickpea. A decrease in catalse activity in the resistant genotype may have enhanced the H_2O_2 above the threshold levels. Even though there was a marginal increase in H_2O_2 degrading enzymes in the resistant genotypes, this might not have contributed significantly to a reduction in H_2O_2 levels, hence promoting SAR. Based on the above studies, we conclude that the catalases of all the resistant genotypes of chickpea were sensitive to SA, whereas all the susceptible genotyeps were insensitive to SA. Hence, inhibition of catalases by SA could be used as a marker to identify which genotype is resistant or susceptible to Foc.

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