Salicylic acid induces resistance to Alternaria blight in crop *Brassica* species

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Abstract

Alternaria blight, caused by *Alternaria brassicae*, is a major disease afflicting *Brassica* crops in India. Reduced bioefficacy and environment costs of recommended fungicides have spawned efforts to develop ecobenign management strategies. Induced resistance, through metabolic enhancement of plants owns defensive responses is one such option. In the present communication, results of experiment to manage alternaria by application of Salicylic acid (SA), a known inducer of systemic acquired resistance pathway, are presented. The experiment comprised SA application @ 50 mg/l and 75 mg/l on two genotypes, *B. napus* cv. GSC 5 and *B. juncea* cv. ELM 079, before as well as after the onset of disease symptoms. SA @ 75 mg/l before disease onset proved better as evidenced by decreased disease severity on leaves; 29.7% in *B. napus* cv. GSC-5 and 32.6% in *B. juncea* cv. ELM 079 as compared to unsprayed control. The disease severity was also lower (24.8% in *B. napus* and 30% in *B. juncea*) when SA was sprayed after the onset of disease SA in both instances, provided better protection than recommended chemical fungicide (Biltox @ 250 mg/l). Greater yield increase (8.4% in *B. napus* and 9.5% in *B. juncea*) was observed when SA was sprayed before the disease onset as compared to SA sprayed after disease onset (7.4% and 7.6% in *B. napus* and *B. juncea* respectively). In both instances the yield was higher than chemical control (4.2% in *B. napus* and 4.4% *B. juncea*) over the unsprayed control. Secondary metabolites like total phenols and O-hydroxy phenols showed increased level, three days after the SA application. Increase being higher when SA was sprayed before the onset of disease symptoms. The activities of defense related enzymes viz., phenylalanine ammonia lyase, peroxidase and polyphenol oxidase also increased, three days after SA spray. Like the secondary metabolites, enzyme activities also showed higher appreciation when SA was sprayed before the onset of disease. Changes in protein polymorphism, following SA application will be discussed.

Key words: *Brassica*, Alternaria Blight, Salicylic acid, Biltox and induced resistance.

Introduction

Alternaria blight, caused by *Alternaria brassicae,* is a menace for rapeseed-mustard crop in India. Yield losses may vary upto 38-45% (Kolte and Singh 1997). The control of disease through chemicals is quite expensive and is not ecofriendly. Under these situations induction/amplification of natural defense mechanism using elicitors appear exciting. In recent years, salicylic acid has been the focus of attention due to its protection inducible properties against various plant pathogens (Raskin, 1992). It is an orthohydroxybenzoic acid which belongs to a group of plant phenolics. It plays an important role as signal for disease resistance (Mauch-Mani and Metraux, 1998) and development of systemic acquired resistance (SAR) allowing plants to defend themselves against pathogens. The SAR response causes rapid accumulation of several pathogenesis related (PR) proteins. Salicylic acid application is also known to increase activities of antioxidative defense enzymes (Fodor *et al* 1997). In this communication we present an account of our efforts to manage *Alternaria brassicae,* a necrotic fungus in *Brassica,* through application of salicylic acid. Biochemical mechanisms involved in this induced resistance were also investigated.

Materials and Methods

Brassica napus cv. GSC-5 and *Brassica juncea* cv. ELM-079 were raised in the fields. Standard agronomic practices were followed throughout the growing season. Salicylic acid (SA) application @ 50 and 75 mg/l were made before or after the onset of disease alongwith Blitox [chemical control (CC)] @ 250 mg/l at intervals of 70, 85 and 100 days. Simultaneously control plots [disease control (DC)] were sprayed with water. The experiment was conducted over two years. Leaf samples (3rd, 4th and 5th leaves from the top) were collected on third day after SA application for the biochemical analysis. Total phenols, orthohydroxy phenols and flavanols were estimated by the standard methods. Activity of peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) was measured by standard methods.

Results

In the present experiments, the effects of exogenous SA application for Alternaria blight control in crop *Brassicas* were studied in comparison to Blitox, the recommended chemical control. Although Blitox and SA application, both resulted in significant decrease in disease severity on leaves and pods, yet disease severity was minimum when SA @ 75 mg/l was sprayed before the on set of disease; 29.7% in *B. napus* cv GSC-5 and 32.6% in *B. juncea* cv ELM-079 as compared to control (Table 1) on leaves and 23% in *B. napus* and 28% in *B. juncea* on pods. Consequent to decrease in disease severity maximum yield was observed in plots sprayed with SA @ 75 mg/l (Table 1) before disease onset (8.4% more in GSC-5 and 9.5% more in ELM-079 compared to controls).

Table 1: Effect of SA application on Alternaria blight severity (%) and yield (kg/ha) in crop *Brassicas*

Treatment	Alternaria Severity (%)				Yield kg/ha	
	Leaves		Pods			
	GSC-5	ELM-079	GSC-5	ELM-079	GSC-5	ELM-079
DC (water)	26.6	35.6	24.3	31.6	791.6	1308.3
CC (250 mg/l)	21.0	32.4	21.3	28.3	825.0	1366.6
SAB (50 mg/l)	19.6	25.0	20.3	23.2	841.6	1416.6
SAB (75 mg/l)	18.3	24.0	18.7	22.7	858.3	1433.6
SAA (50 mg/l)	20.3	26.6	20.0	24.3	833.3	1391.3
SAA (75 mg/l)	20.0	24.6	19.3	23.3	850.0	1408.3
CD (5%)	2.49	1.79	2.02	1.45	NS	NS

DC: Diseased control-water; CC: Chemical control-Blitox; SAB: Salicylic acid spray before disease onset; SAA: Salicylic acid spray after disease onset

At the biochemical level, the investigated phenolic enzymes namely, peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) showed significant upscaling in their activities in leaves after SA spray at the concentration of 50 mg/l and 75 mg/l, before the onset of disease. However, the increase in activities was higher at 75 mg/l as compared to disease control (DC) and chemical control (CC) (Table 2). Peroxidase is known to help in H_2O_2 supported lignification of cell wall, thereby, restricting the invasion of pathogen to cause disease (Sreedhara *et al* 1995). Polyphenol oxidase and phenylalanine ammonia lyase have been reported to aid the synthesis of quinones (with greater toxicity) from phenols and biosynthesis of phenylpropanoid units for synthesis of lignins and flavonols respectively. These also have a role in restricting the spread of the pathogen. Increase in the level of these defensive enzymes in response to elicitor treatment has been previously recorded in many other crops also (Li *et al*, 1991). SA is also known to induce expression of number of defense related proteins (Hunt & Ryals, 1996; Sticher *et al* 1997 and Yang *et al* 1997). The level of secondary metabolites such as total phenols and orthohydroxyphenols also increased dramatically, being maximum at 75 mg/l SA when applied before onset of disease. The increase in flavonol content was not very significant. Presence of phenols and their oxidative products in plant tissue is considered toxic to growth and development of pathogen (Mandavia *et al*, 2000).

Table 2: Effect of SA application on activities of defensive enzymes and phenolics in crop *Brassicas*.

Parameters	GSC-5							ELM-079						
	DC (water)	CC(250 mg/l)	SAB (50 mg/l)	SAB (75 mg/l)	SAA (50 mg/l)	SAA (75 mg/l)	CD (5%)	DC(water)	CC (250 mg/l)	SAB (50 mg/l)	SAB (75 mg/l)	SAA (50 mg/l)	SAA (75 mg/l)	CD (5%)
POD(ΔE min^{-1} mg $protein^{-1}$)	1.57 ±0.02	1.65 ±0.02	1.89 ±0.04	2.65 ±0.04	1.71 ±0.02	1.93 ±0.02	0.04	1.62 ±0.04	1.92 ±0.04	2.93 ±0.05	4.34 ±0.04	1.98 ±0.04	3.14 ±0.04	0.05
PPO(ΔE min^{-1} mg $protein^{-1}$)	0.05 ±0.003	0.07 ±0.003	0.09 ±0.002	0.18 ±0.004	0.08 ±0.005	0.15 ±0.004	0.01	0.06 ±0.002	0.07 ±0.004	0.12 ±0.003	0.22 ±0.003	0.10 ±0.002	0.19 ±0.004	0.004
PAL (µg t-cinnamic acid formed hr^{-1} mg $protein^{-1}$)	40.98 ±2.28	50.44 ±2.06	60.09 ±1.71	83.41 ±1.95	53.45 ±2.31	58.19 ±2.03	3.02	49.29 ±2.40	58.04 ±2.11	61.24 ±2.35	95.45 ±2.30	59.19 ±2.30	70.14 ±2.65	3.50
Total phenols (mg/gm dry tissue)	4.84 ±0.84	5.04 ±0.24	7.19 ±0.22	8.33 ±0.25	6.09 ±0.35	6.91 ±0.23	0.62	5.52 ±0.12	6.67 ±0.12	6.83 ±0.09	7.24 ±0.12	5.93 ±0.11	6.28 ±0.15	0.18
O-hydroxy phenols (mg/gm dry tissue)	1.41 ±0.11	2.45 ±0.14	2.85 ±0.12	3.31 ±0.17	2.51 ±0.10	2.68 ±0.07	0.178	1.48 ±0.11	2.50 ±0.12	2.97 ±0.12	3.53 ±0.12	2.53 ±0.08	3.13 ±0.10	0.16
Flavonols (mg/g dry tissue)	1.36 ±0.06	1.34 ±0.04	1.38 ±0.04	1.48 ±0.05	1.36 ±0.04	1.42 ±0.03	0.07	1.38 ±0.07	1.46 ±0.07	1.48 ±0.08	1.50 ±0.05	1.42 ±0.05	1.39 ±0.07	NS

DC: Diseased control-water; CC: Chemical control-Blitox; SAB: Salicylic acid spray before disease onset; SAA: Salicylic acid spray after disease onset

Conclusion

It was evident from the results that SA when applied @ 75 mg/l before the onset of disease provided better protection against alterneria blight as compared to standard chemical control. These results were supported by noticeable induced appreciation in activities of several defensive enzymes and proteins.

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