RESEARCH ARTICLE

Biochemical basis of resistance in wheat against Karnal bunt caused by *Tilletia indica*

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ABSTRACT: The biochemical basis of Karnal bunt resistance was evaluated in four resistant (ALDAN, CMH77.308, H567.71 and HP1531) and two susceptible (PBW343 and HD2967) genotypes. The parameters studied included total phenols and defense related enzymes (peroxidase, phenylalanine ammonia lyase and polyphenol oxidase). The level of total phenols and activity of the defense related enzymes was more in resistant as compared to the susceptible genotypes. However, there was variation within the resistant genotypes. Maximum level of total phenols was observed in CMH77.308 at 3 days after inoculation while maximum peroxidase activity was observed in H567.71 after 2 days of inoculation. The activity of polyphenol oxidase was highest after 3 days of inoculation in the resistant genotype HP1531 while maximum activity of phenylalanine ammonia lyase was observed in H567.71 after 3 days of inoculation.

Keywords: Karnal bunt, peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, *Tilletia indica*, total phenols, wheat

Karnal bunt (KB) or partial bunt of wheat incited by *Tilletia indica* has been reported from several wheat growing countries of the world including India (Rush *et al.*, 2005). Due to its partial nature, yield losses are few but quality is a serious issue with the import of KB infected grains. The quarantine regulations or zero tolerance by many countries makes it a serious non-tariff barrier to international trade.

Being a seed, soil and air-borne disease, it is difficult to manage it. There are conventional approaches such as crop rotation, sowing of disease free seeds and adjustment of the time of irrigation to minimize KB infection (Singh, 1985). Similarly, chemical control of the disease is very much effective but is neither eco-friendly nor cost effective (Singh *et al.*, 1985). Deployment of host plant resistance is the most effective and economical method for KB management but it is a tedious task owing to labour intensive screening procedures. Nevertheless, differential resistance at biochemical level would help to understand nature of Karnal bunt resistance and its further exploitation for effective disease control. Several attempts have been made to know the basis of biochemical resistance of wheat diseases (Johnson and Lee, 1978; Moerschbacher *et al.*, 1989; Patykowski *et al.*, 1988; Rengel *et al.*, 1994). However, very few workers have reported this aspect with respect to KB (*T. indica*) - wheat host pathogen interactions. Therefore, the present study was planned to elucidate the biochemical basis of resistance in KB resistant and susceptible genotypes.

MATERIALS AND METHODS

Material

The present investigation was conducted in the experimental fields and laboratories of Department of Plant Breeding & Genetics and Department of Biochemistry, Punjab Agricultural University, Ludhiana during the period 2013-2015. Sowing of four Karnal bunt resistant (ALDAN, HP 1531, CMH 77.308 and H567.71) and two susceptible (HD 2967 and PBW 343) wheat genotypes was done in the last week of November. Sowing was done at a spacing of 22.5 cm in 2 x 2 m² beds. After sowing of wheat, all the cultural operations were done according to the standard practices (Anonymous, 2014).

Pathogen isolation, multiplication and maintenance

Twenty isolates of *T. indica* were collected from all over the Punjab. Isolation, purification, mass multiplication and maintenance of all the isolates were done on Potato Dextrose Agar (PDA) medium. Inoculum was prepared by mixing all the available isolates in equal proportions. For preparing the inoculum suspension, sterilized distilled water was added to the test tubes and with the help of glass rod, the fungal sporidia were gently scrapped to remove allantoid secondary sporidia. Inoculum suspension was then filtered through double layer of muslin cloth and its concentration was adjusted at more than 10,000 sporidia per ml.

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Table 1. Total phenolic content in resistant and susceptible wheat genotypes after inoculation with *T. indica*

Genotype	Total phenolic content (mg g ⁻¹ dry weight) after days					
	0	1	2	3	4	5
ALDAN	18.80	37.27	43.60	50.07	36.03	31.37
CMH77.308	26.90	37.43	48.00	78.83	57.09	33.90
H567.71	25.73	43.50	45.40	53.47	38.77	24.33
HP1531	22.17	45.77	55.80	68.07	48.80	27.97
HD2967	12.57	18.13	21.46	24.60	15.93	14.93
PBW343	11.33	16.03	26.10	19.57	16.23	13.47
CD (p=0.05)	Genotype (A) = 1.87, Days after inoculation (B) = 1.87, AxB =4.57					

Artificial inoculation of the genotypes and collection of samples

In each of the six parents, 25 plants were inoculated at boot leaf stage by syringe inoculation method (Aujla *et al.*, 1982). The inoculated ears were used for estimation of defense related enzymes after one day interval till five days of inoculation.

Biochemical analysis

Estimation of total phenols, peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was done by following standard methods given by Swain and Hills (1959), Shannon *et al.* (1996), Zauberman *et al.* (1991) and Burrell and Rees (1974), respectively.

RESULTS AND DISCUSSION

The results obtained in the present study with respect to total phenols and defense related enzymes against *T. indica*, the causal agent of Karnal bunt are presented and interpreted individually as follows:

Total phenolic content

An increase in total phenolic content after inoculation was noticed in all the wheat genotypes. This was followed by a decrease after the peak concentration. The peak concentration in all the genotypes was achieved after 3 days of inoculation (DAI) except PBW 343 which had the maximum level of total phenols after 2 DAI. A perusal of the data presented in Table 1 showed that among the resistant genotypes, total phenol content was maximum (78.8 mg g⁻¹ dry weight) in CMH77.308 at 3 DAI followed by HP1531 (68.07 mg g⁻¹ dry weight), H567.71 (53.47 mg g⁻¹ dry weight) and ALDAN (50.07 mg g⁻¹ dry weight). PBW343 and HD2967, being the susceptible checks exhibited lower phenolic content (26.10, 2DAI and 24.60, 3DAI mg g⁻¹ dry weight, respectively) as compared to resistant genotypes.

Activity of defense related enzymes

Peroxidase activity: Four genotypes viz., ALDAN, CMH 77.308, HP 1531 and HD 2967 exhibited maximum

peroxidase activity 3 DAI whereas it was 2 DAI in H 567.71 and PBW 343 (Table 2). The level of peroxidase (POD) activity was statistically at par in H567.71 (178.1 units min⁻¹ g⁻¹ fresh weight, 2DAI), and CMH 77.308 (176.8 units min⁻¹ g⁻¹ fresh weight, 3DAI) irrespective of DAI with *T. indica*. In HP 1531, the level of POD activity (174.8 units min⁻¹ g⁻¹ fresh weight, 3DAI) was at par with CMH 77.308 but it was different from H567.71. In susceptible genotypes, the increase was significantly less than resistant ones.

Polyphenol oxidase activity: A noticeable difference was observed in polyphenol oxidase (PPO) activity in the resistant as compared to the susceptible genotypes of wheat to *T. indica* (Table 2).

Considering all the genotypes, peak activity of the enzyme was observed 3 DAI except PBW 343 which revealed maximum PPO activity 2 days after inoculation. Among the resistant genotypes, maximum PPO activity was observed after 3 DAI in HP 1531 (21.6 units min⁻¹ g⁻¹ fresh weight) and H567.71 (19.4 units min⁻¹ g⁻¹ fresh weight), and it was minimum in ALDAN (14.3 units min⁻¹ g⁻¹ fresh weight). Among the susceptible genotypes, HD 2967 had more level of PPO (10.4 units min⁻¹ g⁻¹ fresh weight) as compared to PBW 343 (9.7 units min⁻¹ g⁻¹ fresh weight, 2 DAI). The results revealed that resistant genotypes showed significantly more level of PPO as compared to the susceptible genotypes.

Phenylalanine ammonia lyase activity: A perusal of the data presented in Table 2 indicated that among the resistant genotypes, H 567.71 (1098.2 µg t cinnamic acid formed hr⁻¹ g⁻¹ fresh weight) and CMH77.308 (1037.2 µg t-cinnamic acid formed hr⁻¹ g⁻¹ fresh weight) showed maximum phenylalanine ammonia lyase (PAL) activity after 3 DAI, while minimum PAL activity (917.1 µg t-cinnamic acid formed hr⁻¹ g⁻¹ fresh weight, 4DAI) was observed in HP1531. Among susceptible genotypes, HD2967 (564.4 µg t-cinnamic acid formed hr⁻¹ g⁻¹ fresh weight, 4 DAI) exhibited more PAL activity as compared to PBW 343 (511.9 µg t-cinnamic acid formed hr⁻¹ g⁻¹ fresh weight, 4 DAI). PAL activity was significantly more in the resistant as compared to the susceptible genotypes. Peak activity of the enzyme was observed at 3 DAI in two genotypes (CMH 77.308, H567.71) and after 4 DAI in remaining four other genotypes (ALDAN, HP 1531, HD2967, PBW 343).

Table 2. Dynamics of defense related enzymes in resistant and susceptible wheat genotypes days after inoculation with *T. indica*

Genotype	Peroxidase activity (units/ min/g fresh weight) days after inoculation (DAI)						Polyphenol oxidase activity (units/ min/ g fresh weight) DAI						Phenylalanine ammonia lyase activity (µg t-cinnamic acid formed/ hr/g fresh weight) DAI					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
ALDAN	63.8	96.1	128.9	146.4	121.8	101.7	8.4	10.2	11.2	14.3	12.7	9.4	364.2	520.5	656.9	843.7	1015.3	844.7
CMH77.308	82.3	122.7	165.6	176.8	156.1	133.4	8.8	12.4	15.7	16.7	13.9	12.7	337.5	507.2	715.0	1037.2	932.4	830.4
H567.71	79.2	124.0	178.1	167.6	149.1	112.2	9.9	13.4	14.2	19.4	17.7	16.0	348.9	549.1	804.6	1098.2	1035.3	902.8
HP1531	81.3	125.7	165.1	174.8	141.9	90.2	9.1	12.8	16.9	21.6	15.6	13.6	328.9	462.4	579.6	839.9	917.1	822.7
HD2967	44.5	59.3	71.9	85.9	65.3	55.9	7.3	9.7	10.3	10.4	8.9	7.6	277.4	344.2	372.8	553.9	564.4	504.3
PBW343	57.1	67.3	77.1	64.4	59.2	47.7	7.5	9.4	9.7	8.7	7.7	7.0	264.1	311.7	360.4	448.1	511.9	498.6
CD (p=0.05)	Genotype (A) = 2.41, Days after inoculation (B) = 2.41, AxB = 5.91						Genotype (A) = 0.24 Days after inoculation (B) = 0.24 AxB = 0.59						Genotype (A) = 12.47, Days after inoculation (B) = 12.47, AxB = 30.55					

The data presented in both the tables (Table 1, 2) showed an increase in the total phenolic acids as well as defense related enzymes viz., POD, PPO and PAL after inoculation with *T. indica* irrespective of the genotypes. However, the level of increase in resistant genotypes was significantly higher than the susceptible ones suggesting a role of these chemicals in resistance against *T. indica*.

Among the resistant genotypes, maximum total phenols were exhibited by CMH77.308. The POD and PAL activity was maximum in H567.71. Likewise, HP1531 had maximum PPO activity after inoculation with the test pathogen. This difference in level of production of these defense related chemicals may be attributed to the genetic potential of the genotypes. However, both the susceptible genotypes were statistically different from each other with respect to production of phenols and defense related enzymes, however, the difference was small.

The results obtained in the present study can be corroborated with the earlier findings. Gill *et al.* (1993) correlated phenol status of wheat with KB resistance. Similarly, Bakhshi *et al.* (1989) reported that the increase in the phenolics in the KB resistant genotypes could be due to the activities of enzymes such as polyphenol oxidase and peroxidase during host pathogen interactions.

Furthermore, Gogoi *et al.* (2001) have also described role of phenols and peroxidase in imparting resistance to wheat against Karnal bunt of wheat. According to them, phenols were induced in the both resistant and susceptible genotypes of wheat after inoculation with *T. indica*, but were synthesized at higher than normal levels in the resistant as compared to the susceptible genotypes only during the early stages of infection by the pathogen, being enhanced upto 6 days in the resistant genotype DWL 5023.

Purwar *et al.* (2012) while investigating the role of defense related enzymes in imparting resistance against Karnal bunt of wheat reported that PAL and PPO activity was significantly higher in all the developmental stages of resistant genotype (HD 29) than the susceptible genotype (WH 542).

Disease resistance in plants is associated with activation of a wide array of defense responses that slow down or halt infection at certain stages of the host pathogen interactions and total phenols and defense related chemicals impart resistance against a pathogen. The present study indicated the possible role of the total phenols and defense related enzymes in imparting resistance against the *T. indica* in wheat.

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