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RESEARCH ARTICLE

Post-infectional phenolic changes in maize due to *Rhizoctonia solani* f. sp. *sasakii* causing banded leaf and sheath blight

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ABSTRACT: A basic study was undertaken to find out the possible role of the phenolics involved in resistance against banded leaf and sheath blight of maize. The phenolic content in all cultivars of maize increased after infection. This increase was more pronounced in resistant cultivars as compared to susceptible cultivars. The highest increase (32.14%) in accumulation of phenolics was noticed in diseased leaves of genotype BVM-4 infected with the *Rf* isolate. Accumulation of total phenol in diseased leaves of 10 genotypes against 5 isolates of *R. solani* varied from 119.67 to 160.33 µg/g fresh weight and in healthy leaves 111.66-124.33 µg/g fresh weight. Further analysis of the data revealed that the disease severity was negatively correlated with the accumulation of phenol having coefficient of correlation r = -0.83.

Key words: Banded leaf and sheath blight, maize, phenols, resistance, *Rhizoctonia solani*

Among fungal diseases, leaf blights of maize caused by various pathogens such as *Rhizoctonia solani*, *Helminthosporium maydis*, *Helminthosporium turcicum* and *Physoderma maydis* are important. In Jharkhand, banded leaf and sheath blight caused by *Rhizoctonia solani* f. sp. *sasakii* (Kuhn) Exner is endemic and in high rainfall areas the attack is usually preceded. Singh and Sharma (1976) recorded a loss in grain yield ranging from 11.8 to 40.5 per cent. As the disease is assuming alarming status, there is an urgent need for devising suitable measures to manage the disease. Resistance present in some selected genotypes has not been commercially exploited to produce resistant genotypes of maize. Among antifungal natural products, phenolics have been considered as one of the most prominent chemical groups with ubiquitous occurrence in plants and microbes (Harborne, 1988; Waterman and Mole, 1994). Therefore, an attempt was made to study the role of total phenols in resistance to banded leaf and sheath blight caused by *R. solani* on ten maize genotypes.

MATERIALS AND METHODS

The experiment was conducted at glasshouse of the Department of Plant Pathology, Birsa Agricultural University, Ranchi during *Kharif* 2006-07. Maize seedlings were raised in pots and after 45 days of sowing, test genotypes namely BVM-1; BVM-2; BVM-4; BVM-5; BVM-6; BVM-7; BVM-8; BVM-9; BVM-10 and Suwan were inoculated separately with five isolates of *R. solani* isolated from research farm of Birsa Agricultural University, Kanke, Hisri Chauli, Bero, Jirabar and Itki villages of Ranchi district. Kanke is situated at 23^0 17' North latitude and 85^0 19' East longitude with an altitude of 625 meter above mean sea level in the Chotanagpur region of Jharkhand state, India. Hisri Chauli, Bero, Jirabar and Itki villages are located at an altitude of 660-700 meter above mean sea level. The isolates were designated as Rf, Hc, Be, Jr and It based on locality from where these isolates were isolated. Inoculations were made by inserting 2 to 3 maize grains covered with fungal growth of the pathogen isolates gently between the rind and the leaf sheath of test plants (Ahuja and Payak, 1978).

After 21 days of inoculation total phenols were estimated by the method of Bray and Thorpe (1954). One gram of healthy as well as infected leaf samples were weighed and grounded separately with a pestle and mortar in 10 cm^2 of 80 per cent ethanol. The homogenates were centrifuged at 10,000 rpm for 20 min. and the resulting supernatant was kept separately. The residue was re-extracted with 5 times of its volume of 80 per cent ethanol, centrifuged and the supernatants pooled. Evaporation of the supernatant was done by agitating for 15 min. at 70^0C. The residue was dissolved in 5 ml of distilled water. Various aliquots (0.2 to 2 ml) were pipetted into test tubes and distilled water was added to bring the volume to 3 ml in each tube. After that 0.5 ml of folin ciocalteu reagent was added to each tube. After 3 min, 2 ml of 20 per cent Na_2CO_3 solution was added to each tube. The contents of each tube were thoroughly mixed and the tubes were placed in boiling water for one minute, after which they were removed and cooled. Absorbance was measured using a spectrophotometer at 650 nm against a reagent blank. A standard curve was prepared using different concentrations of catechol. From the standard curve the concentrations of phenols in the sample were observed and expressed in terms of µg / g material. The disease severity was recorded 30 days after inoculation using 1-5 scale (Vimla and Mukherjee, 1987) as 1.0 (up to 10%) = resistant (R); 2.0 (10.1-25.0%) = moderately resistant (MR); 3.0 (25.1-50%) = moderately susceptible (MS); 4.0 (50.1-75.0%) = susceptible (S); 5.0 (>75.0%) = highly susceptible (HS).

RESULTS AND DISCUSSION

Changes in the level of total phenols were determined in healthy and infected leaves of 10 genotypes of maize.

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Quantification of total phenol of healthy and diseased leaves and disease severity as well as reaction of each of the 10 genotypes inoculated with different isolates of *R. solani* are presented in Tables 1-3 and Fig. 1-2.

As compared to healthy leaves, genotype BVM-1 accumulated 22.03 per cent higher phenols in samples inoculated with the *Be* isolate; samples infected with the *Hc* isolate resulted in the least increase (8.15%) in accumulation of total phenols. In diseased samples of genotype BVM-2 highest concentration with maximum increase (18.50%) in total phenols over healthy samples was recorded against *Jr* isolate. However, only 6.5 per cent increased accumulation was noticed in samples infected with the *Hc* isolate. The accumulation of phenol in genotype BVM-4 inoculated with the Rf isolate was found highest (32.14%) as compared to healthy samples and only 9.89 per cent increased accumulation of total phenols was recorded due to infection caused by Hc isolate.

Infection caused by the Hc isolate to BVM-5 showed maximum level of total phenols. But the healthy samples of the same genotype accumulated 15.72 % less total phenols as compared to diseased samples infected with *Hc* isolate. A little increase in total phenol accumulation was observed in the samples inoculated with the *Be* isolate. Diseased samples of genotype BVM-6 revealed increased accumulation of total phenols by 18.83 per cent upon infection caused by the Jr isolate as compared to healthy samples. Infection by the Hc isolate resulted in only 5.98 per cent increased accumulation. The Jr isolate inoculated on genotype BVM-7 resulted in a higher (16.12%) production of total phenols as compared to healthy samples, only 8.07 per cent increased accumulation was recorded in leaf samples because of infection caused by the Rf isolate. The per cent increase in accumulation of total phenols in diseased samples of genotype BVM-8 inoculated with Hc isolate was higher (28.65%) as compared to healthy samples and only a 12.67 per cent increase in the accumulation of total phenols was recorded in leaf samples infected with It isolate. Infection of the BVM-9 genotype with the *Be* isolate showed increased accumulation of total phenols by 26.76 per cent in comparison to healthy samples. But diseased samples infected with the Rf isolate resulted in increased accumulation by only 9.02 per cent as compared to healthy samples. There was a 25.95 per cent increase in the accumulation of phenols in genotype BVM-10 inoculated with the Rf isolate compared to healthy samples; only 7.92 per cent increase accumulation was recorded in leaf samples infected by Hc isolate. In the Suwan genotype, infection by the It isolate resulted in a 22.25 per cent increased accumulation of total phenols as compared to healthy samples, but disease samples infected with the Hc isolate accumulated 7.25 per cent increased total phenols (Table 1 & Fig. 1).

The inoculation of genotype BVM-1 with all 5 isolates of the pathogen revealed that isolate Hc caused above 66.00 per cent disease severity. The isolates Jr, It and Rf showed disease severity of approximately 15.00 per cent. The isolate Be caused only 7.60 per cent severity. With respect to genotype reaction, BVM-1 was susceptible to isolate Hc, moderately resistant to isolates Jr, It and Rf and resistant to Be. Against genotype BVM-2, isolate Hc caused maximum disease severity and therefore susceptible to isolate Hc. Against isolates Be, It and Rf, the reaction was moderately resistant because the disease severity ranged from 15.00 to 16.50 per cent. The inoculation of genotype BVM-4 with the isolates revealed that Hc isolate caused above 24.00 per cent disease severity and this genotype showed moderately resistant reaction to this isolate. Isolates Jr and It also caused moderately resistant reaction and the remaining two isolates Be and Rf caused resistant reaction. Inoculation of the host genotype BVM-5 with five isolates of *R. solani* revealed that this genotype was highly susceptible to isolates Hc and Rf. The isolate Jr caused susceptible reaction. The interactions of host genotype BVM-6 with the isolates Hc and It yielded high disease severity. Against two isolates Be and Rf, the reaction was moderately resistance. The inoculation of BVM-6 with the isolate Jr revealed that this isolate caused 6.60 per cent severity and showed resistant reaction against this isolate. In genotype BVM-7, isolate Rf caused maximum disease severity (74.60%) followed by Be (63.40%). Against the isolates Jr and Hc, resistant and moderately resistant reactions were recorded in BVM-7, respectively. The inoculation of genotype BVM-8 with 5 isolates revealed that isolate Be caused maximum blight severity (16.10%) followed by It isolate (15.00%). With the remaining isolates the damage caused was least and, therefore, resistant. The genotype BVM-9 showed susceptible reactions to isolate Rf and moderate resistance to isolates Hc, Jr and It. The genotype showed resistance reaction to isolate Be as the disease severity was only 6.10 per cent. The inoculated plants of

Table 1. Estimation of total phenols in maize genotypes inoculated with *Hc*, *Be*, *Jr*, *It* and *Rf* isolates of *R. solani*

Isolate	Concentration of total phenols (µg/g) in different genotypes									
	BVM-1	BVM-2	BVM-4	BVM-5	BVM-6	BVM-7	BVM-8	BVM-9	BVM-10	Suwan
Hc	131.00	128.00	133.33	140.00	128.33	129.00	143.66	134.33	131.67	133.34
Be	154.33	136.66	144.67	122.67	134.34	122.67	134.00	150.00	141.67	135.67
Jr	135.33	147.34	140.67	132.34	148.66	129.66	155.67	127.00	144.33	141.34
It	139.67	142.33	137.00	125.00	133.00	126.67	136.33	141.34	134.66	152.00
Rf	142.66	133.00	160.33	119.66	141.67	120.67	139.67	135.00	153.66	142.33
Control	120.33	119.67	121.33	112.34	120.66	121.00	118.33	122.00	124.33	111.66
Av. Increase (%)	16.84	14.87	18.03	11.15	13.71	12.60	17.24	16.12	15.74	13.36
CD at 1%	3.12	4.72	3.03	4.92	5.97	4.90	3.94	3.50	4.41	3.68

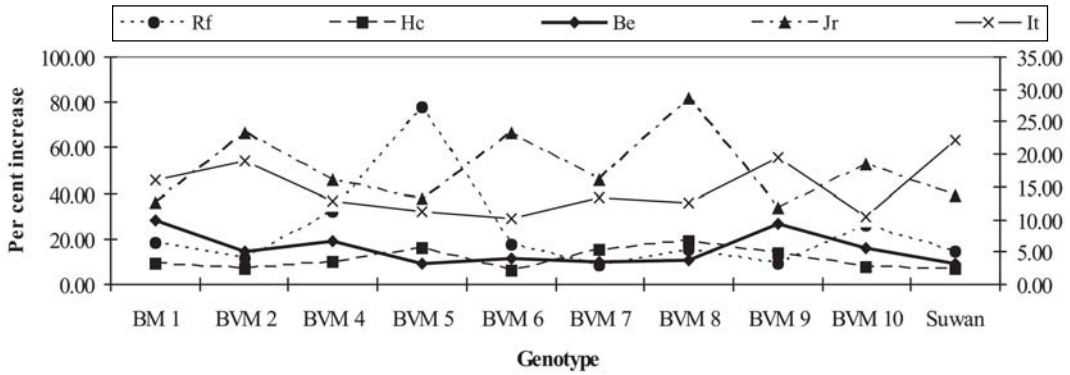

Fig. 1. Per cent increase in phenol accumulation in different genotypes inoculated with different isolates of *R. solani*

genotype BVM-10 caused a disease severity of 74.70 per cent against isolate Hc and, therefore, this genotype showed susceptible reaction. Isolates Be and It also showed susceptible reaction and moderately resistant reaction to isolate Jr. Resistant reaction was recorded in this genotype upon the infection caused by isolate Rf. The genotype Suwan was found resistant to isolates It and Rf and moderately resistant against isolates Be and Jr. The genotype showed moderately susceptible reaction to isolate Hc as the disease severity was 43.50 per cent (Table 2).

Further interpretation of the host x pathogen interactions revealed that none of the host genotypes were highly susceptible to isolates Be, Jr and It. Against Hc isolate, genotype BVM 6 showed highly susceptible reaction. Against isolate Be, none of the genotypes showed highly susceptible reaction but three of them showed moderately resistant and five of them moderately resistant reactions. The reactions between isolates and genotypes were interesting that none of the genotypes were highly susceptible except BVM-5. The difference in amount of disease produced on different maize genotypes by the pathogen isolates was significantly different from each other and among the genotypes, BVM-4 was least susceptible.

Further analysis of the data revealed that the disease severity was negatively correlated with the accumulation of phenol having coefficient of correlation r = -0.83 (Fig. 2). Highest phenol accumulation of 160.33 µg/g of leaf tissue was recorded in genotype BVM-4 against isolate Rf, which showed least disease severity of 2.5 per cent whereas least phenol accumulation of 128.00 µg/g was recorded in genotype BVM-2 against isolate Hc recording highest disease severity of 73.20 per cent.

Synthesis of aromatic substances is a major defense mechanism of plants. These substances include phenols, phenolic acids, flavanoids, tannins and lignins (Harborne, 1988; Mahadevan, 1991; Waterman and Mole, 1994). Synthesis of phenolics in plants is altered due to wounding and infection by pathogens (Dubeler *et al.*, 1997). Conversion of phenols to non-toxic compounds is widespread among microorganisms. Pathogens achieve this by producing oxidative enzymes. The presence of a high concentration of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction (Farkas and Kirlay, 1962). After infection by a pathogen, plant cells synthesise phenol oxidising enzymes that oxidise phenols into toxic quinines, which play a crucial role in disease resistance (Vidyasekaran, 1988; Ashry and Mohamed, 2011).

Estimation of total phenol in healthy and diseased leaves of 10 genotypes indicated varying levels of phenol in leaves infected by different isolates of the pathogen. The

Table 2. Disease severity of maize genotypes inoculated with different isolates of *R. solani*

Genotype	Disease severity (%) and host reaction produced by different isolates				
	Hc	Be	Jr	It	Rf
BVM-1	66.80 (S)	7.60 (R)	15.50 (MR)	15.00 (MR)	4.40 (R)
BVM-2	73.20 (S)	16.50 (MR)	2.70 (R)	14.60 (MR)	43.40 (MS)
BVM-4	24.70 (MR)	3.30 (R)	13.20 (MR)	15.00 (MR)	2.50 (R)
BVM-5	15.00 (MR)	15.00 (MR)	62.10 (S)	16.20 (MR)	93.30 (HS)
BVM-6	93.00 (HS)	13.40 (MR)	6.60 (R)	70.40 (S)	14.60 (MR)
BVM-7	14.30 (MR)	63.40 (S)	2.70 (R)	60.20 (S)	74.60 (S)
BVM-8	6.20 (R)	16.10 (MR)	2.70 (R)	15.00 (MR)	23.40 (MR)
BVM-9	20.00 (MR)	6.10 (R)	16.30 (MR)	14.60 (MR)	61.80 (S)
BVM-10	74.70 (S)	63.90 (S)	15.00 (MR)	67.20 (S)	3.30 (R)
Suwan	43.50 (MS)	15.00 (MR)	14.60 (MR)	2.70 (R)	16.20 (MR)

CD (P = 0.05) Isolates = 4.06; Genotypes = 5.75 and; Isolates x genotypes = 12.86
CV (%) = 26.84

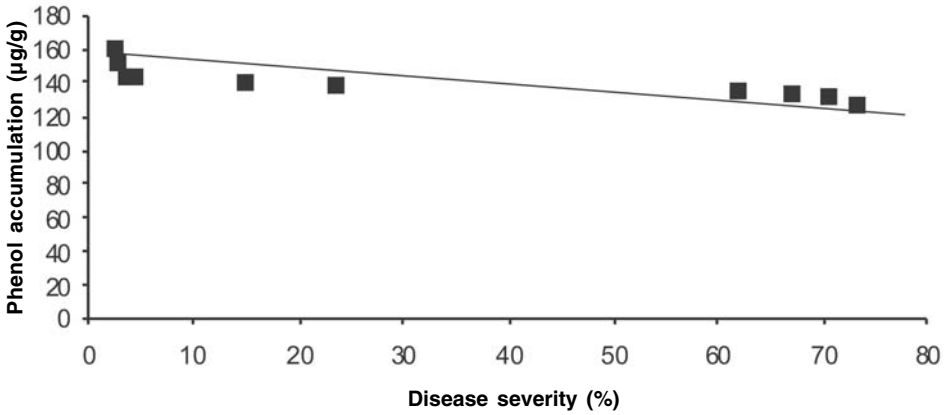

Fig. 2. Correlation of phenol accumulation with disease severity in different genotypes inoculated with different isolates of *R. solani*

results suggested that the accumulation of total phenol was higher in infected genotypes as compared to healthy. The increase in total phenols may probably be because of the activity of peroxidase and polyphenol oxidase enzymes. The increase in phenolics in the diseased samples revealed that phenolics play an important role in inducing resistance against further invasion of the pathogen as they are toxic to the pathogens.

In many cases, a close correlation has been found between the concentrations of phenolic compounds and plant resistance. These observations have led to the conclusion that phenolic substances contribute to the resistance of plants against pathogens. Opinions on the role of phenolics in host resistance have been documented in several reviews (Farkas and Kirlay, 1962; Hare, 1966; Kosuge, 1969; Gangopadhayay and Lal, 1986; Sindhan and Jaglan, 1988; Bajaj, 1988; Kuc, 1992; Sukhwal and Purohit, 2003; Ashry and Mohamed, 2011). Our data showed that increased disease resistance was associated with total phenol in infected leaves. The cause and effect relations for disease resistance are not clear but the possible use of these phenols as a marker for disease resistance is worth exploring.

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