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Biochemical and histochemical parameters associated with slow blighting of spot blotch (*Bipolaris sorokiniana* (Sacc.) Shoem.) in wheat (*Triticum* spp.)

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Abstract

Spot blotch caused by *Bipolaris sorokiniana* is a destructive disease of wheat (*Triticum* spp.) in warm and humid wheat growing regions of the world. Area under the disease progress curve (AUDPC) in field experiment was used to find out the role of total phenol content (TPC), phenylalanine ammonia-lyase (PAL) activity and lignin deposition in the development of slow blight. Ten recombinant inbred lines (RILs) of spring wheat obtained from the cross between the susceptible parent 'Sonalika' and the resistant parent 'Yangmai 6' along with their parental genotypes were scored for disease severity (DS), AUDPC and lesion size in a field experiment on spot blotch. These lines were also evaluated for TPC, PAL and lignin deposition. The minimum DS, AUDPC and lesion size were recorded in the resistant parent 'Yangmai 6' (13%, 116.04 and 0.23 cm^2, respectively) and RILs (18%, 113.3 and 0.27 cm^2, respectively in RIL83 and RIL89) which associated with an elevated level of phenol content (395 mg g^{-1} fresh weight (wt) at 48 hours after inoculation (hai), PAL (928.41 μmoles cinnamic acid (cna) mg^{-1} fresh wt) and higher quantity of lignin (84%). While DS, AUDPC and lesion size were comparatively higher in the susceptible parent 'Sonalika' as well as susceptible RILs (100%, 938.27 and 3.43 cm^2, respectively). Mean TPC (133.5 mg g^{-1} fresh wt 24 hai, respectively), PAL (248.8 μmoles cna mg^{-1} fresh wt) and lignin (10%) were significantly lower in the susceptible genotype 'Sonalika' and suscptible RILs. The study indicated that enhanced level of TPC, PAL and higher lignin deposition led to the development of slow blighting of spot blotch in terms of lower AUDPC and smaller lesion size in resistant RILs of wheat.

Key word: area under disease progress curve, *Bipolaris sorokiniana*, lignin, phenolics, phenylalanine ammonia-lyase.

Introduction

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum*, teleomorph *Cochliobolous sativus*) is an important wheat disease in warmer and humid growing regions of the world such as Eastern India, South East Asia, Latin America, China and Africa (Joshi et al., 2007). Yield losses were estimated to be 18–22% in India (Saari, 1998). Hence, search of effective non-fungicidal control of spot blotch disease is of utmost importance. The best, long term, economically and environmentally safe method for sustainable disease control is the use of resistant varieties. Slow disease development is characterized by several components like low area under the disease progress curve, smaller lesion size and less lesion number (Bashyal et al., 2011). However, no single component is sufficient to determine the resistance since the mechanism is a complex phenomenon. Most plants produce a broad range of secondary metabolites that are toxic to pathogens, either as part of their normal growth and development or in response to biotic stresses. It has been well documented in several pathosystems that phenolic compounds like phytoalexins or phytoanticipins, or physical barrier, i.e. lignins can play an important role in disease resistance, thus preventing plant tissue colonization (Nicholson, Hammerschmidt, 1992). Rapid accumulation of phenols at the infection site, slow the growth of the pathogen and allow activation of phytoalexins or other stress related substances (Matern, Kneusel, 1988). A study has indicated that total phenol contents (TPCs) were significantly higher in resistant varieties compared to the susceptible ones to *Alternaria triticina* (Mishra et al., 2011). Phenylalanine ammonia-lyase (PAL) being the first step in the phenylpropanoid biosynthesis pathway, also plays an important role in biosynthesis of the different families of phenolics (coumarins, flavonoids, lignins), phenolic derivatives and its activity being correlated with the level of synthesis of the phenolic compound (Podile, Laxmi, 1998). The induction of PAL activity preceding an increase in the

phenolics content has been observed in response to fungal infection in a number of systems (Mazeyrat et al., 1999; Pereira et al., 1999). An active role of PAL and phenolics in expression of resistant reaction in various crop species was reported (Nicholson, Hammerschmidt, 1992). The target should be to identify the biochemical compounds involved in resistance, in order to use them as molecular markers in plant breeding programmes or to design appropriate control strategies. Differences in biochemical components, i.e. TPC, PAL and lignin deposition have been used as markers for preliminary selection of different plant species resistant to different pathogens. These enzymes have been correlated with the defense activities against pathogens in several plant species (Thilagavathi et al., 2007). Peltonen and Karjalainen (1995) reported that PAL activity got enhanced in the leaves of resistant cultivars of barely at 24–32 and 40 hours after inoculation of *B. sorokiniana.* Fortification of the cell walls by the intensification of lignin and the accumulation of cell wall bound phenolic compounds to many plant pathogens was reported (Niemann et al., 1991). Therefore, studying the mechanisms at the cellular and biochemical levels is expected to give a better understanding about resistance operating in wheat against *B. sorokinana*. The present study aims to find out the role of PAL, TPC and lignin in the development of slow blighting of spot blotch in the recombinant inbred lines (RILs) of wheat. This study also explores the possibility of using these factors as components of resistance with phenotypic parameters for efficient selection of spot blotch resistant genotypes of wheat.

Materials and methods

Plant materials and field trial. Ten recombinant inbred lines (RILs) of F_{10} progeny of the cross 'Yangmai 6' × 'Sonalika' (Kumar et al., 2009) along with their parents were evaluated in the field at the Agricultural Research Farm of Banaras Hindu University, Varanasi, India (North-Eastern Plains Zone, 25.2 N and 83.0 E) during the crop seasons 2009–2010 and 2010–2011. Each year, the lines were evaluated in three randomized complete block design. Each line was sown in single row of 3 m under irrigated condition. Row to row and plant to plant distance was 25 and 5 cm, respectively. To secure the highest possible disease pressure, sowing was carried out during the fourth week of December which allows the post-anthesis growth stage (GS) 50 (Zadoks et al., 1974) to coincide with warm temperature conducive to the disease in the second week of February to first week of March. Agronomic practices recommended for normal fertility (120 kg N, 60 kg P_2O_5, 40 kg K_2O) were followed. The full dose of P_2O_5 and K_2O was applied at the time of sowing. Nitrogen was given as split application: 1/2 at sowing, 1/4 at first irrigation (21 days after sowing), and 1/4 at the time of second irrigation (40 days after sowing).

Inoculation of the pathogen. A pure culture of the most aggressive isolate of *Bipolaris sorokiniana* (NABM MAT1; NCBIJN128877, Banaras Hindu University, India) was used to create artificial inoculation (Joshi, Chand, 2002). The pathogen was multiplied on wheat (*Triticum* spp.) grain and a spore suspension adjusted to 10^{-4} spore ml^{-1} of water using haemocytometer and was uniformly sprayed at GS 50 during evening hours.

Grouping of recombinant inbred lines (RILs). Based on phenotypic features of the parental lines, the RILs were grouped into 'Sonalika' and 'Yangmai 6' type to avoid the confounding of the background effect on the disease reaction. The clubbed shaped, short, compact and golden colour ear was the characteristic feature of 'Yangmai 6' while triangular, long, less compact and red colour ear with pseudo black chaff was associated with 'Sonalika' (Table 1).

Table 1. Disease severity (DS), area under disease progress curve (AUDPC) and lesion size for the parental lines 'Sonalika' and 'Yangmai 6' and recombinant inbred lines (RILs) of spring wheat (*Triticum aestivum* L.) against *Bipolaris sorokiniana*

Genotype/RIL	DS %	AUDPC	Lesion size	Reaction	Parental type
'Sonalika'	98	844.44	3.43^{a}	S	ST
'Yangmai 6'	11.11	103.70	0.23^{h}	R	YT
76	29.63^{h}	217.28^{fg}	0.90^{e}	MR	ST
83	18.0^{l}	133.3^{i}	0.60^{fg}	R	YT
89	19.11^{l}	173.0^{hi}	0.27^{h}	R	YT
91	25.11^{l}	183.0^{i}	0.30^{h}	R	ST
94	37.03^{g}	222.22^{gh}	1.00^{e}	R	YT
95	43.21^{e}	321.0^{f}	0.80^{ef}	MR	ST
90	25.0	335.80^{e}	0.47^{gh}	MS	ST
133	89.0^{b}	760.5^{c}	1.60^{cd}	S	YT
134	83.3^{c}	723.5^{d}	1.63^{cd}	S	ST
136	97.0^{a}	806.4^{c}	2.17^{b}	S	YT
$LSD_{0.05}$	0.674	60.588	0.259		

Note. S – susceptible, R – resistant, MR – moderately resistant, MS – moderately susceptible; ST – 'Sonalika' type, YT – 'Yangmai' 6 type. Means values with different letters (small case letter) are significantly different ($P < 0.05$) among RILs whereas the means with same letters are not significantly different at ($P < 0.05$) in each column in the table.

Phenotyping of RILs for disease reaction. RILs for spot blotch were categorized into four groups of reaction types. The RILs that scored less than 30% disease severity (DS) and 1–200 AUDPC were considered resistant; between 40 and 50 DS and 201–350 AUDPC as moderately resistant between 60 and 70 DS and 351–400 AUDPC as moderately susceptible, and those higher than 80% DS and >500 for AUDPC as susceptible (Joshi et al., 2004).

Disease assessment. DS was scored using double digit (DD, 00–99) scale (Saari, Prescott, 1975) in the field under artificial inoculation of ten RILs of wheat along with their parental varieties 'Sonalika' and 'Yangmai 6' at three different growth stages (GS) viz. GS 63 (beginning of anthesis to half complete), GS 69 (anthesis complete) and GS 77 (late milking) on Zadoks scale (Zadoks et al., 1974). The first digit (D1) indicates vertical disease progress on the plant and the second digit (D2) indicates severity measured in diseased leaf area. The DS percentage for each score was based on the following formula:

% severity = (D1/9) (D2/9) 100.

AUDPC was based on DS at GS 63, GS 69 and GS 77and was calculated based on the percent severity estimations corresponding to the disease ratings (Joshi, Chand, 2002; Joshi et al., 2004):

$$AUDPC = \sum_{i=0}^{n-1} [\{(Y_i + Y_{(i+1)}) / 2\} \times (t_{(i+1)} - t_i)],$$

where Y_i – disease level at time t_i, $t_{(i+1)} - t_i$ – time (days) between two disease scores, n – number of dates at which spot blotch was recorded.

Measurement of lesion size in parents and RILs. Five lesions were selected randomly from the tagged flag leaf of the RILs and the parents when the DS in 'Sonalika' was >50% on flag leaf. Lesion size (cm^2) was measured by length and width of lesion produced by the pathogen (Bashyal et al., 2011).

Estimation of total phenol contents (TPCs). TPC was determined according to Kofalvi and Nassuth (1995) method. Flag leaves (0.5 g) were collected from resistant, moderately resistant, moderately susceptible and susceptible RILs at 0 (healthy), 24, 48, and 72 hours after inoculation (hai) in all the three replications. Fresh leaves (0.5 g) were extracted in 50% methanol for 90 min at 80°C. The extract was centrifuged at 14000 g for 15 min. The pellet was saponified with 2 ml of 0.5 N NaOH for 24 h at room temperature to release the bound phenolics, neutralized with 0.5 ml 2 N HCl and centrifuged at 14000 g for 15 min. The supernatant was taken for bound phenolic determination using the Folin-Ciocalteu's assay. One hundred microliters of the methanol and NaOH extracts were diluted with water to make volume 1 ml and mixed with 0.5 ml 2.0 N Folin-Ciocalteu's reagent and 2.5 ml of 20% Na_2CO_3. After 20 min incubation at room temperature, absorbance of samples was measured at 725 nm with a UV-spectrophotometer. Phenolic concentration in the extracts was determined from standard curve prepared with gallic acid.

Estimation of phenylalanine ammonia-lyase (PAL). PAL was estimated according to the procedure of Dickerson et al. (1984). Flag leaves collected from the three replications in the field representing resistant, moderately resistant and susceptible RILs at 0 (healthy), 24, 48 and 72 hai. One gram leaf samples of inoculated leaf tissue was crushed in 10 ml of borate buffer with the help of chilled pestle and mortar. The tissue pulp was then centrifuged at 12000 rpm at 4°C for 10 min and the supernatant collected and used as enzyme extract. Enzyme extract (0.2 ml) from each treatment, was transferred into a separate tube containing 2.5 ml of borate buffer and 1 ml of 0.1 mM phenylalanine (pH 8.8) and incubated for 30 minutes at 32 ± 2ºC. Enzyme reaction was stopped by addition of 0.5 ml 1 M trichloroacetic acid. Absorbance of samples was measured at 290 nm with the help of spectrophotometer. The amount of produced cinnamic acid was determined from trans-cinnamic acid standard curve and PAL activity was expressed as µmoles cinnamic acid produced g^{-1} fresh weight (wt).

Histochemical (lignin deposition). Lignin deposition in resistant and susceptible RILs was conducted using phloroglucinol-HCl (Wiesner) test (Stange, McDonald, 1999). Three flag leaves were taken for each RIL from the three replications. Leaf sections after clearing were soaked in 10% w/v phloroglucinol solution in 95% ethanol for 3 min, drained and placed in a drop of 10 M HCl on a slide, covered with a coverslip, sealed with paraffin, and observed until the leaf veins turned purple-red. A four points scale was used to objectively assess lignification by categorizing into 0 – no lignification, 1 – low lignification, 2 – medium lignification, 3 – high lignification based on the density of colour

Statistical analysis. *Arc Sine* transformation was applied on the data as it was not normally distributed. Analysis of variance (*ANOVA*) for DS, AUDPC, lesion size, TPC, PAL and lignin deposition was performed using *PROC GLM* of *SAS* software, version 9.2 (SAS Institute Inc., Cary, USA, 2010). LSD at 0.05 was used to differentiate DS, AUDPC, lesion size, TPC and PAL activity and lignin deposition among RILs and between different time points of TPC and PAL. Phenotypic correlation coefficient between spot blotch DS, AUDPC, TPC, PAL activity and lignin was calculated using *PROC CORR* of *SAS* software, version 9.2 (SAS Institute Inc., Cary, USA, 2010).

Results and discussion

Phenotypic variation. No line was more susceptible than 'Sonalika', which recorded the highest DS and AUDPC value (100% and 938.27, respectively). While the resistance parent 'Yangmai 6' recorded the minimum (13% and 116.04, respectively). DS and AUDPC ranged from 18% and 113.3, respectively in RIL 83 to 97% and 806.4, respectively, in RIL 136 (Table 1). AUDPC is generally used to evaluate the resistance of plant species to the pathogens (Jeger, Viljanen-Rollinson, 2001). In barely genotypes, AUDPC and lesion size were found to be the first principal components for spot blotch resistance and can be used for selection of resistance (Bashyal et al., 2011). Negative correlation was found between DS with TPC, PAL and lignin (−0.51, −0.53 and −0.95, respectively) and AUDPC with TPC, PAL and lignin (−0.52, −0.53 and −0.96, respectively) (Table 2).

Table 2. Pearson's correlation coefficients (N = 22) among disease severity (DS), area under disease progress curve (AUDPC), lesion size, lignin deposition, phenylalanine ammonia-lyase (PAL) and total phenol content (TPC) based on two years of testing of wheat recombinant inbred lines derived from 'Yangmai 6'/'Sonalika' and exposed to *Bipolaris sorokiniana*

	DS	AUDPC	Lesion size	Lignin	PAL
AUDPC	0.982**				
Lesion size	0.908**	0.911**			
Lignin	−0.948**	−0.957**	−0.819**		
PAL	−0.530*	−0.526*	−0.490*	0.677*	
TPC	−0.513*	−0.519*	−0.486*	0.580*	0.785**

** – significant at 0.001, * – significant at 0.05

Lesion size. The largest lesion size measured 3.43 cm^2 in the susceptible parent 'Sonalika' while the resistant parent 'Yangmai 6' displayed the minimum size 0.23 cm^2. Among 'Sonalika' background RILs, resistant RIL 91 recorded the minimum lesion size (0.30 cm^2) while the largest lesion size (1.63 cm^2) was found in the susceptible RIL 134. Amongst 'Yangmai 6' background RILs, the minimum lesion size (0.27 cm^2) was recorded in resistant RIL 89 and the largest (2.17 cm^2) in RIL 136 (Table 1). Negative and significant correlation was reported between lesion size and TPC (−0.49) and PAL (−0.49). High and negative correlation between lesion size and lignin deposition (−0.82) was found, which indicates that higher lignification was associated with smaller lesion size that recorded in resistant RILs (Table 2). Phenolics, phytoalexins, and other compounds were synthesized in cells surrounding the lesion. Callose and lignin fortified the cells and pathogenesis-related proteins (PRs) are also induced at the infection sites (Lattanzio et al., 2006) which would resulted in restricted lesions in the resistant RILs.

Total phenol content (TPC). TPC showed variation in all the three time points of estimation. There was a significantly different increase in the mean TPC of the inoculated leaves with maximum increase of 143% and minimum increase of 18% over uninoculated plants of all RILs (Table 3). RIL 89 (R) recorded the peak TPC (395 and 365 mg g^{-1} fresh wt at 24 and 48 hai with percent increase of 105% over uninoculaed leaves. The minimum TPC was reported in the MS RIL 90 (125.5, 133.5 and 171.5 mg g^{-1} fresh wt, respectively) in both uninoculated and inoculated conditions at 24 and 48 hai. Generally, TPC level in the R and MR RILs was higher and accumulated faster at 24 hai and reached a peak at 48 hai then declined slightly at 72 hai but in MS and S RILs the mean TPC was lesser then declined drastically at 72 hai. TPC has increased significantly in the resistant parent 'Yangmai 6' at 48 and 72 hai (256.5 and 152.5 mg g^{-1} fresh wt, respectively) compared to the susceptible parent 'Sonalika' (198.5 and 55 mg g^{-1} fresh wt, respectively) (Table 3).

Table 3. Mean of total phenol content (TPC) in the resistant, moderately resistant, moderately susceptible and susceptible recombinant inbred lines (RILs) of spring wheat (*Triticum aestivum* L.) against *Bipolaris sorokiniana*

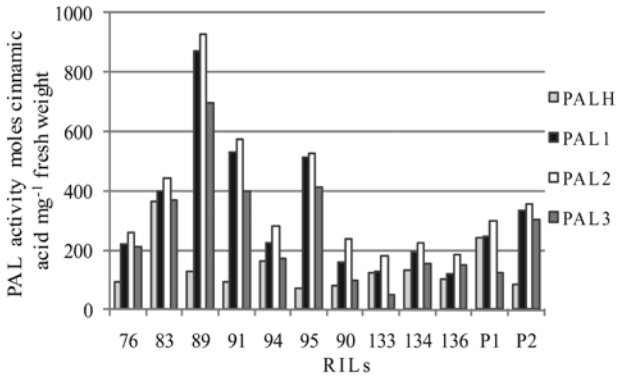
Genotype/RIL	TPC (mg g^{-1} fresh weight)				% change in 48 hai over uninoculated condition	Reaction
	0 hai	24 hai	48 hai	72 hai		
76	157.5^{nopq}	255^{ef}	287^{d}	257.5^{e}	82	MR
83	101^{uvw}	200^{gh}	246^{ef}	162.5^{mnop}	143	R
89	192^{ghi}	365^{b}	395^{a}	165.5^{lmnop}	105	R
91	189^{bij}	302^{d}	323^{c}	115^{tu}	71	R
94	128^{st}	171^{lmno}	202.5^{gh}	108^{uv}	58	R
95	146^{qr}	167.5^{lmnop}	187.5^{hijk}	91.5^{w}	28	MR
Mean	152.25	243.42	273.5	150	82	
90	$125.^{5st}$	133.5^{rs}	171.5^{klmno}	97^{vw}	37	MS
133	161^{nopq}	173^{jklmn}	190^{hi}	70.5^{xy}	18	S
134	202^{gh}	206.5^{g}	240^{f}	60.5^{xy}	18.8	S
136	135^{rs}	156^{opq}	181.5^{ijkl}	75^{x}	34	S
Mean	155.88	167.25	195.75	75.75	27	
Grand mean^{A}	154.1^{C}	205.34^{B}	234.63^{A}	112.9^{D}		
P1	171^{lmno}	177.5^{ijklm}	198.5^{gh}	55^{y}	14	S
P2	131^{rst}	171^{lmno}	256.5^{e}	152.5^{pq}	49	R
Cv %	4.56	LSD 0.05	4.75^{A} 16.45^{B}			

Notes. A – LSD for TPC at different time points 0, 24, 48 and 72 hai (hours after inoculation) in (upper case letter), B – LSD for TPC between RILs, TPC mean values with different letters (small case letter) are significantly different ($P < 0.05$) among RILs across the table whereas the TPC means with same letters are not significantly different at ($P < 0.05$). MR – moderately resistant, R – resistant, MS – moderately susceptible, S – susceptible.

Negative and significant correlation was observed between DS and AUDPC with TPC (−0.51 and −0.52, respectively) (Table 2). Phenolic compounds as potential defense responses are characterized by the early and rapid accumulation of phenolics at the infection site, resulting in the effective isolation and limiting the progress of the pathogen (Chérif et al., 1992). Resistance mechanisms have been associated with expression of PAL activity and accumulation of phenolic compounds in many hosts (Nicholson, Hammerschmidt, 1992). TPC level of wheat has been correlated with host resistance to a variety of diseases; Karnal bunt (Gogoi et al., 2001) and *Alternaria* blight (Mishra et al., 2011). The expression of resistance to spot blotch in wheat may also be influenced by growth stages and if not taken care, early maturing genotypes will appear more susceptible than late maturing ones on a particular date (Joshi, Chand, 2002). This often leads to wrong judgment while screening for resistance in the early maturing genotypes which are considered susceptible compared to the late maturing ones which turned susceptible much later. In this study, resistance in early maturing RILs showing phenological background of susceptible parent 'Sonalika' were used to understand the function of different components of resistance. The peak TPC was recorded at 48 hai. TPC was higher in resistant RILs in both parental backgrounds than in susceptible ones.

Despite low phenol content in 'Sonalika', resistant RILs in 'Sonalika' background recorded higher level of TPC.

Phenylalanine ammonia-lyase (PAL) activity. High level of variation was observed among RILs of both backgrounds under uninoculated and inoculated condition. In the 'Yangmai 6' background RILs, maximum PAL activity was recorded in the resistant RIL 89 at 24, 48, and 72 hai (871.79, 928.41 and 697.81 µmoles cna mg^{-1} fresh wt, respectively) (Fig. 1). The maximum per cent increase in PAL activity (626%) was found in RIL 89 followed by RIL 95 (614%). In 'Sonalika' background RILs, the resistant RIL 91 recorded the peak of PAL activity at 24, 48, and 72 hai that was 500% over uninoculated. In general, the mean PAL activity of the R and MR after inoculation at 24, 48 and 72 hai was higher than MS and S RILs. Under uninoculated condition, PAL activity was significantly lower (83.8 µmoles cna mg^{-1} fresh wt) in the resistant parent 'Yangmai 6' compared to the susceptible 'Sonalika' (243.4 µmoles cna mg^{-1} fresh wt), PAL increased in both the parents at 24 and 48 hai but declined at 72 hai. 'Sonalika' displayed mild increase from uninoculated to inoculated condition at 24, 48 and 72 hai in the order 243.4, 248.8 and 301.2 µmoles cna mg^{-1} fresh wt, respectively. 'Yangmai 6' showed four times increase (334.6 µmoles cna mg^{-1} fresh wt) at 24 hai compared to uninoculated state. Following 48 hai, 'Yangmai 6' continued to show high (357.53 µmoles cna mg^{-1} fresh wt) PAL activity and did not decline significantly at 72 hai (308.7 µmoles cna mg^{-1} fresh wt). On the other hand, the susceptible 'Sonalika' showed significantly lower PAL activity (123.2 µmoles cna mg^{-1} fresh wt) at 72 hai (Fig. 1). PAL catalyses phenylalanine to trans-cinnamic acid which is the first step in the biosynthesis of phenylpropanoids leading to diverse groups of plant secondary metabolites including lignins, phyto-alexins, and flavonoids (Hahlbrock, Scheel, 1989). Over-expression of PAL effectively reduced DS



PALH – 0 hai, PAL1 – 24 hai, PAL2 – 48 hai, PAL3 – 72 hai, P1 – 'Sonalika', P2 – 'Yangmai 6'; * – LSD between PAL (healthy), PAL1, PAL 2 and PAL3 (8.25), ** – LSD between PAL level in genotypes (28.58)

Figure 1. Means of phenylalanine ammonia-lyase (PAL) activity in parental genotypes and recombinant inbred lines (RILs) of spring wheat (*Triticum aestivum* L.) against *Bipolaris sorokiniana*

in tobacco infected with a virulent fungal pathogen. This apparently resulted from the production of one or more phenylpropanoid compounds. Reduction in lesion size, and reduced lesion number, has also been observed on PAL over expression plants following infection with the virulent bacterial pathogen *Pseudomonas syringae*, suggesting that PAL over-expression might be a general strategy for operating disease resistance. PAL over-expression in tobacco developed significantly fewer and smaller lesions following infection with the virulent fungal pathogen *Cercospora nicotianae* (Shadle et al., 2003). Faster kinetics of PAL enzyme may lead to rapid cell wall fortification by lignin deposition that was observed higher in the resistant RILs compared to the susceptible. Quick lignin deposition at infection site checks the further spread of pathogens and less damage to the cell. This might be one of the reasons for low recovery of enzyme at 48 hours in resistant lines when compared to the susceptible ones. In addition, PAL was found in higher level in resistant RILs of 'Sonalika' background whilst it showed low expression in the susceptible parent 'Sonalika'. The present findings indicated that the phenol content and PAL genes were co-transferred or co-inherited into the resistant RILs. Das et al. (2003) reported 30-fold increase of PAL activity in resistant genotype of wheat to spot blotch at 12 hai, whereas in susceptible genotype, the activity was marginal. An association between phenylpropanoid metabolism and host resistance involving lignification and papilla formation has been reported (Das et al., 2003). TPC and PAL activity were rather delayed or remained unchanged during the compatible interaction in susceptible plants. There existed a positive correlation between the host resistance and the amount of phenols and elevated enzyme activities while it was lower in susceptible lines. The positive association of higher phenols and enzymes with resistance could be of immense value for early and quick identification of resistant genotypes during screening of large populations (Jabeen et al., 2009). PAL enzyme reached its peak earlier in resistant RILs when compared to susceptible RILs and, was synthesized leading to a diverse group of plant secondary metabolites including lignins. Although these physiological changes were observed in both resistant and susceptible RILs, their faster kinetics and accumulation in the resistant RILs led to different expressions, response and symptoms. Susceptible plants often take longer to activate their defense response after infection by a pathogen. In some instances they do not respond at all (Moerschbacher et al., 1999). The latter might be due to the fact that the signal transduction leading to the response is in some way blocked by the attacking pathogen (Moerschbacher et al., 1999).

Histochemical (lignin deposition). Lignin deposition was higher in the leaves of R parent 'Yangmai 6' and also in the resistant (R) and moderately resistant (MR) RILs compared to the susceptible. The susceptible RILs, irrespective of their phenological backgrounds showed no detectable or weak lignification.

Table 4. Lignin deposition level and percent lignified cells in the tissue of parental and recombinant inbred lines (RILs) ('Yangmai 6'/'Sonalika') of wheat against *Bipolaris sorokiniana*

Genotype/ RIL	Level of lignification	Lignified cells %	Reaction	Parental type
'Sonalika'	1	10	S	ST
'Yangmai 6'	3	79	R	YT
91	3	77	R	ST
76	2	53	MR	ST
90	2	48	MR	ST
95	3	59	MR	ST
134	1	5	S	ST
83	3	76	R	YT
89	3	84	R	YT
94	2	67	R	YT
133	0	0	S	YT
136	1	6	S	YT
$LSD_{0.05}$		2.59		

0 – no lignification, 1 – low lignification, 2 – medium lignification, 3 – high lignification; ST – 'Sonalika' type, YT – 'Yangmai 6' type; S – susceptible, R – resistant, MR – moderately resistant

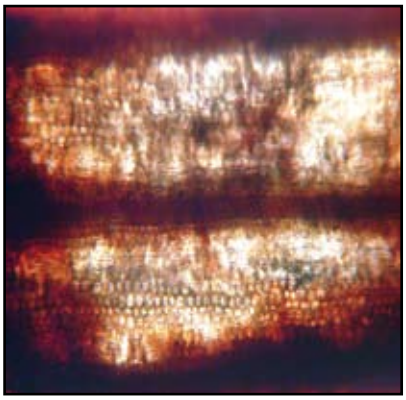
The percent of lignified cell ranged from 77% in resistant RIL 91 to 5% in the susceptible RIL 134 (Fig. 2 a) among RILs of 'Sonalika' background (Table 4).

The range in 'Yangmi 6' background was varied from 84% in the resistant RIL 89 (Fig. 2 b) to 0% in susceptible RIL 133 (Table 4). There was high negative and significant correlation between lignin and DS (−0.95), AUDPC (−0.96) and lesion size (−0.82) whereas positive correlation was found with PAL (0.68) and TPC (0.58) (Table 2). Lignification is a mechanism for disease resistance in plants. During defense responses, lignin or lignin-like phenolic compound accumulation was shown to occur in a variety of plant-microbe interactions. Lignification renders the cell wall more resistant to mechanical pressure applied during penetration by fungal appressoria as well as more water resistant and thus less accessible to cell wall-degrading enzymes (Nicholson, Hammerschmidt, 1992). This would restrict the pathogen progress and consequently the lesion size is restricted to small infected areas. In conclusion, polygenic form of resistance (Kumar et al., 2009) is based on several biochemical components including PAL, phenolic content and lignin deposition. Positive association was found between phenolic compound accumulation and the resistance in resistant RILs. This rapid synthesis of phenolic compounds causes rapid cell wall fortification

a

a) weak lignification accompanied by spore germination and penetration in susceptible RIL 59

b



b) heavy lignification surrounding the site of attempted penetration in resistant RIL 89

Figure 2. Lignin deposition in susceptible RIL 59 (a) and resistant RIL 89 (b) of wheat against *Bipolaris sorokiniana*

in the resistant RILs of wheat which restricts the infection progress, resulting in low AUDPC and smaller lesion. Slow blighting in wheat is also not entirely dependent on any single biochemical parameter studied as was also suggested by Agrios (2007). Rather various biochemical factors that restricted the progress of the pathogen by their antifungal toxic metabolites and also lead to rapid cell wall fortification play a significant role in producing slow blighting in resistant RILs.

Conclusions

1. In the present investigation, 10 selected recombinant inbreed lines (RILs) of wheat (*Triticum* spp.) were tested for two years for slow blighting caused by the spot blotch (*Bipolaris sorokiniana*). The resistant RILs showed low disease severity (DS), low area under disease progress curve (AUDPC) and smaller lesion size as compared to susceptible RILs. Background effect, i.e. early maturity, short height influenced very little in the presence of resistance gene. This study confirmed that agronomically adopted susceptible wheat cultivars can be improved by incorporating spot blotch resistance.

2. The spot blotch scoring in the field is often influenced by the environmental factors and identification of resistance in genotypes is difficult. The presence of phenol, phenylalanine ammonia-lyase (PAL) and lignin in significantly higher quantity in RILs in the present investigation indicated that these can also be used as resistant parameters for the identification of resistance with conventional field screening.

3. Among the various histopathological parameters related to resistance under laboratory and field, the number of appressoria can be used as an indicator of resistance.

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References

Agrios G. N. 2007. Plant Pathology (5th ed.). New York, USA, p. 952

Bashyal B. M., Chand R., Prasad L. C., Joshi A. K. 2011. Partial resistance components for the management of spot blotch pathogen *Bipolaris sorokiniana* of barley (*Hordeum vulgare* L.). Acta Phytopathologica et Entomologica Hungarica, 46 (1): 49–57 http://dx.doi.org/10.1556/APhyt.46.2011.1.6

Chérif M., Benhamou N., Menzies J. G., Bélanger R. R. 1992. Silicon-induced cellular defence reactions in cucumber plants attacked with *Pythium ultimum*. Physiological and Molecular Plant Pathology, 41: 411–425 http://dx.doi.org/10.1016/0885-5765(92)90053-X

Das S., Aggarwal R., Singh D. V. 2003. Differential induction of defense related enzymes involved in lignin biosynthesis in wheat in response to spot blotch infection. Indian Phytopathology, 56 (2): 129–133

Dickerson D. P., Pascholati S. F., Hagerman A. E., Butler L. G., Nicholson R. L. 1984. Phenylalanine ammonia-lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiological Plant Pathology, 25: 111–123 http://dx.doi.org/10.1016/0048-4059(84)90050-X

Gogoi R., Singh D. V., Srivastava K. D. 2001. Phenols as a biochemical basis of resistance in wheat against Karnal bunt. Plant Pathology, 50: 470–476 http://dx.doi.org/10.1046/j.1365-3059.2001.00583.x

Hahlbrock K., Scheel D. 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology, 40: 347–369 http://dx.doi.org/10.1146/annurev.pp.40.060189.002023

Jabeen N., Ahmed N., Ghani M. Y., Sofi P. A. 2009. Role of phenolic compounds in resistance to chilli wilt. Communications in Biometry and Crop Science, 4 (2): 52–61

Jeger M. J., Viljanen-Rollinson S. L. H. 2001. The use of area under disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics, 102: 32–40 http://dx.doi.org/10.1007/s001220051615

Joshi A. K., Chand R. 2002. Variation and inheritance of leaf angle and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). Euphytica, 124: 283–291 http://dx.doi.org/10.1023/A:1015773404694

Joshi A. K., Kumar S., Chand R., Ortiz-Ferrara G. 2004. Inheritance of resistance to spot blotch caused by *Bipolaris sorokiniana* in spring wheat. Plant Breeding, 123: 213–219 http://dx.doi.org/10.1111/j.1439-0523.2004.00954.x

Joshi A. K., Kumari M., Singh V. P., Reddy C. M., Kumar S., Rane J., Chand R. 2007. Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (*Triticum aestivum* L.). Euphytica, 153: 59–71 http://dx.doi.org/10.1007/s10681-006-9235-z

Kofalvi S. A., Nassuth A. 1995. Influence of wheat streak mosaic virus infection phenylpropanoid metabolism and the accumulation of phenolic and lignin in wheat. Physiological Molecular Plant Pathology, 47: 365–377 http://dx.doi.org/10.1006/pmpp.1995.1065

Kumar U., Joshi A. K., Kumar S., Chand R., Röder M. S. 2009. Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. Theoretical and Applied Genetics, 118: 783–792 http://dx.doi.org/10.1007/s00122-008-0938-5

Lattanzio V., Lattanzio V. M. T., Cardinali A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in Research, p. 23–67

Matern U., Kneusel R. E. 1988. Phenolic compounds in plant disease resistance. Phytoparastica, 16: 153–170 http://dx.doi.org/10.1007/BF02980469

Mazeyrat F., Muuzeyar S., Courbou I., Badaoui S., Roeckeldrevet P., Tourvielle D. T., Ledoigt G. 1999. Accumulation of defense-related transcripts in sunflower hypocotyls (*Helianthus annuus* L.) infected with *Plasmopara halstedii*. European Journal of Plant Pathology, 105: 333–340 http://dx.doi.org/10.1023/A:1008770008117

Mishra V. K., Biswas S. K., Rajik M. 2011. Biochemical mechanism of resistance to *Alternaria* blight by different varieties of wheat. International Journal of Plant Pathology, 2: 72–80 http://dx.doi.org/10.3923/ijpp.2011.72.80

Moerschbacher B. M., Mierau M., GraeBner B., Noll U., Mort A. J. 1999. Small oligomers of galacturonic acid are endogenous suppressors of disease resistance reactions in wheat leaves. Journal of Experimental Botany, 50: 605–612 http://dx.doi.org/10.1093/jxb/50.334.605

Nicholson R. L., Hammerschmidt R. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology, 30: 369–389 http://dx.doi.org/10.1146/annurev.py.30.090192.002101

Niemann G. J., van der Kerk A., Niessen M. A., Versluis K. 1991. Free and cell wall-bound phenolics and other constituents from healthy and fungus infected carnation (*Dianthus caryophyllus* L.) stems. Physiological Molecular Plant Pathology, 38: 417–432 http://dx.doi.org/10.1016/S0885-5765(05)80110-9

Peltonen S., Karjalainen R. 1985. Phenylalanine ammonia lyase activity in barley after infection with *Bipolaris sorokiniana* or treatment with its purified xylanase. Journal of Phytopathology, 143: 239–245 http://dx.doi.org/10.1111/j.1439-0434.1995.tb00606.x

Pereira L. F., Goodwin P. H., Erickson L. 1999. The role of phenylalanine ammonia-lyase gene during Cassava bacterial blight and Cassava bacterial necrosis. Journal of Plant Research, 112: 51–60 http://dx.doi.org/10.1007/PL00013858

Podile A. R., Laxmi V. D. V. 1998. Seed bacterization with *Bacillus subtilis* AF1 increase phenylalanine ammonia lyase and reduces the incidence of fusarial wilt in pigeonpea. Journal of Phytopathology, 146: 255–259 http://dx.doi.org/10.1111/j.1439-0434.1998.tb04687.x

Saari E. E. 1998. Leaf blight disease and associated soil-borne fungal pathogens of wheat in south and south East Asia. Duvellier E. et al. (eds). *Helminthosporium* blights of wheat: spot blotch and tan spot. CIMMYT, Mexico D.F., p. 37–51

Saari E. E., Prescott J. M. 1975. A scale for appraising the foliar intensity of wheat diseases. Plant Disease Report, 59: 377–380

Shadle G. L., Wesley S. V., Korth K. L., Chen F., Lamb C., Dixon R. A. 2003. Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of l-phenylalanine ammonia-lyase. Phytochemistry, 64: 153–161 http://dx.doi.org/10.1016/S0031-9422(03)00151-1

Stange R. R., McDonald R. E. 1999. A simple and rapid method of determination of lignin in plant tissues – its usefulness in elicitor screening and comparison to the thioglycolic acid method. Postharvest Biology and Technology, 15: 185–193 http://dx.doi.org/10.1016/S0925-5214(98)00076-3

Thilagavathi R., Saravanakumar D., Ragupathi N., Samiyappan R. 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in green gram. Phytopathology Mediterranean, 46: 157–167

Zadoks J. C., Chang T. T., Konjak C. F. 1974. A decimal code for the growth stages of cereals. Weed Research, 14: 415–421 http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x

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Kviečių (*Triticum* spp.) biocheminiai ir histocheminiai rodikliai, susiję su rudadėme dryžlige (*Bipolaris sorokiniana* (Sacc.) Shoem.)

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Santrauka

Bipolaris sorokiniana sukeliama rudadėmė dryžligė yra žalinga kviečių (*Triticum* spp.) liga šiltuose ir drėgnuose jų auginimo regionuose. Siekiant išsiaiškinti bendro fenolių kiekio, fenilalanino amonio liazės ir lignino įtaką rudadėmės dryžligės vystymuisi, lauko bandymų metu buvo taikytas ligos pažeisto lapo ploto indeksas (AUDPC). Rudadėmės dryžligės tyrimų lauko bandymo metu, siekiant nustatyti ligos intensyvumą, AUDPC ir dėmių dydį, vertinta dešimt vasarinio kviečio rekombinantinių inbredinių linijų (RIL), gautų sukryžminus dryžligei jautrią tėvinę veislę ‘Sonalika’ su atsparia tėvine veisle ‘Yangmai 6’, ir jų tėviniai genotipai. Taip pat tirta šių linijų bendras fenolių, fenilalanino amonio liazės ir lignino kiekis. Vertinta atsparios tėvinės veislės ‘Yangmai 6’ (atitinkamai 13 %, 116,04 ir 0,23 cm^2) ir RIL 18 % (atitinkamai 113,3 ir 0,27 cm^2, RIL83 ir RIL89) minimalus ligos išsivystymas, AUDPC ir dėmių dydis. Tai susiję su didesniu fenolio (395 mg g^{-1} žalios masės svorio po inokuliavimo praėjus 48 valandoms), fenilalanino amonio liazės (928,41 µmol mg^{-1} cinamono rūgšties žalios masės svorio) ir lignino (84 %) kiekiu. Ligos išsivystymas, AUDPC ir dėmių dydis buvo didesni jautrios tėvinės veislės ‘Sonalika’ ir jautrių RIL (atitinkamai 100 %, 938,27 ir 3,43 cm^2). Bendras vidutinis fenolių (atitinkamai 133,5 mg g^{-1} žalios masės svorio po inokuliavimo praėjus 24 valandoms), RIL (248,8 µmol mg^{-1} cinamono rūgšties žalios masės svorio) ir lignino (10 %) kiekis buvo žymiai mažesni jautrios veislės ‘Sonalika’ ir jautrių RIL. Tyrimas parodė, kad dėl didesnio bendro fenolių, RIL ir lignino kiekio rudadėmė dryžligė vystėsi lėčiau, o atsparių RIL AUDPC ir dėmių dydis buvo mažesni.

Reikšminiai žodžiai: *Bipolaris sorokiniana*, fenilanalino amonio lipazė, fenoliai, ligninas, ligos pažeisto lapo ploto indeksas.