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Molecular basis of resistance in wheat varieties against spot blotch disease

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Abstract: During present investigation, among the six wheat genotypes tested against six isolates of *Bipolaris sorokiniana*, the genotype BOW'S' showed resistance response against three isolates, namely, BS-D-1, BS-DWR-K-2 and BS-K-4, whereas moderately resistance response against remaining 3 isolates *i.e.* BS-F-3, BS-P-5 and BS-V-6.The genotype A-9-30-1 showed almost highly susceptible response against each isolate except BS-D-1 which exhibited susceptible reaction on this genotype. Thus, it is clear that genotype BOW 'S' has broad genetic base for resistance, whereas genotype A-9-30-1 has no gene for resistance against these six isolates tested. Remaining five genotypes showed varying response, ranging from highly susceptible, susceptible, moderately susceptible, moderately resistant and resistant against various isolates of *B. sorokiniana* tested.

Keywords: Resistance, Spot blotch, Primer, Dendrogram, Protein Profiling

INTRODUCTION

Wheat (*Triticum aestivum* L. emend. Fiori and Paol.) is the world's most widely cultivated food crop for nearly 38 per cent of the world's population. This crop contributes 33 to 37 % to the national food grain production and will continue to play a crucial role in the food security of the country. The total production of wheat in the world is about 600 million tonnes. The demand for wheat will grow faster than any other major crop as it is estimated that around 1,050 million tonnes of wheat will be required globally for ever growing population by 2020 (Kronstad, 1998), while at the same time Indian demand will be between 105 to 109 million tonnes (Shoran *et al*., 2005). Among the wheat diseases, wheat rusts, loose smut, karnal bunt and foliar blight/ spot blotch are important one, which cause substantial reduction in yield as well as deterioration in grain quality. Three foliar blights *viz.*, leaf blight, blotches and spots caused by *Alternaria* spp., *Bipolaris* spp. and *Drechslera* spp., respectively, have now become important diseases. Among these spot blotch has now emerged as one of the most serious production constraints in Uttar Pradesh and other northern states causing yield losses of nearly 40.9 per cent and grain weight reduction of 26.1 per cent (Singh *et al*., 2002). Management of this disease through host resistance has become a prime concern of scientist and farmers as well. Managing any pathogen through host resistance requires a comprehensive knowledge of prevalent races of the target pathogen, which can be achieved by exploring the virulence diversity in pathogen population as well as the genetic diversity of host. Variability in the spot blotch pathogen *Bipolaris sorokiniana* at pathogenic, morphological and physiological level have been reported (Hetzler *et al*., 1991, Mariatte *et al*., 1998 and Chauhan *et al*., 2007). However, the information so far available on the variability of *B. sorokiniana* isolates seems to be inadequate. The spot blotch resistant gene in wheat are not known to interact in a gene for gene manner, but resistant genotypes are known to show significant reduction in disease development and intensity as compared to the susceptible cultivars (Joshi and Chand, 2002 and Joshi *et al*., 2002) . It is essential to learn as much as possible about the variability in fungi, pathogenic to plants. and also to record the disease reaction of different wheat genotypes to identify sources of resistance against particular disease. Such type of study are not only important for understanding population dynamics of specific pathogen, rather it is equally important for success of any breeding programme for development of disease resistant varieties and to formulate integrated disease management strategy. Such information may also help in marker assisted selection for disease resistance in crops.

MATERIALS AND METHODS

Collection and maintenance of isolates: The pathogen, *Bipolaris sorokiniana* was isolated from wheat leaves showing spot blotch symptoms (irregular shape big size brown colour spot). Diseased leaves were collected from different wheat growing areas of north India infected with spot blotch disease on Potato Dextrose Agar (PDA)

Table 1. Disease severity and reaction of genotypes of wheat inoculated with six isolates of *B.sorokiniana*.

Genotypes	Spot blotch severity (%) exhibited by various isolates					
	BS-D-1	**BS-DWR-K-2**	**BS-F-3**	**BS-K-4**	**BS-P-5**	**BS-V-6**
BOW 'S'	4.99 (R)	8.87 (R)	28.45 (MR)	9.51 (R)	24.89 (MR)	11.57 (MR)
HUW 234	29.58 (MR)	67.10 (S)	80.79 (HS)	46.85 (MS)	67.98 (S)	50.95 (S)
PBW 343	30.45 (MS)	52.92 (S)	64.39 (S)	29.64 (MR)	58.88 (S)	47.60 (MS)
PBW 443	30.40 (MS)	49.28 (MS)	61.85 (S)	30.74 (MS)	68.25 (S)	55.91 (S)
K 9107	25.96 (MR)	25.84 (MR)	70.92 (HS)	70 .55 (HS)	68.52 (S)	48.35 (MS)
A-9-30-1	58.32 (S)	74.92 (HS)	96.85 (HS)	75.09 (HS)	91.53 (HS)	80.75 (HS)
Mean	29.95	46.48	67.20	43.73	63.34	49.18

medium under aseptic condition and Purified through monoconidial isolation (Maraite *et al.*, 1998). Monoconidial isolates maintained from different places were designated accordingly (Table 1) and were used for artificial inoculation.

Inoculation and incubation: Experiment for observing reactions of wheat varieties, (BOW'S', HUW- 234, PBW 343, PBW 443, K-9107 and A-9-30-1) weather they are resistant or susceptible , was carried out at 42 to 45 days old plants in glass house. These varieties were selected because they exhibit varying degree of reactions (resistance to highly susceptible) under Kumarganj conditions. Five seedlings of each genotypes were raised in plastic pots. Two hours before inoculation, the plants were kept in humidity chamber, fitted with mistifier to maintain film of water on leaf surface. The plants were inoculated with spore suspension of each test isolate of *Bipolaris sorokiniana* after pin pricking the leaves. The inoculum was obtained from 20 days old culture of each test isolates, which was grown as mass culture on sterilized sorghum grains. Plants were sprayed with inoculum containing about 70000 to 80000 conidia per milliliter of water and two drops of Tween 20 (polyoxyethelene sorbitan monolaurate) per 100 ml for adherence of spore suspension on plant surface. After inoculation, plants were kept in a moist chamber maintained at 90 to 100% RH for about 48 hours. Subsequently, plants were transferred to another chamber of the glass house where temperature may have been between 20-30^{0}C (month of February 1st fortnight). Disease rating was done, 21 days after inoculation, when lesions attained its maximum size using the rating scale given by Dubin (1992).

Protein profiling of wheat varieties: Protein profiling from leaves of six different wheat varieties was done by method given by Laemmli (1970). Presence of protein was estimated according to Lowery *et al.* (1951) method. Appearance of blue color indicated the presence of protein. The concentration of protein was estimated by optical density at 595 nm which was measured by a Spectrophotometer (2000 Spectran). The isolated Protein was loaded with loading dye into the wells casted in separating gel (5%) and run for 8-10 hours with a supply of 25 mA and 160 Volt Current. In Electrophoresis unit Vertical slab gel type electrophoresis unit (Glass plate 18 x 9 x 0.1 cm.) including Power Pack (ATTO AE 6220, PVT. JAPAN). The Gel was stained by Gel staining solution (coomassie brilliant blue R-250) for 15-20 hr. and then was destained by 10% menthanol and 5% acetic acid for final volume of 100ml to get proper resolution of protein bands.

DNA profiling of wheat varieties and RAPD analysis: Genomic DNA was isolated from young leaves collected and transported in an ice box from the field to the laboratory and subsequently ground in liquid nitrogen using morter and pestle. Total DNA was isolated according to the protocol described by CTAB method Doyle and Doyle (1990) with slight modification. Wheat leaves were homogenized in liquid nitrogen transfer to 50 ml polypropylene centrifuge tube containing 15ml pre-warmed (65^{0}C) DNA extraction buffer. The suspension was incubated for 1hr at 65^{0}C. The mixture was emulsified with an equal volume of chloroform:isoamyl alcohol (24:1) for 5-10 min by inversion followed by centrifugation at 15,000rpm for 15 min. The aqueous phase was transferred to a fresh centrifuge tube with a wide bore pipette and 0.6 volume of iso-propanol was added to it and mixed by gentle inversion. The precipitated DNA was washed twice by 70% alcohol. The pellet was dried under vacuum and dissolved in 1ml of TE buffer. The DNA was purified and its quality and quantity was verified spectrophotometrically.

RAPD analysis was performed following the method given by Kaur *et al.*(2007). All DNA amplification was carried out in a Thermal cycler (Bio Rad) which was programmed for 1 cycle of 4 min at 94^{0}C followed by 45 cycles of 1 min. 94^{0}C, 1min.at 37^0 C and 2 min at 72^{0}C. The last extension cycle was programmed at 72^{0}C for 7 min.

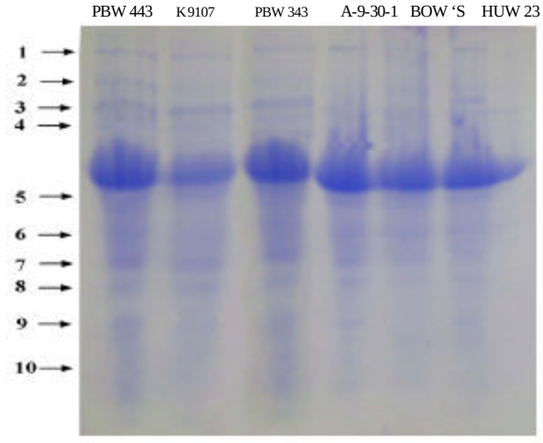

Plate 1. *Protein profiling of six wheat genotypes.*

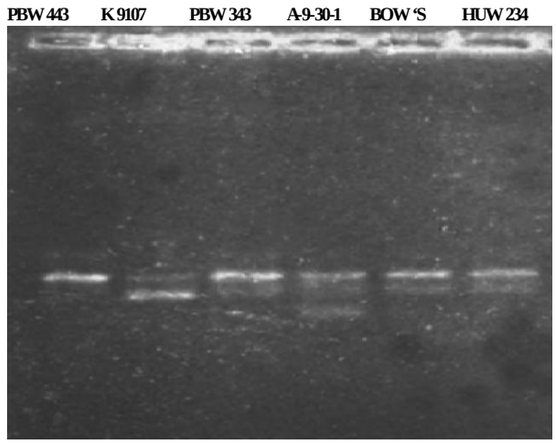

Plate 2. *DNA amplification of wheat genotypes by RAPD.*

The PCR products were resolved in 1.2% agarose gel after staining in Ethidium Bromide and Photographed using Gel documentation System.

All gels showing DNA bands scored twice independently. Band presence was indicated by 1 and its absence was indicated by 0. Presence and absence of unique and shared polymorphic as well as monomorphic products were used to generate similarity coefficient. The similarity coefficient were then used to construct a dendrogram UPGMA (Unweighted Pair Group method with arithmetical averages) using a Computer Programme NTSYSPC Version 2.1.

RESULTS AND DISCUSSION

Disease reactions of wheat varieties: The disease reaction of the six different isolates of *B. sorokiniana* (BS-DWR-K-2, BS-D-1, BS-P-5, BS-K-4, BS-F-3 and BS-V-6) on six different host genotypes of wheat have been given in table-1. The genotype PBW 343 was found to be moderately resistant against isolate BS-K-4, moderately susceptible against BS-D-1 and BS-V-6 and susceptible against remaining three isolates i.e. BS-DWR-K-2, BS-F-3 and BS-P-5. The genotype PBW 443 was found to be moderately susceptible against BS-D-1, BS-DWR-K-2, and BS-K-4 and susceptible against rest three isolates i.e. BS-P-5, BS-F-3 and BS-V-6. The genotype K 9107 was found to be moderately resistant against BS-DWR-K-2 and BS-D-1 moderately susceptible against BS-V-6, susceptible against BS-P-5 and highly susceptible against BS-K-4 and BS-F-3 isolates. The genotype A-9-30-1 was found to be susceptible to highly susceptible against all the isolates.

Protein profiling of wheat varieties: Protein profiling of six wheat varieties resulted in total 46 protein bands and 10 banding patterns (Plate 1). Among these protein bands, the 1st and 10th protein bands were monomorphic with respect to all the varieties except BOW 'S' means, with respect to BOW 'S' there was polymorphism in the 1st and 10th band. In the screening experiment, BOW 'S' was the only variety which showed resistant or moderately resistant reaction against all the six isolates of *B. sorokiniana*. Thus the evidence is indicating towards the correlation of 1st and 10th protein bands with susceptibility against spot blotch Similarly the 3rd and 4th protein bands, which were monomorphic in relation to wheat varieties, BOW 'S', HUW-234, PBW-443, PBW-343 and A-9107 but showed polymorphism in relation to he variety A-9-30-1 (susceptible or highly susceptible). Thus the evidential proofs indicate that the IIIrd and IVth protein bands may be correlated with resistance against *B. sorokiniana*. It is also clear that wherever the 1st and 10th protein bands are present they are conferring susceptibility and wherever the IIIrd and IVth protein bands are present they are conferring resistance. Wherever 1st and 10th bands and IIIrd and IVth bands present together, such varieties are either moderately resistant or moderately susceptible, while presence of only 1st and 10th bands confers absolute susceptibility. Similarly presence of IIIrd and IVth bands alone provide absolute resistance. There is no earlier report with respect to difference in protein profiles of resistant and susceptible wheat varieties against *B. sorokiniana* causing spot blotch of wheat. However some

Table 2. Showing sequence and no. of bands amplified by primers.

Primer	Sequence	Total no. of bands Amplified
AA-09	AGATGGGCAG	21
W-11	CTGATGCGTG	31
W-02	ACCCCGCCAA	35
V-07	GAAGCCAGCC	12
AH-18	GGGCTAGTCA	29
W-01	CTCAGTGTCC	57
R-04	CCCGTAGCAC	48
Total		233

Table 3. Similarity coefficient matrix of six wheat varieties.

	A-9-30-1	**K 9107**	**PBW 343**	**PBW 443**	**BOW 'S'**	**HUW 234**
A-9-30-1	1.0000000					
K 9107	0.6326531	1.0000000				
PBW 343	0.6538462	0.7021277	1.0000000			
PBW 443	0.6274510	0.6041667	0.7291667	1.0000000		
BOW 'S'	0.5918367	0.5652174	0.6956522	0.8750000	1.0000000	
HUW 234	0.5918367	0.5652174	0.6595745	0.8750000	0.8421053	1.0000000

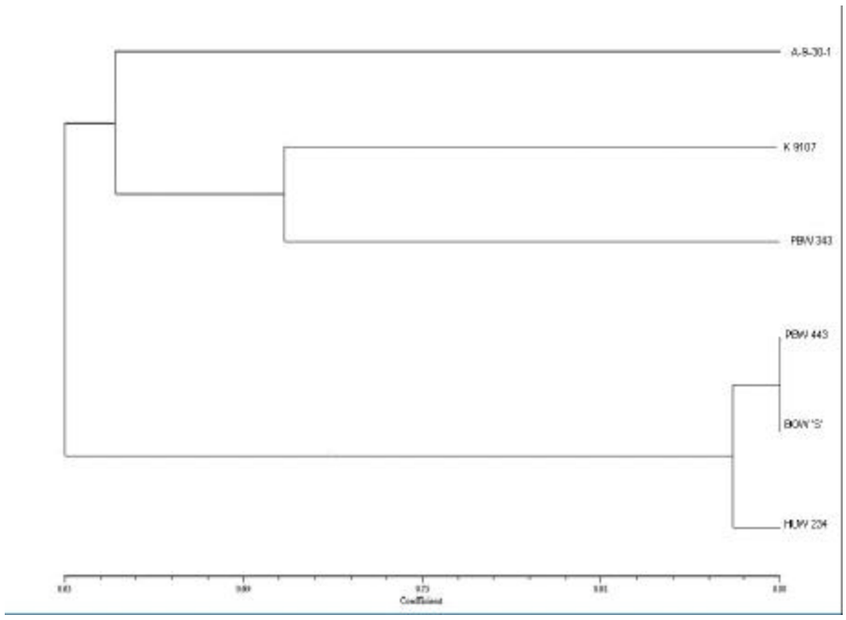

Fig. 1. *Dendrogram prepared from RAPD data of six wheat varieties.*

more repeated experiments are required which will help to confirm the exact role of individual protein bands in providing either resistance or susceptibility or conferring other phenotypic traits.

DNA profile of wheat varieties: In total 30 primers were used. Out of these 30 Operon primers only 7 primers gave amplification. Details of 7 primers are given in table-2.

A total 233 fragments resulted from primer amplification with DNA of 6 wheat varieties (Plate-2). There were PCR products common to all the susceptible and resistant wheat varieties. However, there was one polymorphic PCR product (1st) with primer AA-09 which was specific to variety PBW-443. The primer W-02 also resulted in one specific PCR product which was only amplified in the variety PBW-343 which was either susceptible or moderately susceptible against majority of the isolates tested. The primer V-07 also resulted in two specific PCR products which amplified in the variety A-9-30-1 which is either susceptible or highly susceptible against all the six isolates. Primer AH-18 also resulted in two susceptibility specific PCR products. However, none of coefficients $\leq 63\%$. Major group II was divided into two subgroups, with A-9-30-1 in one subgroup and K-9107 and PBW 343 in another subgroup, the varieties in subgroup two were having $\leq 70\%$ similarity.

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