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Thirty five isolates of *B. sorokiniana* Sacc were collected from naturally infected wheat leaves grown in two locations in North Bengal. *B. sorokiniana* isolates exhibited variability in terms of morphology and virulence. Among these, one isolate (WH.PBW.IP.04) after completion of Koch's postulate was further identified by 18 S rDNA sequencing and also immunologically characterized. Fifteen wheat genotypes were screened for spot blotch resistance. Susceptible and resistant reactions were evaluated on the basis of appearance of infection on leaves following detached leaf inoculation technique and also in one month old potted wheat plants in glass house after 12,24,48,72 and 96 hr of inoculation. Out of ten susceptible genotypes CWL-6702 was found to be highly susceptible whereas CWL-6726(MUNAL 1) out of five genotypes showing most resistance against Spot Blotch. This was further confirmed by Dot-blot and ELISA using antibody of *B. sorokiniana.* Conidial germination was comparatively high in wheat leaves of susceptible genotypes. Increased accumulation of defense enzymes (chitinase, â-1,3 glucanase and phenyl alanine ammonia lyase) were observed in pathogen treated wheat leaves in respect to control.

Key words: Wheat, *Bipolaris sorokiniana*, spot blotch resistance, defense enzymes, Dot-blot, ELISA

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important food crop after rice in the world. A large number of wheat varieties have been released in the post-green revolution phase and new ones are continuously being added to the list every year. In India, Spot blotch has been a serious problem in north-eastern region as well as in north-western parts also (Singh and Srivastava, 1997). Due to wide spread losses, spot blotch is considered as the most important disease of wheat in the warm and humid regions of the world (Saari, 1998). In India, Spot blotch of wheat caused by *Bipolaris sorokiniana* Sacc. (syn. *Helminthosporium sativum* Pamm., King & Bakke) causes up to 36% loss under favourable conditions (Anonymous, 1997). The classification and identification of *Bipolaris* species is based on morphological characteristics. But recently, molecular biology techniques- PCR assays, RAPD, AFLP have been used to study the virulence and molecular diversity of *B. sorokiniana* isolates (Muller *et al,* 2005; Jahani *et al*, 2008; Zhong and Stefenson, 2001). Mondal (2000) reported that infected seeds, and soils infested either with conidial suspension or colonized grains may serve as potential sources for the survival of *B.*

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sorokiniana resulting in germination failure, seedling mortality and Spot blotch development in wheat. Histopathological studies have indicated that the pathogen causes profound changes in infected leaf tissues and toxins play an important role in pathogenesis. Pathogenic variability is of crucial significance in disease management where host resistance is the major component. Siddique *et al,* (2006) have reported that resistant genotypes showed significant reduction in disease as compared to the susceptible cultivars. Many sources have been identified as resistant to Spot blotch (Duveiller and Gilchrist, 1994). Kumar *et al,* (2010) identified quantitative trait loci for resistance to spot blotch caused by *B. sorokiniana* in wheat lines Ning 8201 and Chirya 3. Under low light intensity, *B. sorokiniana* may colonize host tissue intercellularly without causing visible damage. Manandhar *et al,* (1999) inoculated rice with *B. sorokiniana* and observed that pathogenesis related transcripts PR-1, PR-2, PR-4, PR-5 as well as peroxidase accumulated by 12h. As of today, no commercial wheat cultivar has been shown to possess effective level of resistance to *B. sorokiniana*. Breeding for resistance demands high priority for which characterization of germplasm for resistance is a prerequisite.

Therefore, the present investigation has been undertaken to screen for resistance of wheat germplasm against one of the foliar fungal pathogens -*Bipolaris sorokiniana* and to determine induction of defense-related proteins during the resistant/susceptible reaction in pathogen. infected wheat plants along with their time course accumulation.

MATERIALS AND METHODS

Collection of wheat genotypes

Fifteen wheat genotypes were obtained from Borlaug Institute for South Asia (BISA), PUSA, Bihar, International maize and wheat improvement center/Centro International de mejoramiento de maiz y Trigo (CIMMYT) unit. These wheat genotypes were CWL- 6702, CWL-6704, CWL-6705, CWL-6706, CWL-6708, CWL-6714, CWL- 6718, CWL-6723, CWL-6712, CWL-6734, CWL-6726(MUNAL1), CWL-6738(FRNCLN), CWL-6047(CIANO T79), SONALIKA and CHIRYA 3.

Isolation and Identification of fungal isolate

Thirty five isolates of fungus causing spot blotch were collected from naturally infected wheat leaves grown in two locations in North Bengal of which twenty-five were obtained from naturally infected wheat field in the research station UBKV, Coochbehar and ten from University of North Bengal. Out of the ten isolates, one isolate (WH.PBW.IP.04), obtained from the infected wheat leaves of PBW 343 after completion of Koch's postulate was identified as *Bipolaris sorokiniana* by 18 S rDNA sequencing. *Bipolaris sorokiniana* was also immunologically characterized by the appearance of intense colour on nitrocellulose paper using antibody (1st, 2nd and 3rd bleed) of that isolate.

Genomic DNA isolation and 18S rDNA amplification by PCR

Genomic DNA was extracted from fungal culture following the method of Stafford *et al,* (2005) with modifica-tions. DNA was precipitated from the aqueous phase with chilled ethanol (100%) and pelleted by centrifuging at 12000 rpm for 15 min, followed by washing in 70% ethanol and centrifugation. The pellets were air dried and suspended in TE buffer (pH 8). After further purifica-tion, DNA was quantified spectrophotometrically and the quality analyzed in 0.8% agarose gel. For ITS-PCR ampliûcation, DNA was ampliûed by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 ml, containing 78 ml deionized water, 10 ml 10 X Taq polymerase buffer, 1 ml of 1U Taq polymerase enzyme, 6 ml 2 mMdNTPs, 1.5 ml of 100 mM forward and reverse primers and 3.5 ml of 50 ng template DNA. PCR programming was as follows- an initial denaturing at 94^0C for 5 min followed by 30 cycles of denaturation at 94^0 C for 60 s, annealing at 59^0 C for 60 s and extension at 70^0 C for 2 min and the ûnal extension at 72^0 C for 7 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 ml) was mixed with loading buffer (8 ml) containing 0.25 % bromophenol blue, 40 % w/v sucrose in water, and then loaded in 2 % agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis. The PCR product was sent for sequencing to SCIENOM, Kerala, India.

18S rDNA sequence and phylogenetic analysis

The 18SrDNA sequences obtained from PCR products were analysed by NCBI-BLAST and aligned

with ex- type isolates sequences from NCBI GenBank for identiûcation. Phylogenetic analysis was done in Mega4 software (Tamura *et al,* 2007) and evolutionary history was inferred by UPGMA Method (Sneath and Sokal, 1973).

Artificial inoculation
Whole plant inoculation

Bipolaris sorokiniana isolate (WH.PBW.IP.04) was selected for screening of wheat genotypes against Spot blotch. The fungal culture was grown on Potato dextrose agar medium (PDA) in petriplates. Seeds of wheat genotypes were sown in 20 cm" earthenware pots (10 seeds/pot) containing sterilized soil and pots were watered regularly. Experiment was conducted under glass house conditions (20-25^{0}C) in three replications. Wheat plants at one month old stage were spray inoculated with conidial suspension (1×10^3 conidia/ml) of 7 day old culture of *B. sorokiniana*.

Detached leaf inoculation

Spore suspension (1×10^3 conidia/ml) of *B. sorokiniana* were placed on 4 cm long leaf segments of all genotypes and kept in moist chamber.

Assessment of Spot blotch disease

Susceptible and resistant reactions were evaluated after 12, 24, 48, 72 and 96h of inoculation, on the basis of appearance of infection on leaves following detached leaf inoculation technique and also in potted one month old wheat plants in glass house. The disease severity was measured in terms of lesion number per leaf and infection index was calculated by following the method (using 0-5 scale) of Adlakha *et al,* (1984). Percentage disease index (PDI) was calculated- [(class rating x class frequency)/ (Total no of leaves x maximum rating)] x 100. The mean PDI was transformed into disease reaction (Adlakha *et al,* 1984) as: 0%= no infection/ immune; 0-10%= resistant response (R); 10.1-20.0%= moderately resistant (MR); 20.1-30.0%=moderately susceptible (MS); 30.1-50.0%= susceptible (S) and >50.0%= highly susceptible (HS).

Extraction and assay of defense enzymes

Enzymes were extracted from leaf tissues using suitable buffers and liquid nitrogen. 0.1M sodium acetate buffer, pH=5 was used as extraction buffer for extraction of chitinase and â-1,3-glucanase enzymes. Phenylalanine ammonia lyase was extracted using 0.1M sodium borate buffer, pH=8.8.

Chitinase (CHT- EC. 3.2.1.39) activity was assayed following the method described by Boller and Mauch (1988). The enzyme activity was expressed as mg N-acetyl glucosamine (GlcNAc) released min^{-1} g^{-1} fresh tissue.

â-1,3-glucanase (GLU -EC.3.2.1.39) was assayed following the method described by Pan *et al,* (1991). Laminarin was used as substrate and the enzyme activity was expressed as µg glucose released min^{-1} g^{-1} fresh tissue.

Phenylalanine ammonia lyase (PAL- EC.4.3.1.5) was assayed following the method described by Chakraborty *et al,* (1993) with modifications. PAL activity was determined by measuring the production of cinnamic acid from L-phenylalanine spectrophotometrically. The enzyme activity was expressed as µg cinnamic acid produced min^{-1} g^{-1} fresh tissue.

Immunological assays

Fungal antigen was prepared following the method described by Chakraborty and Purkayastha (1983). Fungal mycelia were crushed in 0.05 M sodium-bicarbonate buffer (pH 9.6) in a chilled mortar and pestle and centrifuged at 10,000 rpm. The supernatant was collected and used as antigen.

Polyclonal antibodies were raised against fungal pathogen (*B. sorokiniana*) in white, male rabbit following the procedure described by Alba and Devay (1985). Before immunization, normal sera were collected from rabbit. Following injection schedule with antigens, blood samples were collected and kept at 37^0 C for 1 h for clotting, followed by centrifugation at 5000 rpm for 10 min at room temp. IgG was purified from the serum as described by Clausen (1988).

Dot immunobinding assay was carried out using PAb raised against *B. sorokiniana* following the procedure suggested by Lange *et al,* (1989).

Western blotting was performed using PAb raised against *B. sorokiniana* following the method of Wakeham and White (1996). Hybridization was

done using alkaline phosphatase conjugate and 5-bromo-4-chloro-3-indoylphosphate (NBT-BCIP) as substrate.

Plate-trapped antigen ELISA was performed follow-ing the method as described by Chakraborty *et al,* (1996).

Fluorescence antibody staining and microscopy

PAb of *B.sorokiniana* and goat antisera specific to rabbit globulins conjugated with Fluorescein isothiocyanate (FITC) were used for indirect immunofluorescence study. Observations were made using a Biomed microscope (Leitzz) equipped with an I-3 filter block ideal for FITC fluorescence under UV light in the dark. Photographs were taken by Moticam Pro 285B.

RESULTS AND DISCUSSION

Morphological characteristics and Molecular identification of fungal isolate

Twenty five and ten isolates of fungus causing spot blotch were obtained from naturally infected wheat leaves grown in field in the research station UBKV, Coochbehar and University of North Bengal. Out of the ten, one isolate (WH.PBW.IP.04), from the infected wheat leaves of PBW 343 variety which was confirmed after completion of Koch's postulate showed bipolar germination, simple conidiophores, either single or clustered and 6-10 with septations (Fig. 1 B and C). This isolate was identified as *Bipolaris sorokiniana* by 18 S rDNA sequencing and deposited in NCBI with accession number KM066949.

Phylogenetic analysis

The sequenced PCR product was aligned with extype isolate sequences from NCBI GenBank for identification as well as for studying phylogenetic relationship with other ex-type sequences. The evolutionary history was inferred using the UPGMA method (Fig. 2). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 488 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Phylogenetic analysis showed that *B. sorokiniana* (WH. PBW.IP.04) isolate with acc. no. KM 066949.1 is closely related to *B. sorokiniana* isolate- GU 345084.1 showing 99% similarity (Fig.2). Multiple sequence alignment revealed that there were regions in the sequences which were not similar and, hence, gaps were introduced in these regions. Presence of regions with similar sequences indicated relationships among the isolates of *B. sorokiniana* (Fig. 3).

Immunological characterization

Bipolaris sorokiniana (KM 066949) isolate was also immunologically confirmed by the appearance of intense colour on nitrocellulose paper using antibody (1st, 2nd & 3rd bleed) of that isolate. However reaction with antigens of *Drechslera oryzae*, *Pestalotiopsis disseminata* and *Colletotrichum gloeosporioides* showed development of light purple colour indicating heterologous reaction with the PAb of *Bipolaris sorokiniana* (Fig.4 A-C; Table 1). Western blot analysis was performed by *B. sorokiniana* antigens against 3rd bleed. Expression of bands were observed in homologous antigen of fungus when probed with 3rd bleed. In western blotting using PAb of *B. sorokiniana* the homologous antigen showed 4 bands ranging from 29.0 to 72 kDa (Fig.5). In immunofluorescence, young mycelia of *Bipolaris sorokiniana* (KM 066949) isolate was

Table 1: Immunodetection of *Bipolaris sorokiniana* (KM 066949)

Mycelial Antigen	ELISA A 405 values	Dot Blot Colour intensity*
Treatments		
Spot blotch pathogen		
Bipolaris sorokiniana	1.648±0.080	+++
Non spot blotch pathogen		
Drechslera oryzae	0.440±0.002	++
Pestalotiopsis disseminata	0.298±0.004	+
Colletotrichum gloeosporioides	0.140±0.001	-

±=S.E; Average of 3 replicates, PAb dilution: 1:1000; Alakaline phosphatase dilution:1:10,000; *Colour intensity- Pinkish red- ++++; Bright pink- +++; Pink- ++; Light pink- +; No colour

Table 2: ELISA and DIBA values of leaf antigens of fifteen wheat genotypes reacted with PAb of *B. sorokiniana*

Antigen Source Leaf Antigens	ELISA A_{405} values	DIBA Colour intensity of dots*	** Disease response
CWL-6702	1.218±0.05	+++	HS
CWL-6704	1.210±0.06	++	S
CWL-6705	1.209±0.09	++	S
CWL-6706	1.119±0.08	++	S
CWL-6708	1.09±0.10	++	S
CWL-6714	1.110±0.05	++	S
CWL-6718	1.200±0.11	++	S
CWL-6723	1.118±0.09	++	S
CWL-6712	1.210±0.10	++	S
CWL-6734	1.209±0.09	++	S
SO (Sonalika)	1.207±0.085	+	S
CWL-6726 (MUNAL 1)	0.885±0.06	+	MR
CWL-6738 (FRNCLN)	0.905±0.09	+	MR
CWL- 6047 (CIANO T79)	0.921±0.08	+	MR
CHIRYA 3	0.901±0.07	+	MR
Mycelial Antigen			
B. sorokiniana	1.648±0.08	++++	-

±=S.E; Average of 3 replicates, PAb dilution: 1:1000; Alakaline phosphatase dilution:1:10,000; *Colour intensity- Pinkish red- ++++; Bright pink- +++; Pink- ++; Light pink- +; No colour; Leaf antigens= Antigens of fifteen wheat genotypes (CWL- Cimmyt wheat Germplasm Line); Mycelial antigen= Antigen of Bipolaris sorokiniana. **Disease response= HS= Highly susceptible; S= Susceptible; MR= Moderately resistant.

probed with 3rd bleed of the fungus labelled with FITC Con A. strong apple green fluorescence was evident in mycelia (Fig. 6).

Assessment of Spot blotch disease

Fifteen wheat genotypes were used for screening against *Bipolaris sorokiniana* (KM 066949) isolate causing Spot blotch disease. All wheat genotypes tested showed varying degrees of resistance and susceptibility. Wheat genotype- CWL- 6702 was found to be highly susceptible whereas CWL-6726(MUNAL 1) exhibited the highest degree of resistance against spot blotch (Fig.7; Table 2). CWL-6704, CWL-6705, CWL-6706, CWL-6708, CWL-6714, CWL- 6718, CWL-6723, CWL-6712, CWL-6734 wheat genotypes were found susceptible and CWL-6738(FRNCLN), CWL- 6047(CIANO T79) and CHIRYA 3 were found moderately resistant. Susceptible and resistant reactions were further confirmed by dot blot and ELISA using leaf antigens of fifteen wheat genotypes, probed with PAbs of *B. sorokiniana* from 3rd bleed. In dot blot, intensity of dots were high in homologous binding, whereas in resistant reactions dot colour intensity was slightly low in comparison to susceptible reactions. Absorbance values for resistant leaf samples were significantly lower than corresponding susceptible leaf samples (Fig.8; Table 2).

In detached leaf inoculation, spore suspension (1×10^3 conidia/ml) of *B. sorokiniana* was used as inoculum. Out of the fifteen genotypes, it was observed that in inoculated leaf surfaces of CWL-6702 genotype conidial germination was comparatively more in comparison to other genotypes after 48 hr of inoculation of spore suspension.

Susceptible and resistant reactions were also evaluated after 12,24,48,72 and 96 hr of inoculation on the basis of appearance of infection on leaves in potted one month old wheat plants in glass house. Disease was established in leaves after 48 hr of inoculation of spore suspension. In CWL-6702 genotype, more infection was noticed in comparison to other selected genotypes (Fig. 1 A) and in CWL-6726(MUNAL 1), comparatively less infection was observed than other genotypes.

Activities of defense enzymes during susceptible and resistant reactions

Out of the fifteen wheat genotypes, highly suscep-

Fig.1: Appearance of disease symptoms (A) in leaves of CWL 6702 susceptible wheat genotype. Microscopic characteristics of fungal isolate- (WH.PBW.IP.04), isolated from the infected wheat leaves of PBW 343; B= Structure of spore under 40X with Bipolar germination; C= Enlarged view of spore showing bipolar germination

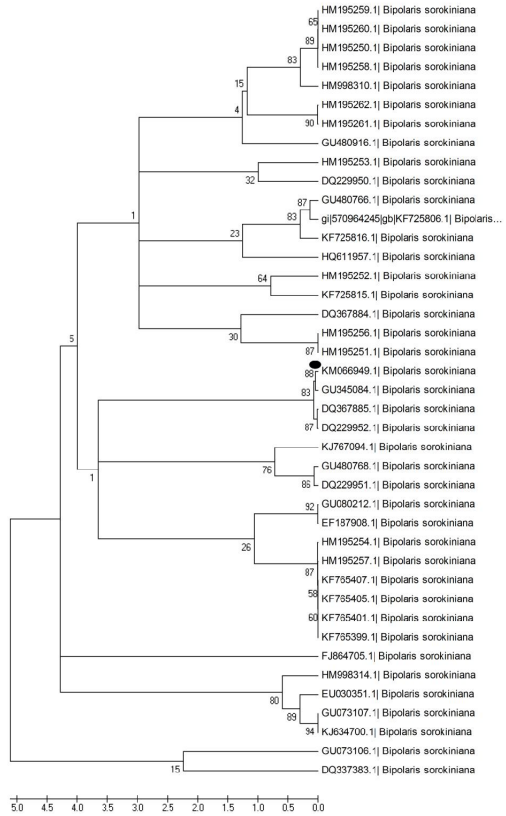

Fig. 2: The phylogenetic analyses conducted using UPGMA method among the isolate of *B. sorokiniana* (KM 066949) with other ex-type strains obtained from NCBI GeneBank database by MEGA4.1 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

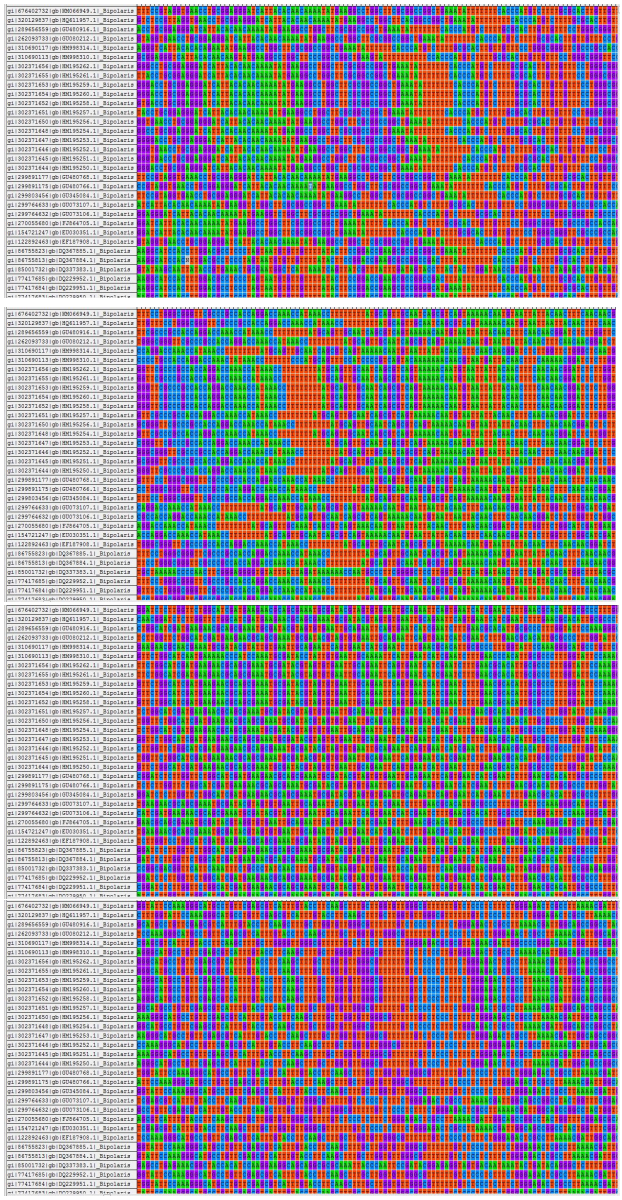

Fig.3: 18S rDNA sequence alignments of *B. sorokiniana* (KM 066949) with other ex-type strains obtained from NCBI GeneBank database. The conserved regions of the gene are demonstrated in different colour.

tible genotype- CWL- 6702 and resistant genotype- CWL-6726 (MUNAL 1) were selected to analyze the changes in the activities of defense enzymes during susceptible and resistant reactions. Increased accumulation of chitinase (CHT), â-1,3 glucanase (GLU) and phenyl alanine ammonia lyase(PAL) were observed in pathogen treated wheat leaves in respective to control. In CWL- 6702 genotype during time course accumulation, PAL activity increased in UI(*B. sorokiniana* treated) samples than control from 12-48 hr. But in 72 and 96 hr PAL activity reduced in UI sample than previous hr treatments. The same trend was observed

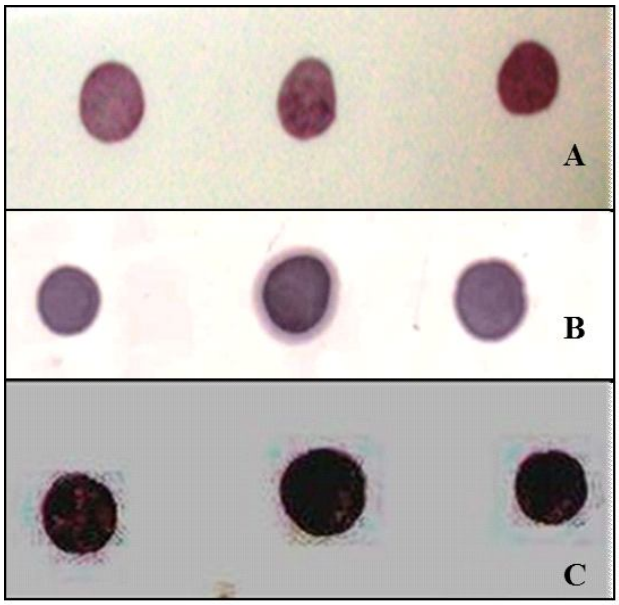

Fig.4: Immunological characterization of *B. sorokiniana* (KM 066949); A= Dot Blot of antigen of *B. sorokiniana* probed with PAbs of *B.sorokiniana* from 1st bleed; B= 2nd and C= 3rd bleed.

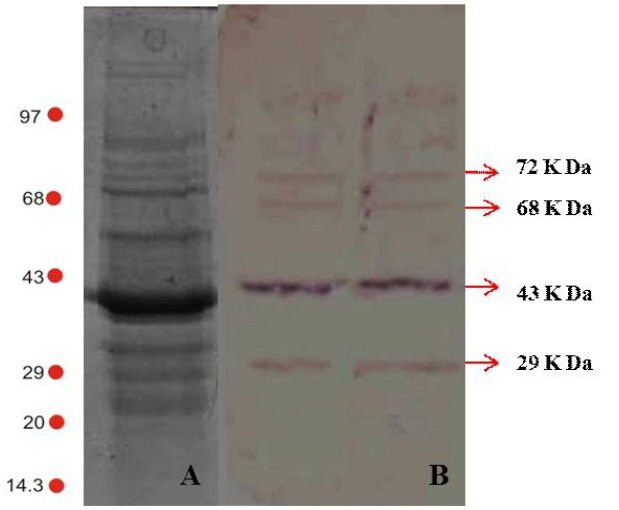

Fig.5: Immunological characterization of *B. sorokiniana* (KM 066949) by SDS PAGE (A) and Western blot (B) analysis.

in case of CHT activity. GLU activity increased in UI samples than control from 12-72 hr. But after 96 hr GLU activity reduced in UI sample than previous hr treatments.

In CWL-6726(MUNAL 1) resistant genotype during time course accumulation, the trend in increase in PAL, CHT and GLU activities were observed up to 96 hr (Fig.9 A-F). But in CWL-6726(MUNAL 1) genotype, PAL, CHT and GLU activity value slighty more in comparison to CWL- 6702 genotype.

Phylogenetic analyses showed *B. sorokiniana* (WH.PBW.IP.04) isolate with acc. no. KM 066949.1 is closely related to *B. sorokiniana* isolate GU 345084.1 showing 99% similarity. Multiple sequence alignment revealed the presence of regions with similar sequences which indicated relationships among the isolates of *B. sorokiniana*. This

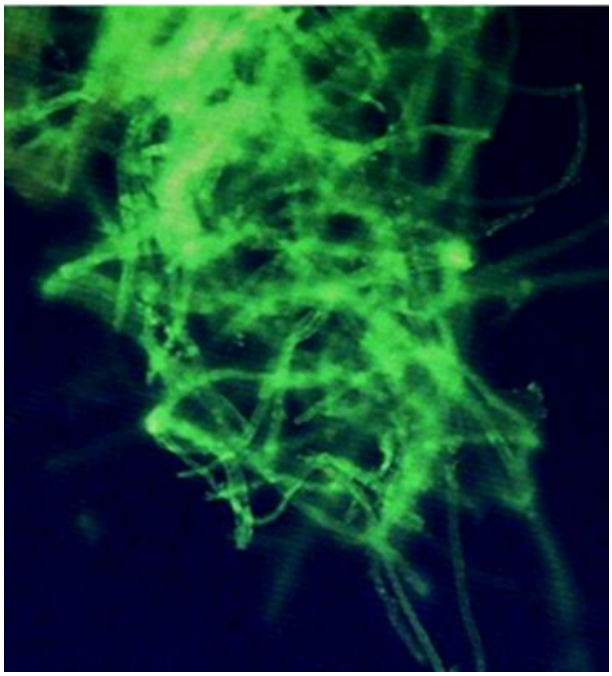

Fig.6: Immunofluorescence of young mycelia of *B. sorokiniana* probed with 3rd bleed of the fungus labelled with FITC Con A.

fungus showed bipolar germination, simple conidiophores, which were either single or clustered and with 6-10 septations. Earlier studies have also indicated a high level of morpho-pathological variability in the pathogen (Chand *et al,* 2003). These authors also mentioned another possible cause of variability in spot blotch pathogen which was suggested to be the variable rearrangement of one to six nuclei per cell. Aggarwal *et al,* (2010) studied molecular variability in *B.sorokiniana* using URP-PCR and grouped the isolates according to their geographic origin. *Bipolaris sorokiniana* (KM 066949) isolate was also immunologically characterized by dot-blot and immunofluorescence of young mycelia by probing with PAbs of that isolate.

After molecular and immunological characterization of *Bipolaris sorokiniana*, fifteen wheat genotypes- CWL- 6702, CWL-6704, CWL-6705, CWL-6706, CWL-6708, CWL-6714, CWL- 6718, CWL-6723, CWL-6712, CWL-6734, CWL-6726(MUNAL 1), CWL-6738(FRNCLN), CWL- 6047(CIANO T79),

SONALIKA and CHIRYA 3 were collected from Borlaug Institute for South Asia (BISA), PUSA, Bihar, CIMMYT unit in order to screen them against spot blotch disease. On the basis of conidial germination on leaf surface in detached leaves as well as appearance of disease symptoms in whole plants, CWL-6702 could be considered as most susceptible and CWL-6726(MUNAL 1) to be most

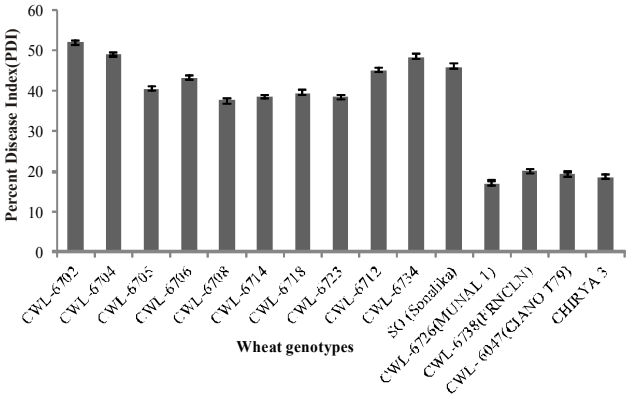

Fig.7: Percent Disease Index of susceptible and resistant wheat genotypes against *B. sorokiniana*.

resistant.

Earlier workers also reported and identified differentially resistant wheat genotypes against *B. sorokiniana* (Ibeagha *et al,* 2005). Joshi *et al,*

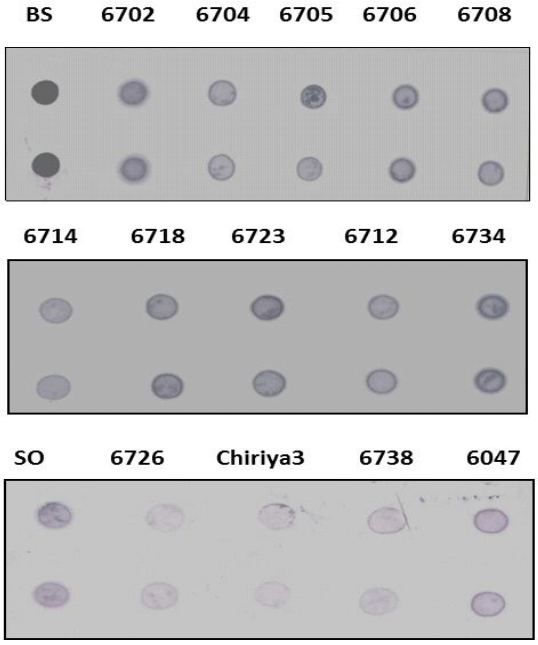

Fig.8: Dot blot with leaf antigens of fifteen wheat genotypes probed with PAbs of *B. sorokiniana*. BS= *Bipolaris sorokiniana*; (CWL 6702- CWL 6734 and Sonalika)= susceptible wheat genotypes; (Chirya 3, CWL6738,CWL 6726 andCWL 6047)= resistant wheat genotypes; CWL= Cimmyt wheat germplasm line

(2003) reported that inheritance of resistance for spot blotch disease in wheat was controlled through additive interaction of more than two genes. Adlakha *et al,* (1984) mentioned that resistance in wheat to *B. sorokiniana* was conditioned by one or two dominant factors.

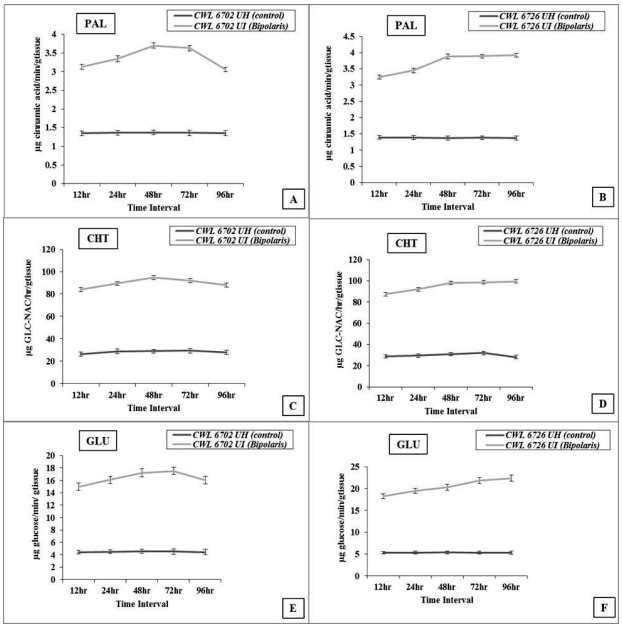

Fig.9: Activities of defense enzymes- A&B= PAL; C&D=CHT and E&F= GLU in pathogen treated leaves in comparison to control in CWL6702 susceptible (A,C&D) and CWL6726 resistant (B,D &F) wheat genotypes.

Highly susceptible genotype- CWL- 6702 and resistant genotype- CWL-6726(MUNAL 1) were selected to analyze the changes in the activities of defense enzymes during susceptible and resistant reactions. Increased accumulation of chitinase (CHT), α-1,3 glucanase (GLU) and phenyl alanine ammonia lyase (PAL) were observed in pathogen treated wheat leaves in respective to control both in susceptible and resistant genotypes. Overall chitinase (CHT), α-1,3 glucanase (GLU) and phenyl alanine ammonia lyase (PAL) activities were observed more in resistant genotype than susceptible genotype. Phenyl alanine ammonia lyase (PAL) activity was also strongly induced in wheat leaves after inoculation with *B. sorokiniana*. Kervinen *et al,* (1998) used gene-specific probes to assess the expression patterns of four different PAL clones (hpa12, hpa13, hpa14 and hpa16) in barley leaves and cell cultures. The genes were all pathogen-responsive, although with considerable variation in their expression level and timing.

The overall results suggest that CWL- 6702 was

found to be highly susceptible whereas CWL-6726(MUNAL 1) exhibited the highest degree of resistance against spot blotch. Increased activities of defense enzymes also observed during susceptible and resistant reactions. The study will pave the way for better understanding the biotic stress resistance and to take suitable measure for induction of systemic acquired resistance in wheat plants in order to achieve integrated management of foliar disease.

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