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DIVERSITY OF *BIPOLARIS SOROKINIANA* ISOLATES FROM WHEAT GROWING AREAS UNDER RICE-WHEAT CROPPING SYSTEM IN PUNJAB, PAKISTAN

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Abstract

A survey was conducted for the assessment of foliar blight of wheat in main rice-wheat cropping areas of the Punjab province, Pakistan. The foliar fungus *Bipolaris sorokiniana* was isolated from leaf samples. Isolates of *B. sorokiniana* were classified according to their aggressive behavior based on disease severity scale. *B. sorokiniana* isolates showed foliar blight symptoms on wheat but not on rice. Random Amplified Polymorphism DNA (RAPD) was used to study the genetic variation within the populations of the fungus. A correlation study was carried out with the help of five primers viz., P1 (5´-AGGAGGACCC-3´), P2 (5´-ACGAGGGACT-3´), PE7 (5´-AGATGCAGCC-3´), P14 (5´-CCACAGCACG-3´) and PE20 (5´-AACGGTGACC-3´). A tree was constructed based on the pattern of bands which highlighted the correlation between morphological, aggressiveness and genetic variations of *B. sorokiniana.*

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop of Pakistan. It is the basic food for most of the people, occupies more farmland than any other crop and mainly grown under irrigated conditions (Bajwa, 1985). A number of diseases are known to occur on this crop. Among these rusts, smuts, bunts, leaf spots or blights, mildews, seedling blight and root rot are reported to be important (Bhatti *et al.*, 1986). Information about the foliar blight of wheat in Pakistan is rather scanty (Bhatti & Soomro, 1996). Survey of wheat crop in the main rice-wheat cropping areas of Punjab was done at the booting stage of the crop. During the survey, foliar blight disease was assessed and many foliar fungi were isolated including *Bipolaris sorokinian. Bipolaris sorokiniana* [*Cochliobolus sativus*] is the causal agent of root rot, foliar blight, seedling blight, head blight and black point of wheat and barley. The disease caused by the fungus is one of the constraints for both crops in warmer growing areas and causes significant yield losses (Aftabuddin *et al*., 1991). High temperature and high relative humidity favor the outbreak of the disease particularly in South-Asia`s intensive irrigated rice-wheat production systems (Aggarwal *et al*., 2000). In the present studies, distribution and severity of foliar blight in rice-wheat cropping areas of Pakistan was carried out and RAPD was used to assess the genetic diversity in the population of *B. sorokiniana.*

Materials and Methods

Assessment of foliar blight in rice-wheat cropping areas of the Punjab

Foliar blight samples of wheat crop were collected from the farmers fields. Sample were taken at 10 points along a diagonal transect (Anon., 1996). In each field, an overall

view of the crop was obtained and general observations of the presence or absence of disease symptoms were also noted. The foliar blight samples were placed in paper bags and transferred to the laboratory for further analysis.

Isolation and preservation of fungi

Diseased foliar samples were cut into small pieces. Leaf sections (1 cm) were surface sterilized with 3% Clorox for 1 min., three times, rinsed with sterilized distilled water and placed on moistened blotting paper in 9 cm diameter Petri dish. The plates were incubated for 2 days, 24 hours at 25ºC under light period and then 24 hours at 18ºC in dark period. After continuous light and dark period the presence of fungi on leaf sections was recorded under stereomicroscope (De Wolf *et al.,* 1998). The cultures were then cut in to small pieces with the help of cork borer, dried in a laminar flow cabinet overnight and stored in sterilized tubes.

Aggressiveness analysis of foliar blight fungi

A total number of 19 isolates of *B. sorokiniana,* isolated from foliar samples of wheat cultures, from different locations of Punjab, were tested for their aggressiveness. A single spore of each *B. sorokiniana* isolate was transferred on PDA and grown in an incubator for 5-6 days at 22-26°C. Inocula were produced by the method used by Lamari & Bernier (1989). Conidia plus mycelial suspension were prepared by flooding the plates with sterile distilled water and scrapping the colony with a glass slide. A drop of Tween 20 was added per 100 ml of suspension and the concentration was determined with a hemocytometer. Eight seeds of wheat and rice varieties were planted in plastic pots containing sterilized soil in a green house. Seedlings were thinned after emergence to five plants per pot and then transferred to controlled-experimental conditions and arranged in Randomized Complete Block Design (RCBD). Seedlings of rice and wheat at the two-leaf stage were sprayed with a suspension of 3.5×10^5 conidia/ml. Control plants were sprayed with distilled water. Rice and wheat plants were covered with plastic bags for high humidity and bags were removed after 30 hours. Disease severity was estimated on inoculated leaves seven day after inoculation using a 0-5 severity scale (Carangal, 1985). The pathogen was re-isolated by the method described as above in pathogenicity. Data was analyzed using the SAS computer software package and disease severity means of all isolates were subjected to an Analysis of Variance using the SAS ANOVA procedure (Carson, 1987).

Genetic characterization of fungi

Each isolate of fungi was grown on potato broth. Mycelium was harvested by filtration through Whatmann No 1 filter paper and frozen at -20°C for few minutes. The frozen mycelia were grounded in liquid nitrogen (Rogers & Bendich, 1985). Phenol Chloroform and isoamyl-alcohol extraction was carried out for the extraction of DNA (Yeates *et al*., 1997).

DNA amplification

For PCR amplification, five 10-mer random primers viz., P1 (5´-AGGAGGACCC-3´), P2 (5´-ACGAGGGACT-3´), P14 (5´-CCACAGCACG-3´), PE7 (5´-AGATGCAGCC-3´) and PE20 (5´-AACGGTGACC-3´). were selected (Altomare *et al*., 1997). The amplification was performed in a thermal cycler program: Cycle-1: 94°C for 10 min., Cycle-2: 97°C for 15 min., 36°C for 1 min., 72° C for 2 min., repeat for 40 times, Cycle-3: 72°C for 10 min., and Cycle-4: 4°C for 30 minutes.

Analysis of amplified products

PCR products were analyzed by gel electrophoresis on 1.4 % agarose and detected by ethidium bromide. After washing of gel, the photograph was taken with UV transilluminator. DNA bands on gels were scored as present (1) or absent (0) for all isolates and species studied. The 0/1-matrics were analyzed with 'PHYLIP' phylogeny inference package version 3.57c.

Results

Aggressiveness of *Bipolaris sorokiniana* for foliar blight on wheat and rice

In aggressiveness test, all isolates were found pathogenic to both the varieties of wheat (Inqalab-91 and Chkwal-86) but not to rice (Basmati-385 and IRRI-6). Aggressive behaviour of isolates of *B. sorokiniana* on wheat was analyzed by Analysis of Variance (ANOVA). There was no significant effect of replications, varieties x replications and replications x isolates interaction. There was a highly significant effect of varieties, isolates and varieties x isolates interaction ($p<0.01$). One large group A of similar isolates was identified by cluster analysis of the mean of the two varieties using the centroid method (Fig. 1). In group A, all the isolates were aggressive.

RAPDs analysis of *Bipolaris sorokiniana*

Five 10-mer RAPD primers were tested on the 12 isolates of *Bipolaris sorokiniana.* Out of 19 isolates only 12 were able to revive and produced mycelium. Five primers distinguished the 12 isolates of *B. sorokiniana*. All isolates were scored for either presence (+) or absence (-) of bands. During data analysis, only very clear bands were scored. Certain faint bands were ignored. All 59 DNA bands produced three groups. The range of *B. sorokiniana* DNA bands was 500bp to 300bp (Fig. 2).

Discussion

The results of the present studies support the findings of Maraite *et al*., (1997) who analyzed infected samples of wheat in hot and humid areas and found that *B. sorokiniana* was found associated with 81% of the analyzed samples. Hafiz (1986) and Kishwar *et al*., (1992) also reported that *B. sorokiniana* is a principal fungus involved in the seedling blight and root rot of wheat in Pakistan. During the present studies *B. sorokiniana* was isolated from Narowal and Gujranwala areas where moist conditions were prevalent. Use of blotter method was found more suitable for the isolation of *B. sorokiniana* as also reported by Duczek (1984).

Isolates distance between cluster centroids

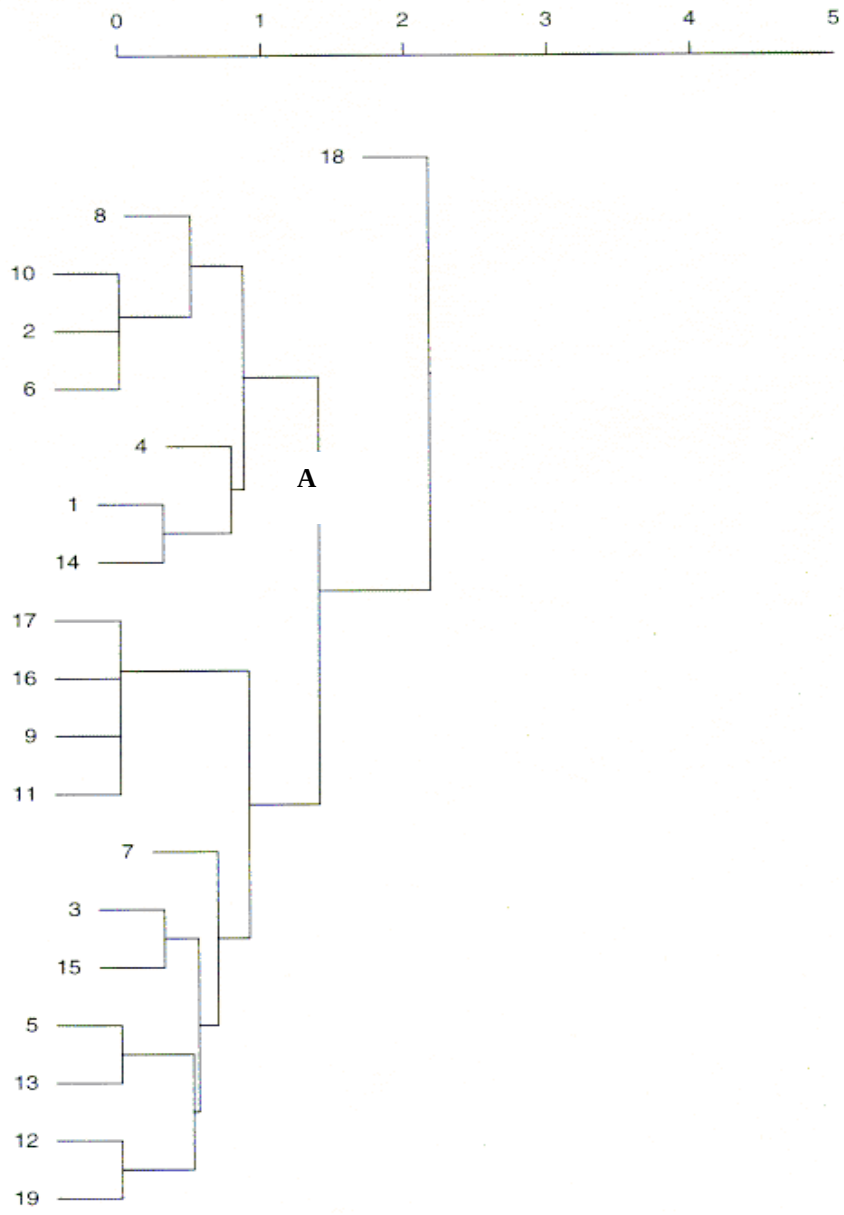

Fig. 1. Dendrogram showing similarity and successive clustering of isolates of *Bipolaris sorokiniana* based on their aggressiveness on two wheat varieties.
* Group A= aggressive isolates.

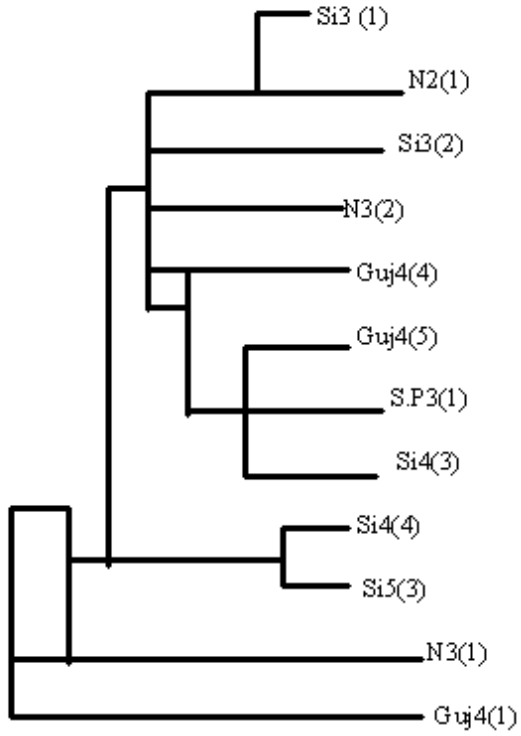

Fig. 2. Phylogenetic dendogram of the isolates of *Bipolaris sorokiniana* based on RAPD fingerprinting.

The molecular fingerprint patterns revealed differences in all the isolated strains of *B. sorokiniana*. In this study 12 isolates of *B. sorokiniana* showed genetic variability. In some of isolates, the amplification profiles of the *B. sorokiniana* were highly polymorphic, presenting genetic differences. On the other hand some of the isolates showed quite monomorphic amplification profiles. Such similar results have been reported by Vicario *et al*., (1995). It indicates the RAPD is a good molecular tool that could be used to characterize the *B. sorokiniana* isolates since aggressiveness study is time-consuming to conduct (Dimond & Horsfall, 1965; Chang, 2002).

References

Aftabuddin, A., W. E. Grey, D. E. Mathre and A. L. Scharen. 1991. Resistance in spring wheat to common root rot, spot blotch and black point caused by *Cochliobolus sativus*. *Proc. First Int. Workshop on Common Root Rot of Cereals.* Saskatoon, Saskatchewan, Canada. 11-14 August 1991. pp: 45-47.

Aggarwal, P. K., K. K. Talukdar and R. K. Mall. 2000. Potential yields of rice-wheat system in the Indo-Gangetic Plains of India. Consortium paper series 10. *Rice-wheat consortium for the Indo-Gangetic Plains*, New Delhi, India. pp: 16.

Altomare, C., O. Petrini, A. Logrieco and A. Bottalico. 1997. Taxonomic relationships among the taxogenic species *Fusarium acuminatum, Fusarium sporotrichiodes* and *Fusarium tricinctum* by isozyme analysis and RAPDs assay. *Can. J. Bot*., 75: 1674-1684.

Anonymous. 1996. *Standard evaluation system for rice*. 4th edition. International Rice Research Institute, Philippines.

Bajwa, M. A. 1985. Wheat research and production in Pakistan. pp: 68-77. In: *Wheat's for more tropical environments*. A proceeding of the international symposium. Mexico, D.F., CIMMYT.

Bhatti, M. A. R and M. B. IIyas. 1986. Wheat diseases in Pakistan. pp: 20-30. In: *Problems and progress of wheat pathology in South Asia*. (Eds.): L.M. Joshi, D.V. Singh and K.D. Srivastava. New Delhi: Malhotra publishing house.

Bhatti, I. M and A. H. Soomro. 1996. Agricultural inputs and field crop production in Sindh. Agricultural Research Sindh, Hyderabad.

Carangal, V. R. 1985. Rice-wheat cropping system in south and southeast Asia. *Proc. Int. Symp. on wheat for more tropical environments*, CIMMYT, El Batan, Mexico. pp: 277-285.

Carson, M. L. 1987. Assessment of six models of host-parasite pathogen interaction in horizontal pathosystems. *Phytopathology*, 77: 241-246.

Chang, S. W. 2002. Relationship of host genotype to *Bipolaris* leaf blight severities and yield components of Adlay. *Plant Disease*, 86: 774-779.

De Wolf, E. D., R. J, Effertz., S. Ali and L. J. Francl. 1998. Vistas of tan spot research. *Can. J.Plant Pathol*., 20: 349-444.

Dimond, A. E and J. G. Horsfall. 1965. The theory of inoculum. Pages 404-419. In: *Ecology of soil-borne plant pathogens*. (Eds.): K.F. Bakerand and W.C. Snyder. University of California. Press, Berkeley, CA.

Duczek, L. J. 1984. Comparison of the common root rot reaction of barley lines and cultivars in northwestern Alberta and central Saskatchewan. *Can. J. Plant Pathol*., 6: 81-89.

Hafiz, A. 1986. *Plant diseases*. PARC, Islamabad.

Kishwar, A., H. Sher, I. Shamim, K. Ali, S. Hassan and S. Iftikhar. 1992. Foot rot diseases of wheat in rainfed areas of North West Frontier Provinces and Punjab. *Sarhad Journal of Agriculture*, 8: 541-545.

Lamari, L. and C. C. Bernier. 1989. Evalution of wheat lines and cultivars for reaction of tan spot (*Pyrenophora tritici-repentis*) based on lesion types. *Can. J. Plant Pathol*., 11:49-56.

Maraite, H., T. Di Zinno, H. Longree, V. Daumerie and E. Duveiller. 1997. Fungi associated with foliar blight of wheat in warm areas. pp: 293-300. In: *Helminthosporium blights of wheat: Spot blotch and tan spot*. Proc. Int. Workshop held at CIMMYT. El Batan, Mexico. (Eds.): E. Duveiller, H.J. Dubin, J. Reeves and McNab.

Rogers, S. O and A. J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molecular Biology*, 5: 69-76.

Vicario, F., G. Vendramin, P. Rossi, P. Lio and R. Giannini. 1995. Allozyme, chloroplast DNA and RAPD markers for determining genetic relationships between *Abies alba* and the relic population of *Abies nebrodenis*. *Theoretical & Applied Genetics*, 90: 1012-1018.

Yeates, C., M. R. Gillings, A.D. Davison and N.V. Altavilla. 1997. PCR ampilification of crude microbial DNA extracted from soil. *Letters in Applied Microbiology*, 25:303-307.

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