Pak. J. Phytopathol., Vol. 34 (02) 2022. 255-261 DOI: 10.33866/phytopathol.034.02.0813

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

CURRENT STATUS OF BROWN LEAF SPOT IN RICE GROWING AREAS OF PUNJAB, PAKISTAN

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A B S T R A C T

Brown leaf spot (BLS), a disease caused by the fungus *Bipolaris oryzae* (teleomorph: *Cochliobolus miyabianus*), is a major economic concern in rice-growing regions around the world including Pakistan, but it has gotten little attention in Pakistan. All well-known Basmati rice cultivars are affected by BLS. During 2020-21, surveys were conducted in nine rice-growing districts of Punjab province, Pakistan, from various fields of rice, infected samples were collected. BLS was found in all of Punjab's surveyed districts. According to the findings, eighty-five rice fields were surveyed, and 81 were found to be infected with BLS. Gujranwala has the highest BLS incidence (51%), Hafzabad (50%), and Narowal (47%). The highest severity rate was 75% in Super rice in district Gujranwala at Nathu Siwiya road, while the lowest was 5% in basmati rice in Gujrat. Based on morphological characterization, the pathogen was identified as *B. oryzae*. The findings showed that BLS has been present in rice-producing regions of Pakistan, particularly in the Basmati region of Punjab, for many years. To reduce inoculum levels and yield losses, resistant Basmati cultivars must be introduced.

Keywords: Rice; Brown leaf spot; BLS; incidence; severity; *Bipolaris oryzae*.

INTRODUCTION

The most critical and important human food crop is rice for global food security (Pérez-Montaño *et al.*, 2014). The 2nd most widely used cereal crop in Pakistan is rice, after wheat and if we compare it on the basis of profit it is the 3rd one after wheat and cotton (Ameen *et al.*, 2014). Pakistan is an agricultural country with ideal temperatures and climate conditions for rice production. It is vital to note that rice is Pakistan's third most significant crop, encompassing about 10% of the country's total land area for cultivation and accounting for around 17% of the country's cereal grain production (Ali *et al.*, 2009). In most parts of the Subcontinent, particularly in Bangladesh and Sri Lanka, it is regarded as a staple dish. China is the world's leading producer of rice (214 million tonnes), with India coming in second with 173 million tons produce (Song *et al.*, 2022). The enhancement of the rice cereal began in Larkana, Pakistan, in 1912. It then relocated to Punjab's Kala Shah Kaku. In different parts of Pakistan, more than fifty varieties have been approved, certified, and farmed. Rice is cultivated in all provinces of Pakistan, but it is mostly cultivated in the districts of Kasur, Sialkot, Sheikhupura, Gujranwala, Nankana Sahib, Sahiwal, Faisalabad, Pakpatan, Gujrat, Toba Tek Singh, and Lahore in Central Punjab. The rice crop accounts for 0.6% of GDP. In Pakistan, the crop is most commonly grown in a variety of meteorological and soil conditions (Azam and Shafique, 2017). Diseases are one of the major limiting factors in rice production, producing an annual output loss estimated to be over 5% (Song and Goodman, 2001; Wang *et al.*, 2018). Pakistan has a total

Submitted: October 02, 2022
Revised: November 22, 2022
Accepted for Publication: December 05, 2022
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rice-growing area of 3034 million hectares. Higher domestic prices and the availability of subsidized inputs contributed to a 2.9% increase in production to 7.410 million tonnes, up from 7.202 million tonnes (Gul *et al.*, 2022). However, due to inadequate disease management, rice yield per hectare is lower than many other countries (Ghazanfar *et al.*, 2009). Brown leaf spot is among the most fetal diseases to rice crops all over the world. Brown leaf spot (BLS) of rice disease is caused by *Bipolaris oryzae*, a pathogen that infects rice and causes physiological and genetic alterations (Arshad *et al.*, 2008). Small, circular, oval-shaped dark brown spots occur as a sign of BLS, and the pathogen influences the grains, glume, panicles, coleoptile, and especially the leaves badly (Terensan *et al.*, 2022). Yield loss is estimated between 4 to 52% (Bedi and Gill, 1960; Aluko, 1975; Fomba and Singh, 1990). This disease is commonly found in the regions where there is water deficiency and a nutritional imbalance, particularly nitrogen shortage (Barnwal *et al.*, 2013). Rice is prone to diseases that adversely affect the yield and quality of the crop, causing severe economic losses (Nazari *et al.*, 2015). The disease-induced loss affects both yield and grain quality (Savary *et al.*, 2005). To control brown leaf spot on rice, the best and most cost-effective option is use of resistant varieties (Bonman, 1992). The current study was designed to determine the status of BLS in Pakistan's major rice-growing areas which will be helpful for the researchers to estimate disease scenarios under different climate change conditions.

MATERIALS AND METHODS

Survey and Sample collection

In the 2020-21, a comprehensive survey was carried out in Punjab province, Pakistan, to determine the frequency, incidence, and severity of BLS in farmer fields. Gujrat, Sheikhupura, Gujranwala, Hafizabad, Lahore, Kasur, Nankana, Sialkot, and Narowal were among the nine major rice-growing districts chosen for these surveys (Figure 1). At five different ends of the sampling arms positioned at a distance of 100 feet, the incidence was recorded in a 1-meter radius. Formulas were used to collected data on disease prevalence and incidence:

$$\text{Prevalence (\%)} = \frac{\text{Locations/fields showing BLS presence disease}}{\text{Total location surveyed}} \times 100$$

$$\text{Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total plant examined}} \times 100$$

Data on severity was collected using a scale (Table 1). (Anonymous, 2002)

Because the disease affects the rice plant at all stages, BLS leaf/panicle samples were obtained at the panicle emergence stage on a visual symptomatic basis (Figure 2A, B and C). The samples were brought to the Crop Diseases Research Institute (CDRI), National Agricultural Research Centre (NARC). The samples were kept in the refrigerator at 4°C until further processing.

Table 1. Brown leaf spot disease severity scale

Scale	Affected leaf area
1	No infection
2	Less than 1%
3	1 – 3%
4	4 – 5 %
5	11– 15%
6	16 – 25%
7	26 – 50%
8	51 – 75%
9	76 – 100%

Isolation and identification of the pathogen: A sterilized scalpel was used to excise segments (5 mm^2) of leaf tissue from the borders of lesions, which were then immersed in a 5% Clorox water solution for 1 minute for surface sterilization. These samples were washed twice in sterilized distilled water, autoclaved filter paper dried it, and then transferred to PDA medium. For 2-3 days, the Petri dishes were incubated at 28°C with 12-hour per day illumination and fungal growth was observed. The morphological properties of purified cultures (colony color, conidia form, conidia color and size, conidial septations, hyphal septations, and color) were noted under the microscope (Figure 3).

Pathogenicity Assay: Rice seedlings (Basmati-385) were grown in an autoclaved soil-filled plastic tray. Thirty-day-old rice seedlings were sprinkled with clean refined water and enveloped with polyethylene sacks for 24 hours before inoculation to create a high-humidity microenvironment. The spore suspension (2×10^4 spores/ml) was prepared in sterile distilled water from the isolated *B. oryzae* fungal isolates that had been growing for 10 days (Nazari *et al.*, 2015). The spore suspension was sprayed onto the healthy plant leaves, which were then covered by plastic bags once more. After 48 hours of incubation, those sacks were takeoff and the plants were moved to the greenhouse. The control plants were kept alive by spraying them with sterile distilled water and incubating them the same way as the inoculated plants. After a one-week gap, the symptoms were observed. The pathogen was re-isolated from damaged leaves and morphological features were compared to previous cultures to complete Koch's postulates.

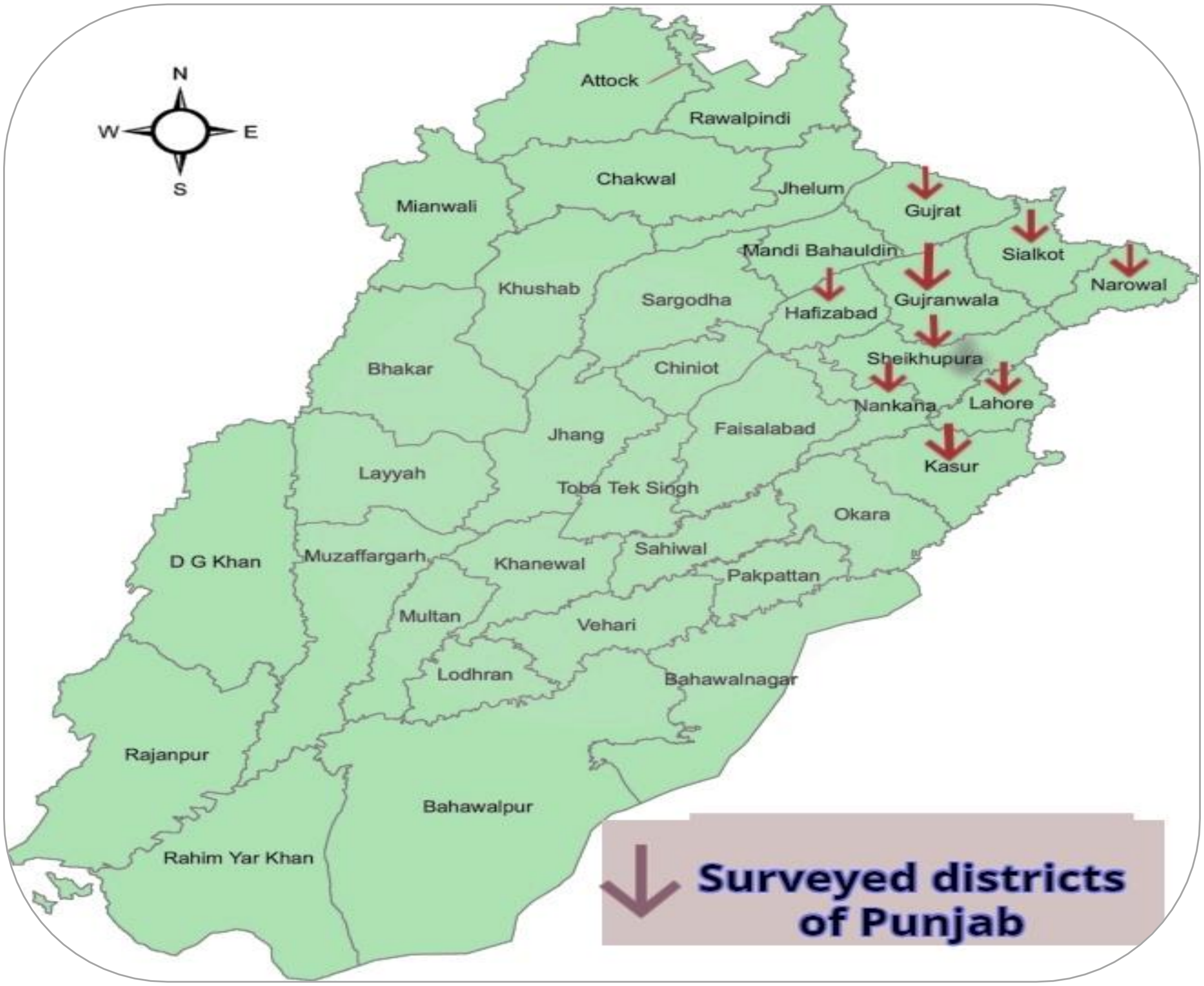

Figure 1. Map depicts the locations in Punjab where BLS disease samples were collected and data on disease prevalence, incidence, and severity was recorded

Figure 2. (A) Brown leaf spot data recording and sample collection (B) and (C), Rice leaves showing brown leaf spots

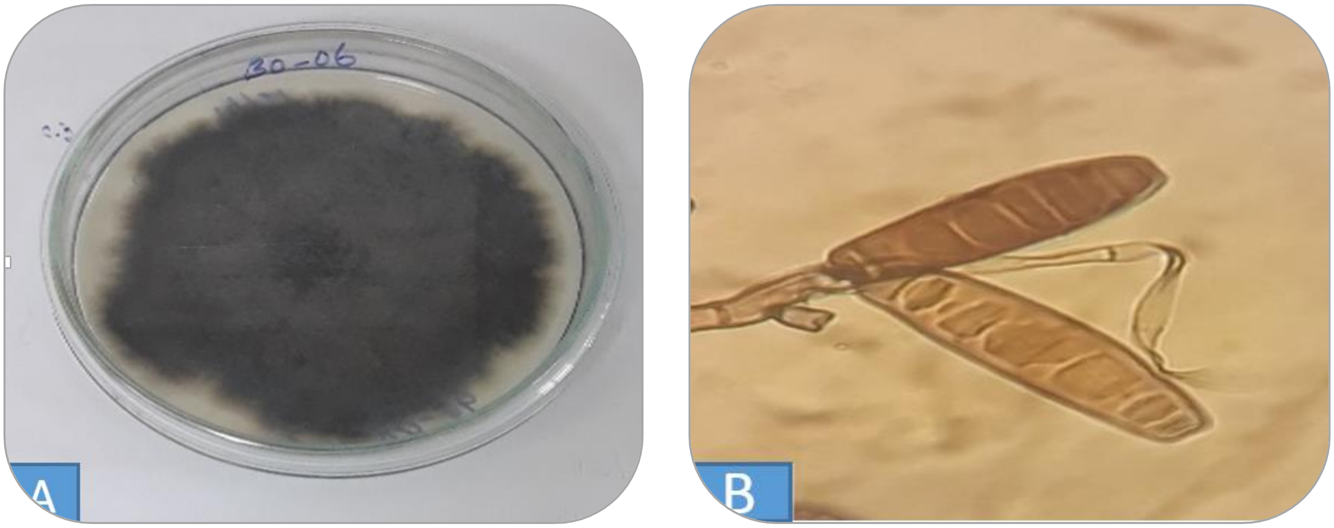

Figure 3. (A) Pure culture of *B. oryzae* after 7-9 days of incubation at 28°C. (B) Conidial spore of *B. oryzae* adhering to a conidiophore

RESULTS

Status of brown leaf spot: Nine rice-growing districts of Punjab were visited for recording the BLS status. Eighty-five rice fields were surveyed, and 81 were found to be infected with BLS. The disease was present in all the surveyed districts (Figure 2A, B and C). Brown leaf spot incidence in Gujrat, Sheikhupura, Gujranwala, Hafizabad, Lahore, Kasur, Nankana, Sialkot, and Narowal was 40%, 35%, 51%, 50%, 32%, 25%, 33%, 35%, and 47% respectively. Disease incidence observed in district Gujarat was 100%, likewise the severity was highest in Gujrat (31%), Sheikhupura (13%), Gujranwala (19%), Hafizabad (12%), and Kasur (22%) respectively (Figure 4).

BLS pathogen *B. oryzae* was isolated and confirmed from diseased samples: Eighty five diseased leaf samples were collected from the entire surveyed areas and processed for isolation and further studies. The suspected *B. oryzae* cultures were identified on the morphological basis by studying the colony characteristics, conidial spore structure. Dark green to brown fungus colonies with grey centers was regularly separated and purified. (See Figure 3A&B.). Conidia of *B. oryzae* shape were generally curved, rarely almost cylindrical, fusoid or obclavate, pale to mid golden brown, 5 to 6 septate with hillum. The conidia showed bipolar germination design. The size of *B.oryzae* conidial dimension were measured by using ocular micrometer lens and average dimension was s 98.41 x 13.31 μm.

Pathogenicity Assay: The isolated *B. oryzae* cultures were also confirmed by pathogenicity assay on the susceptible host (Bas-385). After two weeks of inoculation, disease symptoms were observed. The symptoms were identical to those observed when samples were obtained from farmer fields. Koch's postulates were fulfilled when the fungus was successfully re-isolated from the infected plants.

DISCUSSION

In the current study, surveys were conducted in several rice growing areas of the Punjab Province of Pakistan, to record the BLS data and diseased sample collection. The pathogen of this disease was identified based on morphological characterization and pathogenicity assay. In each growing season brown leaf spot is a devastating disease that contaminates millions of hectares of rice fields. Breeders still need to improve the mechanisms of resistance to brown leaf spot disease (Barnwal *et al.*, 2013). The results revealed that all BLS was present in all the surveyed districts with varying intensity Gujranwala (51%) and Hafizabad (50%), had the highest disease incidence. According to Akinbile *et al.* (2015) climate change, particularly the suitable atmosphere is helpful in the upgrading of brown spot disease percentage. The greatest level of disease incidence was computed during the current survey in Dwalatnagar's field. Gujarat has 100%, Gujranwala has 100%, and Wazirabad has 80%. Variations in disease occurrences could be caused by changes in inoculum quantity, environmental conditions, and traditional behaviors. According to Groth and Bond (2007), the number of inoculums, infection during the growth stage, climate circumstances, and resistance types all influence disease incidence and severity. Gujrat (31%), Sheikhupura (13%), Gujranwala (19%), Hafizabad (12%), Lahore (30 %), Kasur (22%), Nankana (18%), Sialkot (17%), and Narowal (17%) had the worst disease severity (17%). Brown spot on rice is becoming more severe in all of Punjab's examined districts. The intensity of the disease was at its peak in the blustry, hotspot, and high-humidity areas, indicating that brown spot disease had invaded a vast area. Rain, cloudy weather, and humid conditions are all conducive to the disease's development (Magar, 2015). Primary infection is caused by seed-borne inoculum, while secondary infection is caused by wind-borne inoculum. The inoculum reservoirs are soil, infested debris, and weed hosts. The appropriate environment for disease growth are 25-30°C for conidial germination and 27-30°C for hyphal development, with a relative humidity of more than 90%. Continuous rains and overcast weather with hot day, i.e. 25-30°C, enhance the disease's quick spread and occurrence (Carvalho *et al.*, 2010). During 2014-2017 the disease occurrence ranged from 1.12% to 14.37% in Punjab districts. The effect of relative humidity (RH) in disease incidence was substantially positively associated. High temperature was not affect the incidence of BLS (Choudhury *et al.*, 2019). Furthermore, these Punjabi districts are the primary producers of Basmati rice, particularly Super Basmati. The other varieties growing in Punjab such as Kainat, Supra, and Basmati-386 are all highly susceptible to BLS that play a key role in producing inoculum at the maturity stage and transferring brown spot inoculum year after year, which can be prevented by correct handling of field sanitation, straw, and rice stubbles (Jabran *et al.*, 2019).

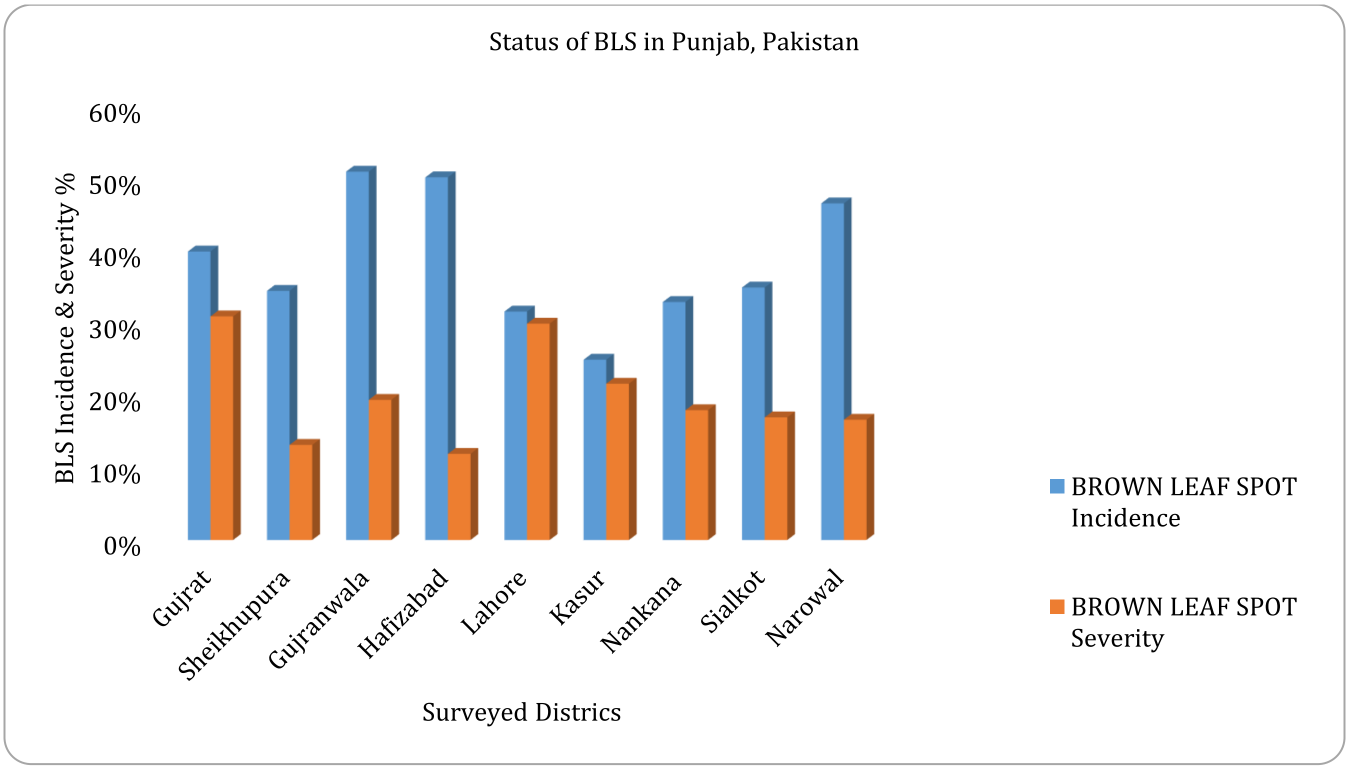

Figure 4. Graphical presentation of BLS incidence and severity in visited Districts of Punjab.

CONCLUSION

According to the findings of this study, brown leaf spot of rice is a profoundly broad malady in Punjab's main rice fields. This disease was more prevalent in the districts of Gujranwala and Narowal. The outcomes of this survey provide helpful information to rice growers, allowing them to use the resistant rice types in and use of appropriate chemical sprays. This could help rice farmers enhance their yield. Further studies are required to identify the other organisms involved in causing brown leaf spot complex and pathogen characterization on the molecular basis.

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Contribution of Authors:

Muhammad F. A. Khan	:	Experiment planned, Conducted Survey, experiment, Data Analysis and Wrote the manuscript
Abid Riaz	:	planned experiment
Ishaq Ahmad	:	Conducted Survey
Saif Ullah	:	conducted experiment
Muhammad U. Raja	:	Planned experiment
Farid A. Shaheen	:	Planned experiment
Rafia Ahsan	:	conducted experiment
Muhammad Zakria	:	Experiment planned, Conducted Survey, Help in Data Analysis, manuscript Writing