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EFFECTS OF SOWING TIMES AND RICE VARIETAL RESISTANCE ON THE SEVERITY OF NARROW BROWN LEAF SPOT IN RELATION TO ENVIRONMENTAL CONDITIONS

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ABSTRACT

The objective of this study was to investigate the effects of sowing times and genetic resistance of 10 commercially important rice varieties on the percent disease severity (PDS) of narrow brown leaf spots (NBLS) caused by *Cercospora oryzae*. Two sowing time treatments (mid-May and mid-June) each year were evaluated for this 2-year study. Rice varieties were drill seeded and arranged in randomized complete block designed plots with four replications for each variety. The effects of environmental factors (high and low temperature, relative humidity, and precipitation) on the NBLS severity of the rice varieties at both sowing times were also evaluated. Disease data was analyzed using computer software GenStat 10th edition. Results: By changing the cultivation timing of rice varieties, B-Chenab, KSK-434, PK-386, B-Kissan, and KSK-133 from mid-May to mid-June, their resistance levels were also changed from a higher to a lower. Weather conditions during the mid-June cultivation of 2019 induced a higher level of disease severity than did the mid-May weather conditions of 2018. The variation in temperatures affected the percent disease severity more as compared to relative humidity and precipitation. High temperature of 31-33˚C and low temperature of 18-21˚C were found to be most effective in enhancing the NBLS development. Minimum NBLS severity of 4.75% and 8.25% was noted on variety B-515 in mid-May and mid-June sowings respectively. Thus, its rating changed from resistant to mildly susceptible by changing sowing timing. Rice variety PK-1121 depicted maximum NBLS severity of 60.25 % in mid-May sowings, rated as very highly susceptible, while severity increased to 79.25% in mid-June sowings, and rated as completely susceptible. It is concluded that the severity of NBLS was affected by the genetic resistance level of rice varieties, sowing times and weather conditions thus consideration of these factors is the key to the integrated management of the NBLS in rice.

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INTRODUCTION

The rice crop is the most important component of Pakistan's rice-wheat cropping system. Rice is an important food and cash crop in Pakistan. The world's best rice (basmati) is produced in Pakistan, with high national and international demand due to its delicious taste and distinctive aroma (Iqbal, 2009; Iqbal *et al.*, 2022). The rice crop was harvested on an area of 35, 37,369 hectares of Pakistan and 13984009 tones produce was obtained in 2021 (FAO, 2022). There is a dire need to overcome yield limiting biotic (insect pests, weeds, and diseases) and abiotic (unusual rainfall, temperature, moisture, and relative humidity, etc.) factors of rice crop to enhance per hectare yield of rice for fast growing human population. Among the biotic factors, fungal infections are the most important due to poor management measures. Rice blast (*Pyricularia grisea*), brown leaf spot (*Bipolaris oryzae*), sheath blight (*Rhizoctonia solani*), and bakanae (*Fusarium moniliforme*) are the most important fungal diseases that affect rice production (Gill *et al.*, 1999; Iqbal, 2009; Iqbal *et al.*, 2013). However, due to climate change in recent years, the environmental conditions become more favorable for the development and spread of once-considered minor fungal diseases. Among them, narrow brown leaf spot (NBLS) caused by *Cercospora oryzae* Miyake has emerged as a major disease for rice production around the globe, causing significant qualitative and quantitative losses (Groth and Hollier, 2010; Groth, 2013; Groth *et al.*, 2014). It's epidemics can cause more than 40% of yield loss (Mani, 2015; Soura *et al.*, 2020; Zhou and Jo, 2014). In severe cases, it can cause damage to more than 50 % of the acreage (Groth, 2013). It is considered a major and most serious disease of rice in some major rice-growing areas of the world (Uppala *et al.*, 2019; Sah and Rush, 1988), including Pakistan (personal observations). Minor infection of *C. oryzae* has been detected from the seed samples of the commercially popular rice variety Basmati-385, collected from different districts of Punjab province of Pakistan (Ilyas and Javaid, 1995). But, there is no report about the serious threat of NBLS in Pakistan earlier and usually this disease was ignored due to its nature of sporadic occurrence. The development of *C. oryzae* on the potato dextrose agar (PDA) media can take 20 to 30 days during the isolation process. Development of mycelium colony of *C. oryzae* can take three to four weeks in the purification process. Due to this long incubation period (20-30 days), several types of other saprophytic fungi and non-target pathogens can cause contamination on the culture media. That's why, isolation, purification and multiplication processes of *C. oryzae* are difficult tasks (Estrada and Ou, 1978; Uppala *et al.*, 2019). So, no scientific work has been reported regarding the spread and management of this disease in the country. But, *C. oryzae* is now considered a serious pathogenic threat in the main paddy cultivating districts of the Punjab province (personal observations). That's why, there was a dire need to manage this rising problem which limiting the rice production. So, studies were planned to find out the resistance source among commercially important rice varieties and evaluation of two sowing times regarding the management of NBLS disease. To find out resistance sources among rice varieties and selection of appropriate sowing time are cost effective and easy ways to manage the disease. For this purpose, field experiments were conducted on 10 rice varieties on two sowing times (mid-May and mid-June) of the season i.e., early and late sowings. The other objective of this study was characterization of the meteorological variables which are favored the NBLS disease development. Therefore, meteorological variables (high and low temperature, relative humidity and precipitation) were characterized regarding the spread of NBLS on the rice varieties.

MATERIALS AND METHODS

Selection of Rice Varieties (RV) and Seed Inoculation

Ten rice varieties (RV), named PK-1121, KSK-133, B-515, KS-282, KSK-434, B-Super, PK-386, B-Punjab, B-Chenab, and B-Kissan, were collected from the Rice Research Institute Kala Shah Kaku (RRIKSK) Sheikhupura (district) to assess their genetic ability of resistance against the infection of NBLS at two different sowing timings (ST). The disease is seed-borne (Sah and Rush, 1988; Uppala and Zhou, 2018). The seeds of 10 rice varieties were inoculated by dipping in a spore suspension at the concentration of10^5 conidial spores/ml (Sah and Rush, 1988; Uppala *et al.*, 2019; Iqbal *et al.*, 2022) for 24 hours and then dried by placing them on blotter papers before sowing.

Experimental Location and Sowing Times

The research trials were conducted at the research area (31.44° N and 73.07° E) of the Plant Pathology Department, University of Agriculture Faisalabad, in 2018 and 2019. The experimental field was sandy-loam soil with a level of uniform fertility. The study selected two sowing times of the season, mid-May (early sowing) and mid-June (late sowing). The experiments were established with the direct dry seeding method on May 18 and June 18, during the years 2018 and 2019.

Layout of Experiments and Rating of NBLS Severity

The experiments were conducted with a randomized complete block design (RCBD) consisting of four replications of each variety in both sowing dates during the two years of study. Each replication of each variety has two lines, and each line was 4 m long and 0.46 m spaced. The recommended agronomic and insect pest management practices were applied for the maintenance of rice crops. To rate the percent disease severity (PDS) of NBLS, 10 plants from each replication of each rice variety were selected and tagged randomly after germination. The PDS of NBLS was recorded from August to October on randomly selected tagged plants at the 2-week intervals in both years. The PDS was noted by visualizing the specific symptoms of NBLS (Estrada and Ou, 1978; Uppala and Zhou, 2018, 2019). The disease started as pinhead spots on the leaf lamina, and then lesions were expanded longitudinally along the mid-rib of leaves. The lesions that appeared on the leaves of resistant varieties were narrower than the lesions developed on the leaves of susceptible varieties, which were broad. The lesions were coalesced and become long along the mid-rib of leaves over time. The final percent disease severity (PDS) was assessed at the full maturity phase of each rice variety before harvesting according to the slightly modified 0-9 disease rating scale (Mani *et al.*, 2016; Iqbal *et al.*, 2022).

Meteorological Data

The meteorological data were obtained from the Meteorology Section, Department of Crop Physiology, University of Agriculture, Faisalabad. NBLS severity trend along with environmental factors [High temperature (HT), low temperature (LT), relative humidity (RH), precipitation (PRP)] was observed to assess which factors were more critical in the disease development on two different sowing times in both years.

Statistical Analysis of Percent Disease Severity Data

The disease data of each experiment was analyzed by a general analysis of variance to pool residual of both years using computer software GenStat 10th edition (Steel and Torrie, 1986; George and Mallery, 2019). The main and interactive means of PDS after analysis of variance (ANOVA were used to determine the significant difference among the treatments by Fisher's protected least significance difference test (LSD) test at the 5 % significance level. The error bars with standard deviation were used to demonstrate the difference from the mean value in a given set of disease data.

RESULTS

Response Assessment of Rice Varieties (RV) at Different Sowing Times (ST)

Sowing timing is considered a critical factor in avoiding or reducing infection of plant diseases because it can significantly affect disease development. The genetic resistance of rice varieties (RV), sowing times (ST), and their interactions (ST × RV) significantly affected the NBLS percent disease severity (PDS) (Table 1).

Table 1. General analysis of variance of final PDS at two sowing times (ST) of ten rice varieties (RV) for the years (Yr) 2018-19.

S.O.V.	M.S.	V.R.	F. Pr.
R stratum	59.86	9.5	
R units stratum			
ST	3600.51	571.45**	<0.001
RV	7345.85	1165.88**	<0.001
Yr	252.51	40.08**	<0.001
ST × RV	99.85	15.85**	<0.001
ST × Yr	35.16	5.58*	0.020
RV × Yr	0.80	0.13	0.999
RV × ST × Yr	0.78	0.12	0.999
CV (R Units Stratum)	8.4%		

** Highly significant F. Pr. ≤ 0.01 and *significant at F. Pr. ≤ 0.05; CV (R stratum)

Narrow brown leaf spot PDS was also significantly affected by year and their interactions with sowing times (Yr × ST). While other interactions of years i.e., Yr × RV and Yr × RV × ST were found non-significant. Figure 1 depicted the trends in NBLS severity on rice varieties due to sowing times in both years. The maximum NBLS disease on all varieties was recorded in the mid-June (MJ) sowing treatment for 2019. It means the environmental conditions of 2019 affected more in trigging the disease progress on all varieties as compared to 2018 for both sowing times. Table 2 depicted that the sowing times affected the disease ratings of rice varieties because the NBLS disease severity was significantly increased in late sowing (mid-June) compared to early sowing (mid-May). The rice variety B-515 was rated as resistant in mid-May sowings but mildly susceptible in mid-June sowings under the high disease pressure. Similarly, the varieties B-Chenab, KSK-434, and PK-386 were rated as mildly resistant in the mid-May sowings while susceptible in the mid-June sowings. The rice variety KSK-133 was ranked highly susceptible in the mid-May sowings and very highly susceptible in the mid-June sowings as depicted in Table 3. The variety PK-1121 showed complete susceptibility in the mid-June sowing while it was very highly susceptible in the mid-May sowings. Figure 1 also demonstrated that minimum severity was noted on resistant variety B-515 and maximum on PK-1121 and KSK-133, respectively, in both sowing times for both years. The NBLS symptoms on the leaves of various rice varieties exhibited their genetic resistance/susceptibility levels against the infection of *Cercospora oryzae* as shown in the Figure 2.

Table 2. Comparative main and interactive (RV × ST) means of PDS at two sowing timings (ST) of ten rice varieties (RV) for the years (Yr) 2018-2019.

		Sowing Times (ST)		
		Mid-May(MM)	Mid-June (MJ)	Means
Rice Varieties (RV)	B-515	4.75^{l}	8.25^{k}	06.50 ^{G}
	B-Chb	17.38^{i}	26.00^{g}	21.69 ^{D}
	B-Ksn	37.75^{f}	51.50^{e}	44.63 ^{C}
	B-Pb	12.88^{j}	19.00^{i}	15.94 ^{F}
	B-Spr	12.75^{j}	19.38^{i}	16.07 ^{F}
	KS-282	14.75^{ij}	22.63^{h}	18.69 ^{E}
	KSK-133	54.88^{d}	70.75^{b}	62.82 ^{B}
	KSK-434	19.38^{i}	25.25^{g}	22.32 ^{D}
	PK-1121	60.25^{c}	79.25^{a}	69.75 ^{A}
	PK-386	18.38^{i}	26.00^{g}	22.19 ^{D}
	Mean	25.32^{B}	34.80^{A}	30.06

LSD for ST: 0.79, RV: 1.76, RV × ST: 2.49, Yr: 0.79, ST × Yr: 1.11

Means having the same letters did not differ significantly by Fisher's protected LSD test at 5 % significance level

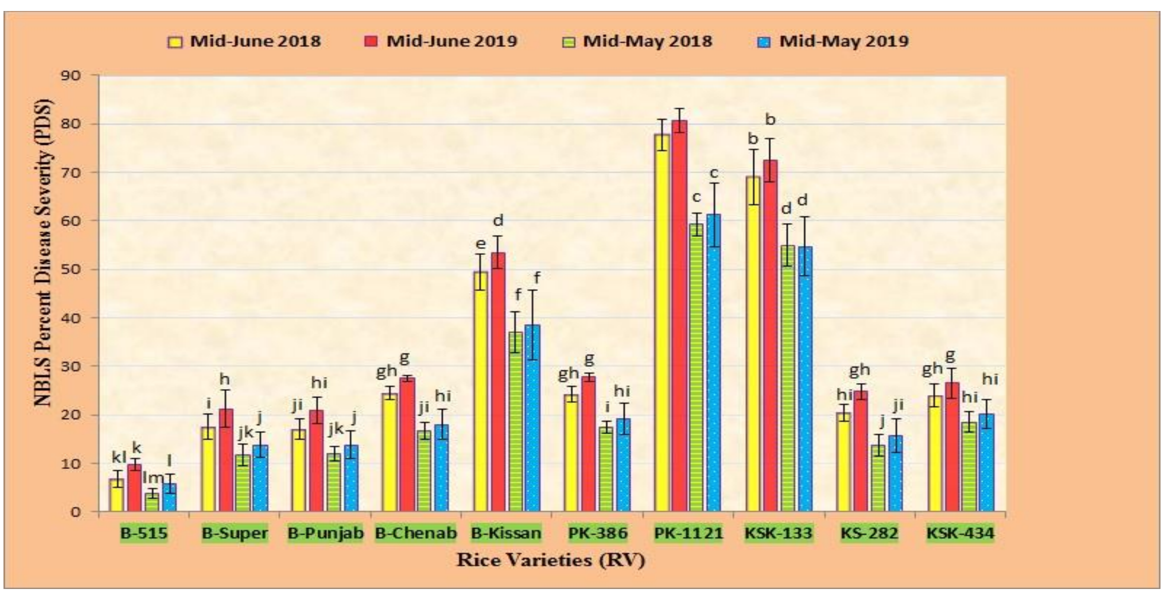

Figure 1. Error bar chart showing the error bars with standard deviation (SD). The PDS means with same letters did not significantly differ. Different sowing times significantly affected the genetic level of resistance/susceptibility of different rice varieties against the NBLS disease in 2018 and 2019

Figure 2. (a-f) Symptoms of the NBLS disease in rice varieties with various level of genetic resistance against the infection of *Cercospora oryzae*.

Effect of Environmental Factors on the Severity of NBLS

The average low temperature (LT) and high temperature (HT) were found most affective for the development of NBLS during the months of September and October. During mid-October, when HT ranged 31-33 ˚C and LT ranged 18-21 ˚C, the PDS was significantly triggered on susceptible and highly susceptible varieties, as shown in Figure 3. Like temperature, the precipitation or moisture and relative humidity also played a significant role in the onset of the NBLS disease. High relative humidity promoted the infection process. The fungal spore germination and dissemination were significantly enhanced by the relative humidity (RH). During the months of September and October, the relative humidity (RH) ranged 57 to 65 % significantly favored the PDS.

Precipitation (PRP) from the first week to mid-October was slightly high, triggering the disease's progress in both sowing times but more in late sowing. The role of temperature looked more critical in disease progression from October to November rather than relative humidity and precipitation.

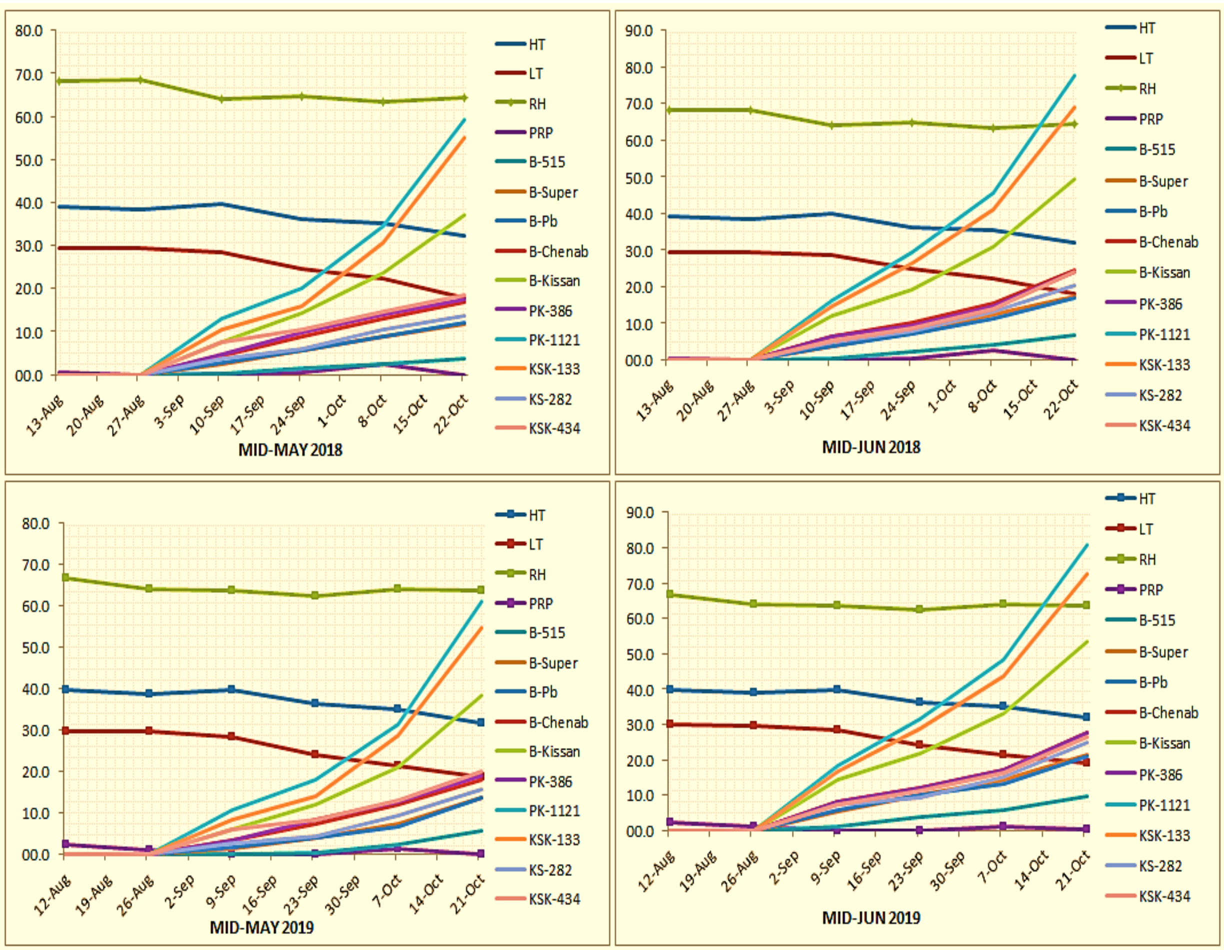

Figure 3. NBLS severity trends on ten rice varieties along with environmental conditions during mid-May and mid-June cultivations in 2018 and 2019.

DISCUSSION

Because sowing timing (ST) has a substantial impact on disease progression, so selection of proper sowing timing is essential for preventing or minimizing the spread of many plant diseases. The NBLS symptoms in this study were appeared earlier on all rice varieties, regardless of their level of genetic resistance/susceptibility in the mid-June sowing treatment. The resistant varieties exhibited less sever NBLS symptoms than the susceptible rice varieties. The rice varieties shown the highest level of NBLS infection rated as highly, very highly, or completely susceptible, and the varieties that depicted less PDS were ranked as very highly, highly and resistant respectively (Zhou and Jo, 2014; Tuli *et al.*, 2017; Uppala and Zhou, 2019) in both sowing times in the both years. The response of same rice varieties was evaluated against the infection of *C. oryzae* under controlled environmental conditions during the 2-years study. The rice variety PK-1121 was rated as completely susceptible and rice variety B-515 was rated as highly resistant. The response of other rice varieties against the infection of *C. oryzae* under controlled conditions was almost likewise as noted in the current field studies in different sowing dates which shown the level of resistant or susceptibility these rice varieties (Iqbal *et al.*, 2022). Rice varieties having complete or partial resistance created hindrance in the course of infection of *C. oryzae* despite the prevalence of favorable environmental conditions. Late sowing promoted the infections (i.e., leaf spot of sesame, *Cercospora* grey leaf spot of maize, peanuts, sugar beet, and carrot) caused by different species of genus *Cercospora* in their respective hosts plants (Lipps, 1995; Bhatia and Munkvold, 2002; Lemtur *et al.*, 2013).

At the reproductive stage, probably the most of the nutrition and energy of rice plant is used and consumed to develop the panicle and florescence; consequently, genetic resistance of plants may be compromised by the attack of plant pathogens. So, more PDS was noted at later growth stages of all rice varieties in both sowing times. In late sowing of rice varieties, the reproductive stage came up early and probably plants have comparatively weak immune system against the infection of *C. oryzae* as compared to early sowing.

The **s**usceptible rice varieties at the later growth stages during the months of September and October were more vulnerable to the infection of *C. oryzae* and exhibited by higher trends in Figure 3. The growth phases and favorable environmental conditions were more coincided during late sowing of rice varieties; so the NBLS disease is significantly triggered on mildly resistant, susceptible and highly susceptible rice varieties. The genetic resistance level of rice varieties is become more compromised by late sowing of rice varieties against the virulence of pathogen that resulted in more disease severity (Mani *et al.*, 2017; Wamishe *et al.*, 2018; Chaulagain *et al.*, 2019).

Because, favorable environmental conditions play a critical role in the onset and progression of all types of diseases (Wamishe *et al.*, 2018). The plant disease management strategies strongly dependent on environmental conditions which have positive, negative, or neutral influence on the epidemics of several plant diseases (Colhoun, 1973). A compatible virulent pathogen and a susceptible host plant must contact for a disease to begin under favorable environmental conditions and this interaction is described as disease triangle (Miedaner and Juroszek, 2021; Agrios, 2005). There is no concept of plant diseases occurrence without suitable environmental conditions.

Figure 4 exhibited the effects of environmental factors on the development of disease and host plant and pathogen interactions (Velásquez *et al.*, 2018). The environmental factors particularly high temperature (HT), low temperature (LT), precipitation (PRP), and relative humidity (RH), significantly affected the rice yield (Osman *et al.*, 2015) and disease parameters (Velásquez *et al.*, 2018). The severity of plant diseases is significantly influenced by temperature. Sometimes, temperature favors the growth of pathogens; while sometimes temperature triggered and enhanced the host plant immune system (Sahar Abdou, 2019).

The rate of inoculum formation, spore germination, and development of hypha are all directly impacted by temperature. The fitness of pathogens to become virulent strongly depends upon suitable temperature (Sahar Abdou, 2019; Miedaner and Juroszek, 2021) which promoted more severity of the NBLS disease on rice varieties at later growth stages during the months of September and October. The variations in temperature may lead certain pathogens to go through an additional one to five life cycles in every season, which enhances the pathogen's virulence to overcome the plant genetic resistance completely or partially.

The weather conditions also directly affected the mechanism of genetic resistance of rice varieties and virulence of pathogenic races of *C. oryzae*. The gene expressions and signaling pathways of host plants are modified according to the modifications in environmental conditions. The favorable environmental conditions for plant resistance enhanced the durability of resistant *R* genes and their products. Sometimes, single *R* gene provides complete or vertical resistance to the plants against the pathogens. But in some cases, several *R* genes collectively control the mechanism of resistance knows as horizontal or multi gene resistance (Agrios, 2005; Pooja and Katoch, 2014; Pokhrel, 2021) that was detected in all rice varieties. The pathogenic races of *C. oryzae* were more fit and virulent due to the less durability of R genes in the late sowing rice varieties and more PDS was recorded.

The pathogen-associated molecular-pattern (PAMP) triggered immunity and effector-triggered immunity (ETI) mechanisms of the rice varieties are influenced by the variation in environmental conditions. The PAMP-triggered immunity (PTI) system of rice varieties might be less effective against the invasion and establishment of conidial spores of *C. oryzae* in late sowing rice varieties as compared to early sowing. The PTI and ETI systems might be activated early in the early sowing (mid-May) and late in the late sowings (mid-June) rice varieties. The conserved molecules of pathogens or microbes (PAMP or MAMP) may early recognized by pattern recognition receptors (PRR) localized in plant plasma membrane which activate the resistance system of plants when the pathogen tried to enter in the plant cell.

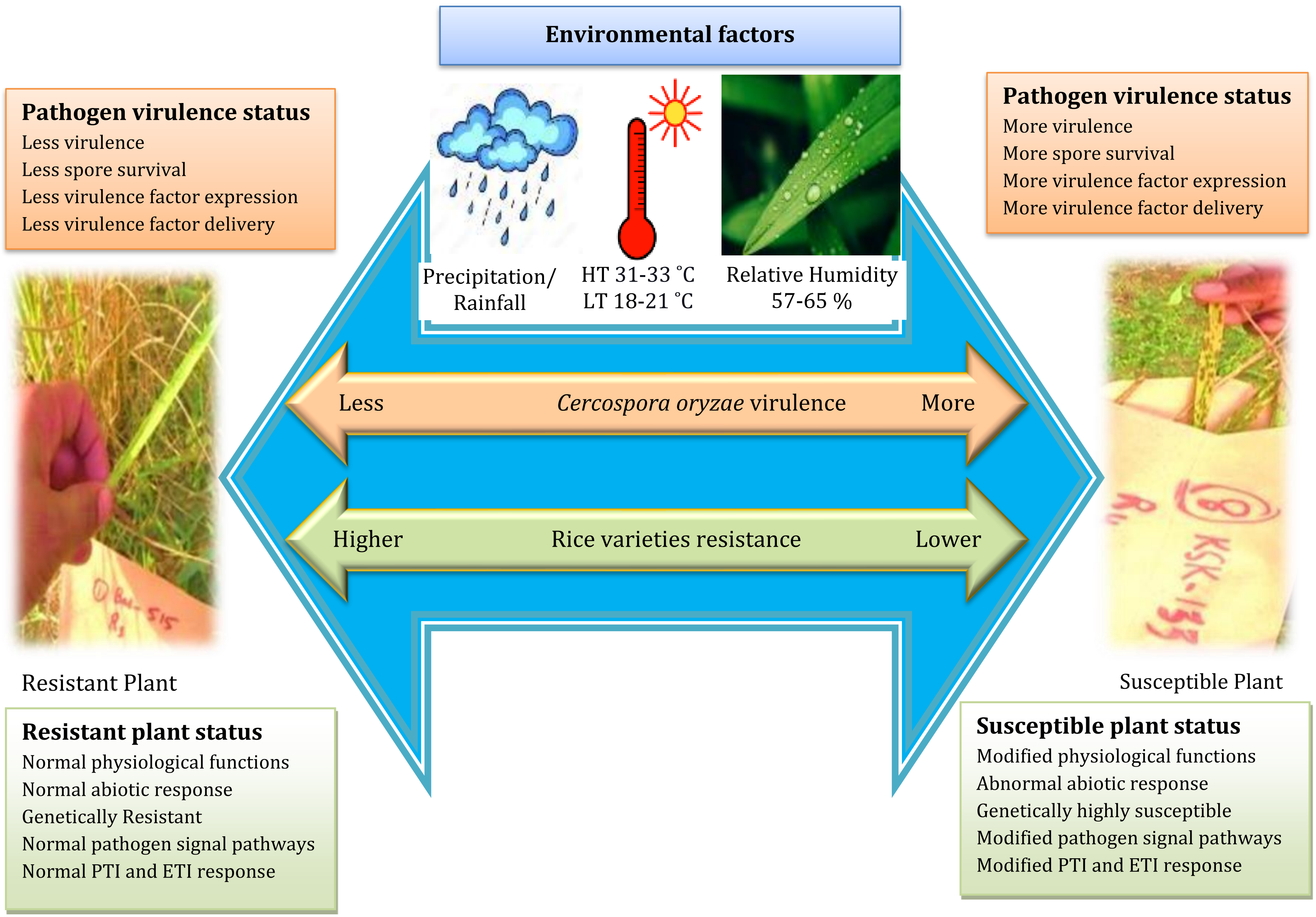

Figure 4.Effect of environmental factors on plant and pathogen interactions.

The PRR might be activated the defense mechanism after recognition of pathogens at particular growth stage of rice varieties in early sowings and guard cells of plants closed the natural openings (stomata) to stop the entrance of pathogens. So, less severity of NBLS disease symptoms was noted in the early sowing of rice varieties. Environmental conditions (temperature, moisture and relative humidity) might be altered the PTI signals short or long term and consequently plants are become more or less susceptible against the attacks of pathogens (Agrios, 2005; Pokhrel, 2021). The PAMP-triggered immunity (PTI) and ETI processes might be quick and fast in resistant rice varieties and week in susceptible rice varieties in both sowing times under favorable environmental conditions. The PTI and ETI processes for activation of genetic resistance in the same rice varieties (B-515, B-Pb, B-Spr, KS-282, KSK-133, PK-1121) responded different in different sowing times (mid-May and mid-June) due to effect of environmental conditions at different growth stages of the same rice varieties. In the late sowing, the growth stages of rice varieties and coincidence of environmental conditions may affect the PTI and ETI systems of rice plants and enhance the severity of NBLS disease. Among environmental conditions, the suitable temperature is the most crucial in activation of ETI mechanism and constantly cell death takes place to overcome the spread of pathogen with in the plant tissues (Pokhrel, 2021) and consequently more NBLS disease symptoms were developed on susceptible rice varieties.

The modification of plant physiological process under certain environmental conditions (LT, HT, RH, and PRP) altered the resistance mechanism of rice plants. Due to modification in physical mechanisms of rice plants for resistance, the same rice varieties were rated different according to the disease rating scale (Table 3) in different sowing times. The modifications in physical mechanism of rice plants for resistance were affected due to changing environmental conditions in different sowing times at a particular growth stage of particular rice varieties.

Table 3. Rating of rice varieties (RV) according to 0-9 disease rating scale sown in mid-May and mid-June (2018-19).

Score	NBLS % (Leaf area infected)	Remarks	Response of mid-May sowing RV	Response of mid-June sowing RV
0	No lesions	Immune		
1	1	Very Highly Resistant (VHR)		
2	1.1-3	Highly Resistant (HR)		
3	3.1-5	Resistant (R)	B-515	
4	5.1-12	Mildly Susceptible (MS)		B-515
5	12.1-25	Mildly Resistance (MR)	B-Chb, B-Pb, B-Spr, KS-282, KSK-434, PK-386	B-Pb, B-Spr, KS-282,
6	25.1-40	S	B-Ksn,	B-Chb, KSK-434, PK-386
7	40.1-65	HS	KSK-133	B-Ksn,
8	65.1-75	VHS	PK-1121	KSK-133,
9	More than 75	CS		PK-1121

The rice variety B-515 was ranked as resistant in mid-May sowing but mildly susceptible in mid-June sowing. But, not all rice varieties showed variations in their resistance/susceptibility level in both sowing times. The response of some rice varieties (B-Pb, B-Spr, and KS-282) was same in both sowing times, although sowing times and growth stages were different. In mid-May (early) sowings, the vegetative (panicle initiation and booting) stages came early and late in mid-June (late) sowing. The reproductive stages of rice varieties came early in mid-June sowings, physical mechanism for resistance might be more affected by the environmental conditions, in late sowing and more PDS was resulted. It was also noted that modifications in physical mechanism of some rice varieties (B-Pb, B-Spr, and KS-282) might be not affected in both sowing times. The physiological process like papillae production, the accumulation of silicon in the sites of aspersorium penetration, increase or decrease in fiber content, increase or decrease in photosynthetic rate, creation of additional epidermal layer, reduced or more nutrient concentrations and modification in the production of resistance-related enzymes lead different changes in genetic resistance of different rice varieties against the pathogen due to influence of environmental conditions. So, the rice variety KSK-133 has shown high susceptibility in mid-May sowing and very high susceptibility in mid-June sowing. Similarly, the rice variety PK-1121, was found very high susceptible in mid-May sowing and completely susceptible in mid-June sowing in both years of study. So, the host plant and pathogen interaction is modified (Ghini *et al.*, 2008). The environmental conditions may favor the pathogens to establish infection at a particular growth phase at late sowing and rice plants probably gets early infections, more infection cycles, and more favorable micro and macro-climatic conditions (Mani *et al.*, 2016). It is also thought that, due to change in environmental factors, the period between reproductions of pathogenic generations is reduced (Ghini *et al.*, 2008) which resulted in more disease severity under favorable environmental conditions.

CONCLUSION

Study characterized the main and interactive effects of sowing times and rice varieties in two years along with different environmental conditions. The determination of most effective range of high and low temperatures, relative humidity and rainfall will be beneficial in adopting precautionary measures to manage/avoiding the disease to become more sever. Sowing times and genetically resistant rice varieties should be considered when deploying diseases management strategies to restrict the NBLS disease severity and incidence. When rice crop is cultivated after the recommended dates, the yield may be low due to increased disease during the heading and grain filling period. The environmental conditions particularly average range of high and low temperatures at particular growth phase of the crop should be strictly monitored to assess the range of expected disease epidemic and adoption of proper management strategy.

AUTHOR CONTRUBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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