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Assessment of environmental variables conducive for epidemiology of rice-blast pathosystem

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Abstract

Rice blast-pathosystem is one of most serious disease of rice caused by *Pyricularia oryzae*. Environmental factors play an important role in sudden outbreaks of rice blast in all rice growing regions of the world. In the current study different environmental factors were explored by correlation and regression analysis to find out their contribution to facilitate the rice blast disease. Maximum disease severity 62.32% and AUDPC (635.35) was calculated on Basmati-2000 while minimum (432.85) was recorded on Basmati-Pak. Environemntal factors viz., Rainfall and relative humidity showed a positive correlation whereas temperature maximum depicted a negative correlation and remained irresponsive in disease development. All of the regression model parameters were significant at $P \leq 0.05$ and Rain fall, relative humidity of morning and evening and wind speed significantly contributed to the development of rice blasting pathosystem except sun shine hours and temperature factor. The coefficient of determination (r^2) of 0.93 proved the significance of overall regression model accuracy. The model developed as full showed 96% variability towards the advancement of the disease.

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Introduction

Rice (*Oryza sativa* L.) is the world's most important crop and a primary source of food for more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Anonymous, 2010a). Globally rice occupies an area of 163 million hectares with a production of 719 million tonnes of paddy (FAO, 2012). In Pakistan, rice was grown on an area of 4.2 million hectares with a total production of 10.1 million tonnes (Anonymous, 2010b) which is very low as compared to developed countries of the world. Rice is known to be attacked by many fungal, bacterial, viral and nematode diseases which cause huge losses annually worldwide. Among the fungal diseases, rice blast caused by *Pyricularia oryzae* is one of the most serious, widely distributed and significant economic importance disease of rice. Rice blast was probably the disease known as rice fever disease in China as early as 1637. It was reported as Imochibyo in Japan in 1704, and brusone in Italy in 1828. It was reported in the USA as early as 1876 and in India in 1913. In Pakistan rice blast is mostly found in all the rice growing areas including district Lahore, Gujranwala, Sheikhupura, Hafizabad and Narowal, Toba Tek Sing, Vehari and place like Gaggo Mandi (Arshad *et al.*, 2008). Blast is known to attack nearly all above ground parts as well as during all growth stages of plant. Recent reports have shown that the fungus has the capacity to infect plant roots also (Sesma & Osbourn, 2004). Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al.*, 1994). Khan *et al.* (2008) reported that most of the basmati varieties cultivated in the country is susceptible to blast. Although blast is capable of causing very severe losses of up to 100%, little information exists on the extent and intensity of actual losses in farmer's fields. Losses of 5 to 10, 8 and 14% were recorded in India in (1960-1961), (Padmanabhan, 1965), in Korea (mid-1970s) and in China (1980-1981) respectively, (Teng, 1986). In Philippines, yield losses ranging from 50 to 85% were reported. (Naque *et al.*, 1983). Some analysts estimate that each year blast destroys rice that could feed 60 million people, at a cost of some $66 billion (FAO, 2012). Khan *et al.* (2002) reported that most of the basmati varieties cultivated in the country are susceptible to blast. The disease is weather driven and the pathogen is highly variable. To reduce damage by this disease, most farmers' use resistant varieties, apply fungicidal chemistries, and avoid excessive amounts of fertilizers. Despite these precautions, rice blast causes severe losses under certain meteorological conditions. This is due to the interacting influences of weather on both the reproductive ability of the parasite and the resistance of the rice plant. Thus, an outbreak of rice blast in a given paddy field cannot be explained solely by the relation between the parasite and meteorological conditions. Many scientists have worked in their own areas on the epidemiology of the rice blast pathosystem. Yet it was first time to study the epidemiology of the rice blast in Multan in Southern Punjab. Hence the present study was conducted with the aim to explore the environmental factors conducive for the epidemiology of rice blast-pathosystem in District Multan, Punjab, Pakistan.

Materials and methods

Study site

The current research was conducted at Agricultural Experimental Farm, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan (30.268 ˚N and 71.495 ˚E, 122 m altitude from sea level) from May to November 2013.

Plant material, Nursery sowing and transplanting

The planting material viz., Basmati-2000, Basmati-385, Shaheen-basmati, Basmati-Pak and Basmati super were collected from National Agriculture Research Center (NARC) Islamabad, and nursery was prepared on raised beds with seeds of each variety in individual small plots of (1 x 1 ft width and length). Forty days old rice seedlings of individual cultivar were transplanted into the field in a Randomized Complete Block Design (RCBD) with three replicates consisted of fifty plants of each variety randomized at 9˝ plant-to-plant and row-to-row distance.

Recommended fertilization with N: P: K at the rate of 58:23:25 kg/acre and routinely cultural practices were performed to sustain a vigorous crop stand.

Pathogen isolation and identification

Rice blast infected plants were observed in the field and identified on the basis of visual symptoms of the disease. To confirm the association of *Pyricularia oryzae*, diseased leaves were brought to the Mycology laboratory, Department of Plant Pathology, Bahuddin Zakariya University, Multan; excised into small pieces 1.0-1.5cm, surface sterilized with 1% sodium hypochlorite solution, counter washed with distilled water and lined on petri plates containing the potato dextrose agar medium.

The inoculated petri plates were incubated at 27 ± 2°C for a week to confirm the pathogen by its cultural and physiological characteristics as described in technical bulletin on seed borne diseases and seed health testing of rice (Nelson *et al.*, 1983).

Disease severity and area under disease progress curve

Rice blast disease severity was assessed in the field on the basis of 0-to-9 class, disease rating scale (IRRI, 1996) wherein class 0 was the highly resistant response of the varieties and no lesion was observed on the plants. Class 1 was the resistant response yet small brown specks of pin head diameter were appeared on the leaves. Class 2 and 3 were the moderately resistant response where at small round to elongated necrotic grey spots with distinct brown margins were visible on both upper and lower leaves. Class 4 and 5 showed the moderately susceptible response where lesions of 3mm or longer size infecting 4-to-10% leaf area were visible while class 6 and 7 consisted of susceptible response of the variety showing 26-to-50% infected leaf area.

Class 8 and 9 showed the highly susceptible response of the disease leading towards 75% or more infection of the leaf area. Disease severity was recorded for the whole cropping season and the data were averaged by the following formula (Anonymous, 1996).

$$\text{Disease Severity index} = \frac{\text{Sum of all the score of individual plants/variety}}{\text{Total No. of plants observed}} \times \frac{100}{\text{Maximum Scale}}$$

Area under disease progress curve (AUDPC) was determined by trapezoidal assimilation of percent disease severity over time taking into consideration the total crop duration evaluated as suggested by Shanner and Finney (1977).

$$AUDPC = \sum_{i=1}^{n-1} [(x_i + x_{i+1})/2]\,(t_{i+1} - t_i)$$

Correlation and linear regression

To study the epidemiology of the rice blast pathosystem; correlation and linear regression among the metrological factors and disease severity was determined. For this, the data regarding the environmental variables i.e. maximum and minimum daily temperature of air, relative humidity of morning and evening, rainfall, wind speed and daily sunshine hours were collected at the Metrological Station, Agricultural Experimental Farm, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan on daily basis for whole cropping season and averaged for the analysis. Metrological factors significantly affecting the disease severity were identified and established by the linear regression analysis whereas the climatic factors were set as independent variables and disease severity served as the dependent variable.

Statistical analysis

All the collected datasets were statistically analyzed by analysis of variance (ANOVA). The dataset were finally subjected to correlation of disease severity with the climatic factors and linear regression analysis to identify the responsive variable using the SAS^{R} 2002, (Statistical analysis system, V.8.0, SAS Institute, Cary, NC).

Results

Conducive environmental conditions facilitated early disease initiation, rapid disease development and highest disease pressure in the highly susceptible genotypes during experiment whereas varieties with delayed initiation of disease, slower rates of disease development, and lower final disease severities were measured. Different rice cultivars grown for the experiment in the field showed varying response to rice blast disease under the influence of significant metrological conditions. Basmati-2000 showed maximum disease severity 62.32% followed by Basmati-385 and Shaheen basmati with 56.51 and 51.80% disease severity indicating the highly susceptible response. Similarly, the disease severity was recorded minimum on Basmati-Pak and Basmati-super with 49.43 and 43.29% showing a susceptible response to rice blast disease. AUDPC was calculated maximum (635.35) on Basmati-2000 and minimum (432.85) on Basmati-Pak following the method suggested by Jeger and Viljanen-Rollinson (2001) based on the integration of disease progress models (Table 1).

Table 1. Response of various rice varieties to rice blast disease epidemic under natural environmental conditions.

Sr. No.	Variety	Disease severity	Response	*AUDPC
1	Basmati-2000	62.32 a	**HS	635.35 a
2	Basmati-385	56.51 b	HS	561.20 b
3	Shaheen Basmati	51.80 c	HS	516.50 c
4	Basmati- Pak	49.43 c	***S	432.85 d
5	Basmati- Super	43.29 d	S	505.60 e

Means followed by the same letter in the disease severity column are not statistically different at ($P < 0.0001$).

* Area under disease progress curve (Means with the same letter in AUDPC column are statistically similar at *($P < 0.0001$)*

** Highly susceptible

*** Susceptible.

Table 2. Weather parameters during assessment weeks of rice blast epidemic in 2013 (08th Aug to 07th Nov).

Weeks	[a]Temp. Max. (°C)	[b]Temp. Min. (°C)	[c]Temp. Avg. (°C)	[d]RH (%)		Rainfall (mm)	Sun Shine (h)	[e]WV (Km/h)
				8.00 am	5.00 pm			
1st	34.50	25.30	29.75	79.10	66.21	0.00	8.51	6.16
2nd	36.21	26.13	31.17	77.11	65.21	0.00	9.33	5.79
3rd	34.01	27.10	30.51	75.01	75.00	2.11	8.33	7.51
4th	34.33	24.02	29.17	83.19	71.00	0.33	9.00	6.41
5th	36.16	26.11	31.13	77.10	53.01	0.00	9.41	4.12
6th	35.77	26.66	30.93	76.61	58.91	0.00	8.14	6.53
7th	34.66	25.33	29.51	91.13	72.21	2.66	5.33	8.91
8th	34.66	25.33	29.51	83.19	64.21	0.00	6.29	7.61
9th	37.11	23.01	30.09	91.10	71.34	3.33	4.00	6.41
10th	31.01	16.11	23.51	70.11	62.10	1.17	8.01	5.19
11th	28.01	16.57	22.41	81.41	62.02	0.33	4.13	2.96
12th	34.11	22.19	28.11	83.11	47.09	2.21	8.21	2.33
13th	33.66	21.91	27.75	79.91	63.11	0.33	7.19	2.66

[a]= Temperature maximum, [b]= Temperature minimum, [c]= Temperature average, [d]= Relative humidity, [e]= Wind velocity.

The overall correlation of meteorological factors with the disease severity on all the genotypes showed the significant effect of rain fall, relative humidity of morning and evening which played a key role for the development of rice-blasting pathosystem. Rainfall and relative humidity showed a positive correlation whereas temperature maximum depicted a negative correlation and remained irresponsive in disease development (Table 2,3).

All of the regression model parameters were significant at $P \leq 0.05$. The metrological data of the rice cropping season 2013 (May to November) clearly indicated that rain fall, relative humidity of morning and evening and wind speed significantly contributed to the development of rice blasting pathosystem except sun shine hours and temperature factor. The coefficient of determination (r^2) of 0.93 proved the significance of overall regression model accuracy (Table 4 and 5).

Table 3. Overall correlation of weekly environmental conditions with rice blast disease severity recorded on five varieties.

Weather variables	[a]Temp. Max. (°C)	[b]Temp. Min. (°C)	[c]RH (M) (%)	[d]RH (E) (%)	[e]RF (mm)	[f]WV (Km/h)	[g]SS (h)	[h]DS
Temp (Max.)	1							
Temp. (Min.)	– .961**	1						
RH (M)	.162*	.163*	1					
RH (E)	.555**	.577**	.617**	1				
RF	.162*	.178*	.031	.145*	1			
WV	.648**	.727**	.338**	.876**	– .175*	1		
SS	.955**	.989**	– .045	.659**	– .181*	.771**	1	
DS	– .924**	– .892**	.097	– .576**	.101	– .677**	– .895**	1

** Correlation is significant at $P > 0.05$ (2-tailed)

* Correlation is significant at $P > 0.01$ (2-tailed)

[a]= Temperature maximum, [b]= Temperature minimum, [c] = Relative humidity morning, [d] = Relative humidity evening, [e]= Rain fall, [f]= Wind velocity, [g]= Sunshine hours, [h]= Diseases severity.

Table 4. Analysis of variance of regression analysis for rice blast disease during (2013).

Source	DF	Sum of square	Mean square	F-value	P-value
Regression	1	59982.481	59982.488	177	(P<0.0001)
Residual	193	10319.123	53.46		
Error	187	9218.851	49.29		
Total	194	70301.606			

* Significant at ($P \leq 0.05$).

With respect to the comparative importance of the climatic factors, rain fall had evidenced the highest percentile contribution (87%) followed by relative humidity of the morning (81%), relative humidity of the evening (61%), wind speed (31%) and temperature minimum (11%) towards the disease development. Based on the Mallow's (Cp) value model 3, 4 and 5 had the best combination of input variables (Table 6).

Regression equation of full model

The regression model was elaborated with the regression equation shown as under:

$\mathbf{y} = 106.902 - 1.91 X_1 + 0.32 X_2 + 0.65 X_3 + 4.14 X_4 - 2.60 X_5$

where, X_1 = Rainfall, X_2 = Relative humidity of the morning, X_3 = Relative humidity of the evening, X_4 = Wind speed and X_5= Temperature minimum.

The model developed as full showed 96% variability towards the advancement of the disease.

Discussion

Different rice cultivars grown for the experiment in the field showed varying response to rice blast disease under the influence of significant metrological conditions. Basmati-2000 showed maximum disease severity 62.32% followed by Basmati-385 and Shaheen basmati with 56.51 and 51.80% disease severity indicating the highly susceptible response. Similarly, the disease severity was recorded minimum on Basmati-Pak and Basmati-super with 49.43 and 43.29% showing a susceptible response to rice blast disease. AUDPC was calculated maximum (635.35) on Basmati-2000 and minimum (432.85) on Basmati-Pak following the method suggested by Jeger and Viljanen-Rollinson (2001) based on the integration of disease progress models. EI-Refaei (1977) described that the susceptibility of rice plants to the rice blast fungus *Magnaporthe grisea* is increased by cold stress. However, the mechanism of this phenomenon is unknown. Our results indicate that low temperature condition is a key factor for suppressing rice plant resistance and making it susceptible to *M. grisea*. Greer and Webster (2001) explained that rice blast, caused by *Pyricularia oryzea*, was first found in California in 1996 during the disease surveys spreading at a moderate rate in rice fields.

Table 5. Coefficients estimates of the regression analysis for rice blast disease during (2013).

Variables	Parameter estimate	Standard error	Sum of square	F-value	Pr > F
Intercept	106.902	49.769	227.452	4.61	*(P<0.0001)**
Temp (Max)	−5.111	0.566	4008.428	8.31	*(P<0.0001)**
Temp (Min)	−2.601	1.525	143.384	2.91	*(P<0.0001)**
RH (M)*	0.328	0.433	28.328	0.57	*(P<0.0001)**
RH (E)*	0.652	0.921	24.731	0.50	*(P<0.0001)**
RF*	1.914	1.337	100.999	2.05	*(P<0.0001)**
WS*	4.145	1.288	510.226	10.35	*(P<0.0001)**
Sun Shine	−3.106	2.793	60.938	1.24	*(P<0.0001)**

* Significant at ($P \leq 0.05$).

Although no effective major resistance genes are known to occur in widely grown commercial rice cultivars, there appear to be differences among the cultivars with respect to field susceptibility to the pathogen. *P. oryzae* was recovered from rice crop residue and commercial seed lots which were suggested as possible sources of initial *P. oryzae* inoculum in rice fields. Meteorological factors also affect the cultivation and soil conditions, which affect the growth of the rice plant.

Table 6. Summary of regression analysis with input variables.

Step	Variable	Number Var. In.	Partial R-square	Model R-square	Mallow's C(*P*)	F-value	Pr > F
1	RF	1	0.875	0.815	29.553	2.673	*(P<0.0001)**
2	RF, RH (M)	2	0.081	0.848	17.000	0.577	*(P<0.0001)**
3	RF, RH (M), RH (E)	3	0.061	0.918	13.307	1.441	*(P<0.0001)**
4	RF, RH (M), RH (E), WS	4	0.031	0.933	11.230	14.911	*(P<0.0001)**
5	RF,RH(M),RH(E),WS, Temp(Min)	5	0.011	0.966	6.745	1.810	*(P<0.0001)**

Mallow's C*p* is a measure of error in the best subset model. Adequate models have low C*p* values which shows the mallows constant.

Recently, these relationships were made still more complicated by the introduction of new fungicides that influence both the parasite and the rice plant (Dean, 1997; Hamer *et al.*, 1988). The overall correlation of meteorological factors with the disease severity on all the genotypes showed the significant effect of rain fall, relative humidity of morning and evening which played a key role for the development of rice-blasting pathosystem. Rainfall and relative humidity showed a positive correlation whereas temperature maximum depicted a negative correlation and remained irresponsive in disease development. The primary inoculum for this disease develops after the pathogen has overwintered inside affected rice straw remaining from the previous year. Conidia are formed when the temperature and humidity rise in the spring. After these conidia infect the rice plants, the fungus develops inside the host tissues, and then induces lesions in which new conidia are formed. An epidemic results when this cycle is repeated many times. The intensity of the epidemic is determined mainly by the abundance of conidia, the influence of environmental conditions on infection processes, and the resistance of the host plants.

These three factors are influenced not only by meteorological conditions, but also by the varieties and cultivation practices used, so that the development of the disease is very complex. When rice blast infects young rice seedlings, whole plants often die, whereas spread of the disease to the stems, nodes or panicle of older plants results in nearly total loss of the rice grain (Talbot, 2003). Different host-limited forms of *P. oryzae* also infect a broad range of grass species including wheat, barley and millet.

Conclusion

Correlation of environmental variables with disease severity showed rain fall, relative humidity of morning and evening as significant parameters for the development of rice-blasting pathosystem.

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Abbreviations

AUDPC= Area under disease progress curve, NARC= National agriculture research center Islamabad, RCBD= Randomized complete block design, IRRI= International rice research institute.

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