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***Full Length Article***

# Evaluation of Virulence of *Xanthomonas oryzae* pv. *oryzae* against Rice Genotypes

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## Abstract

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*, arguably is the most important disease of rice (*Oryza sativa* L.) worldwide due to its growing concern, wide spread and destructive nature and lack of understanding its control. The present study was carried out for screening of rice germplasm against *X. oryzae* pv. *oryzae* and determination of phenolic contents regarding the physiology and biochemistry of diseased plants. Out of the 40 cultivars tested none of the variety showed significantly resistant response against the virulence of pathogen; only six were found moderately resistant, eight were graded as moderately susceptible, while 19 showed susceptible and six as highly susceptible response. Significantly highest disease severity (68.08%) was recorded on TN1, whereas minimum (11.92%) was calculated on Mehak-2006. All the basmati varieties were found to be susceptible to highly susceptible against the disease and showed disease severity within a range of (51.21 to 65.05%), while the KSK genotypes presented (32.62 to 45.68%) disease severity. The total phenolics concentration significantly varied in response to treatment and showed negative correlation with the resistance of the variety. The highest phenolics compounds (183.42 mg $100^{-1}$) were produced in Mehak-2006, while the phenolics of TN1 were not in the range of gallic acid standard. It appeared that resistant varieties showed higher concentration of phenolics than the susceptible ones, which played important role in reducing the disease severity. Thus, the study suggests that the exploitation of secondary metabolites in breeding programs and integrated management strategies could open the avenues to avoid future disease epidemics. 

**Keywords:** Rice; *Xanthomonas oryzae* pv. *Oryzae*; Genotype; Phenolics; Resistance

## Introduction

Rice is world's important crop and a major source of nutrition for about two-thirds of mankind by providing 21 % of per capita energy (Smith and Bruce, 2000; Zafar *et al*., 2004). Similar to cereal grains, rice is also rich in nutrient i.e., carbohydrates, proteins, fatty acids and many bioactive non-nutritive compounds, known as antioxidants, including phenolics which exists in relatively high concentration and accumulate in response to wounds and invasion of pathogen (Frei and Becker, 2004). The total cultivation area under rice, worldwide is 170 million hectares which is almost 17% of all available crop land worldwide with the average production of 490.5 million tonnes (Anonymous, 2013a). Pakistan ranks 11$^{th}$ for the world rice production with the cultivation area of 2.3 million hectares and an average production of 5.5 million tonnes (Anonymous, 2013b). Besides the fact that rice is an important crop but the average production is too low in Pakistan from the rest of the world top rice producing countries in general and in particular far below from many neighboring countries because this crop is threatened to many constraints of biotic and abiotic disorders (Khan *et al*., 2009). Bacterial leaf blight (BLB) of rice (*Oryza sativa* L.), caused by *X. oryzae* pv. *oryzae* (Ishiyama, 1922; Swings *et al*., 1990) is a major bacterial disease of significant importance, prevalent in rainfed, irrigated, deep water, temperate and tropical rice growing areas of the world (Mew, 1987). The disease has become a serious threat to rice crop in South East Asia and particularly to Japan causing 25–30% losses, often rising up to 50–60 % while in India, Philippines and Indonesia losses were recorded up to 10–81% in some genotypes (Ahmad and Singh, 1975). During the 1960–70's, by the introduction of TN1 and IR 8 bacterial leaf blight became common in the Asian rice growing areas (Amna, 2008).

In Pakistan, the disease was first noticed in 1977 at Rice research Institute, Kala Shah Kaku on rice cultivars Palman, Basmati 198 and IRRI-6 (Mew and Majid, 1977; Ahmad and Majid, 1980). Increasing incidence of disease has been reported from Khayber Phukhtoon Khaw (KPK), Sindh and Punjab especially in Kallar belt of Punjab (Akhtar and Sarwar, 1986; Akhtar and Akram, 1987). Bacterial leaf blight is a vascular disease, causes systemic

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infection and symptoms are noticed at tillering stage which comprises of two phases leaf blight and kresek phase (Ou, 1985; Akhtar *et al*., 2008). The most characteristic symptoms of the disease are yellow lesions with wavy margins at the leaf blade which normally extend to sheath; later on these lesions acquire whitish straw color followed by the bacterial ooze from infected leaves in warm and humid climates (Mew, 1987). The destructive manifestation of the disease can be observed during the kresek phase wherein the entire plants wilt and become pale yellow at early tillering stage, resulting in total crop failure (Mew *et al*., 1993). The disease is so important because its presence predisposes rice to many other diseases and it has the potential to become the serious threat for rice crop in Pakistan mainly due to the lack of information about the pathogen and effective control strategy (Waheed *et al*., 2009). In 1997, 2002, 2006, 2007 and 2008 epidemics of disease were recorded in rice zone of Northern and central Punjab due to the famous basmati super variety and absence of resistant cultivars (Khan *et al*., 2009). Akhtar *et al*. (2008) reported the prevalence of bacterial blight in four provinces of Pakistan with maximum disease incidence in Punjab. It has been reported that all the commercial basmati varieties normally cultivated in Punjab were highly susceptible to bacterial leaf blight (Cheema *et al*., 1998; Khan *et al*., 2000a, b; Akhtar *et al*., 2008; Ali *et al*., 2009).

A number of diseases of various crop plants are controlled by numerous chemistries, but they are not fit and not environment friendly (Liu, 2007). Struggle for survival has enabled the crop plants to develop complex response systems which provide a line of defense to plants. The presence and accumulation of phenolics in plants is the most useful weapon in their defense against the pathogens (Abid *et al*., 2008). The plants synthesize secondary metabolites, chemically heterogeneous compounds including phenolics, flavanoids, tannins and lignins which play important role in the reduction of disease severity (Tiaz and Zeiger, 2002). Beside any control measure of the disease, resistant varieties offered an economically sound alternative for the management of the crop plants. No work has been done on bacterial blight of rice in Multan region so the prime objective of study was to explore the source of resistance in forty rice genotypes against bacterial leaf blight and their relation with the potential role of phenolics in restricting the disease severity.

## Materials and Methods

### Study Site

Study was carried out 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

A total of 40 cultivars were used in these studies. The seed was obtained from National Agriculture Research Centre, Islamabad and Rice Research Institute, Kala Shah Kaku which comprised of one local and two international highly susceptible checks viz., Basmati super, IR 6 and TN 1.

### Experimental Design

The present studies were conducted in the year 2013–2014. For nursery preparation, seeds of each variety were sandwiched separately within a fine layer of crushed farm yard manure (FYM) in forty small plots of (1 x 1 ft width and length) on raised beds in clayey loam soil, covered with wheat straw and watered with sprinkler thrice a day whereas nursery was flooded first time after one week of sprouting. Forty days old plants were transplanted into the field in a Randomized Complete Block Design (RCBD) with three replicates. Each replication represented ten plants of each variety randomized at 9″ plant to plant and row to row distance in each line and susceptible check of basmati super was used as a spreader line after each two test lines. Plants were given fertilization according to the recommended doses of N: P: K at the rate of 143.2: 56.81: 61.75 kg $ha^{-1}$, respectively, while normal cultural practices were performed to maintain a healthy crop stand.

### Sample Collection and Pathogen Isolation

Current studies were carried out in the rice cropping season 2013. The disease was observed in patches in the field with characteristic symptoms of yellow to white water soaked stripes with wavy margins at the edges and on the leaf blade. The samples were collected from the experimental research farm, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, kept in plastic bag and brought to Laboratory of Department of Plant Pathology, Bahauddin Zakariya University, Multan. Diseased tissues of (0.5–1 cm) from the collected samples were excised with sterilized scalpel, leaf surface was disinfected in 1% sodium hypochlorite (NaOCl) solution and washed twice in sterilized distilled water, later dried on sterilized blotter paper and positioned into sterilized petri plates lined with Nutrient Agar (Bio Basic Inc.) at 28 ±1°C temperature for 72 h. Bright yellow, circular and viscous colonies of the bacterium (*X. oryzae* pv. *oryzae*) which developed subsequently in petri plates were cultured on fresh nutrient agar and grown at 28 ± 1°C for three days (Wilson *et al*., 1967; Devadath and Dath, 1970).

### Field Screening of Rice Germplasm for BLB Resistance

**Inoculum preparation and inoculation of plants:** Bacterial culture maintained at –20°C was revived on nutrient agar slants at 28 ± 1°C for 48 h, transferred to fresh nutrient agar slants and incubated for 48 h at 28 ± 1°C.

Each NA slant suspended with distilled water was transferred to volumetric flasks, prior to inoculation. Bacterial concentration was adjusted to about $10^8$cfu/ ml at the wavelength of 600 nm using the spectrophotometer (Goto, 1992). Inoculation was done after 21 days of sowing by clipping method with sterilized scissors (Kauffman *et al*., 1973). The inoculation of 1200 plants was completed in the evening time to favor the entry of bacteria into infection courts in the presence of enough moisture on leaf surface.

**Disease Assessment**

Development of bacterial leaf blight on rice plants was assessed fourteen days after inoculation, the time on which control plants produced fully susceptible lesions. BLB on rice plants in the field were rated on the basis of 0–9 scale (Chaudhary, 1996). Class 0 was the highly resistant response in which no lesions were visible on leaves. Class 1 was the resistant response exhibited 0.1–10.0% lesion area while class 3 and 5 were moderately resistant to moderately susceptible response covering 10.0–25.0 and 25.1–40.0% lesion area, while on the other hand Class 7 and 9 consisted of susceptible to highly susceptible response ranging from 40.1–60.0 and 60.1–100% lesion area on rice plants. The disease severity index of BLB was calculated from September 2013 to November 2013 by the formula as mentioned below and all the data set was averaged to find out the response of rice cultivars (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 for each genotype, taking into account the total crop duration evaluated (Madden *et al.* 2007).

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

Where, *n* represents number of dates on which disease was recorded; *Xi*, Percent disease severity on the *ith* date; and $(t_{i+1}-t_i)$, duration between two successive assessments.

**Estimation of Phenolics Compounds**

The leaf samples of individual variety were collected from the field, kept into the ice box and brought to the laboratory for examination of total phenolics contents. The modified Folin–Ciocalteu assay was used in this investigation (Singleton *et al*., 1999). Gallic acid (Sigma-Aldrich, USA) stock solution, (1 mg/10 mL) and working standard concentrations of 0, 10, 25, 50 (ppm) were made in distilled water to prepare the gallic acid calibration standard curve. A sample of 0.5 g was homogenized in 2.0 mL 70% ethanol (Merck, Germany) to prepare the plant extract and incubated for two hrs in water bath at 65ºC. The samples were centrifuged at 14000 rpm for five minutes in centrifuge tubes in (Centurion, UK). An amount of 4 mL of Folin – Ciocalteu phenol reagent (Sigma – Aldrich, USA) was added to one ml of supernatant taken from the sample, kept for seven min at room temperature and later on sodium carbonate was added in the treated sample. Test tubes were shaken vigorously and incubated in darkness for two hrs. Optical density of test sample of each variety was determined at 765 nm using the spectrophotometer (UV 300, ORI, Germany). Total phenolics concentration (w/ v), absolute weight was determined on the basis of standard concentration of gallic acid (Kelsey and Harmon, 1989).

**Statistical Analysis**

All the collected data sets were statistically analyzed and subjected to analysis of variance (ANOVA). Treatments means of all data sets were compared by the least significant difference (LSD) and Duncan's Multiple Range (DMR) test for multiple mean comparison at ($P \leq 0.05$) by using the statistical software package (SAS, 2002). The varieties were grouped into different levels of resistance by construction of dendrogram using the cluster analysis (Minitab 1.5).

## Results

Infected leaf samples showing bacterial blight symptoms were collected and the causal bacterium was isolated on NA from the fresh lesions on the leaves which are better isolation material. The bacterium produced light yellow, raised and circular colonies on the medium.

**Disease Severity Assessment**

Analysis of variance indicated significant ($P < 0.05$) variation among all the varieties at different locations for bacterial blight resistance in the natural environment of Multan. Out of the forty genotypes, eight cultivars showed moderately susceptible response, 19 varieties were grouped as susceptible, six varieties proved to be moderately susceptible against the virulence of pathogen and six cultivars gave highly susceptible response. Significantly highest disease severity (68.08%) was recorded in the international susceptible check TN1 responded as highly susceptible and minimum disease severity (11.92%) was observed in Mehak 2006, which proved to be moderately resistant. All KSK varieties showed disease severity within a range of (32.61 to 45.68%) and gave a susceptible response while basmati varieties showed maximum disease severity against the virulence of the pathogen. The AUDPC was calculated to show the disease progress throughout the whole season in the field (Table 1). The dendrogram showed five different groups among the forty cultivars, group one and two included twenty varieties which were susceptible to highly susceptible in their response; while cluster three, four and five indicated twenty varieties while were moderately susceptible to moderately resistant in their response to disease (Table 2; Fig. 1).

**Table 1:** Rice genotypes used in bacterial leaf blight evaluation, disease severity with AUDPC and their response

| Genotype | Percent disease | | Scale for infection rate | Response resistance/[d] | Clip inoculation 30 plants/variety |
|---|---|---|---|---|---|
| | Severity[a] | AUDPC[b] | | Susceptible | (Percent plant infection) |
| KSK133 | 45.24 a-g | 444.47 fgh | 7 | S | 73.33 c-e |
| KSK-434 | 42.68 a-i | 425.51fgh | 7 | S | 73.33 c-e |
| KSK-463 | 45.26 a-g | 454.72fg | 7 | S | 73.33 c-e |
| KSK-282 | 47.16 a-f | 472.13efg | 7 | S | 73.33 c-e |
| KSK-14 | 32.61 c-j | 316.38 ij | 5 | MS | 50.00 gh |
| IR-6 | 62.26 ab | 632.81 abc | 9 | HS | 93.33 ab |
| IR-9 | 60.93 abc | 617.96 abc | 9 | HS | 90.00 a-c |
| SHUA-92 | 15.83 hij | 155.91 k | 3 | MR | 33.33 hi |
| KARGHNI TORH | 15.12 ij | 148.12 k | 3 | MR | 30.00 i |
| JAJAI-77 | 26.26 e-j | 261.82 j | 5 | MS | 50.00 gh |
| PS-2 | 44.63 a-h | 444.17 fgh | 7 | S | 76.67 b-d |
| SATHRA | 29.58 e-j | 280.39 ij | 5 | MS | 50.00 gh |
| F. MALAKAND | 18.77 f-j | 177.45 k | 3 | MR | 33.33 hi |
| JP-5 | 41.13 a-i | 413.12 gh | 7 | S | 73.33 c-e |
| PK-386 | 45.46 a-g | 456.96 fg | 7 | S | 70.00 d-f |
| SARSHAR | 29.82 e-j | 296.33 ij | 5 | MS | 50.00 gh |
| DILROSH-97 | 30.17 d-j | 290.74 ij | 5 | MS | 53.33 gf |
| MALHAR-346 | 42.37 a-i | 419.91 fgh | 7 | S | 76.67 b-d |
| PAKHAL | 43.64 a-i | 435.34 fgh | 7 | S | 73.33 c-e |
| SHANDAR-2006 | 31.91 d-j | 303.71 ij | 5 | MS | 50.00 gh |
| MEHAK-2006 | 11.92 j | 117.68 k | 3 | MR | 33.33 hi |
| SWAT-1 | 42.23 a-i | 417.85 gh | 7 | S | 56.67 e-g |
| SWAT-2 | 44.34 a-h | 440.76 fgh | 7 | S | 70.00 d-f |
| DR-82 | 32.22 c-j | 319.67 ij | 5 | MS | 50.00 gh |
| DR-83 | 47.03 a-f | 470.65 efg | 7 | S | 70.00 d-f |
| DR-92 | 48.86 a-e | 487.97 efg | 7 | S | 70.00 d-f |
| BASMATI-385 | 51.21 a-e | 509.95 efg | 7 | S | 70.00 d-f |
| RACHNA BAS[e] | 17.73 g-j | 170.15 k | 3 | MR | 30.00 i |
| DOKRI BAS | 16.05 hij | 157.82 k | 3 | MR | 30.00 i |
| BASAMTI PAK | 54.73 a-e | 552.03 cde | 7 | S | 73.33 c-e |
| BASMATI-370 | 49.84 a-e | 496.88 efg | 7 | S | 73.33 c-e |
| PUSA BASMATI | 36.72 b-j | 356.71 hi | 5 | MS | 50.00 gh |
| BASAMTI 515 | 58.76 a-d | 598.56 cd | 7 | S | 76.67 b-d |
| PUNJAB BAS | 47.03 a-f | 470.65 efg | 7 | S | 70.00 d-f |
| BASAMTI 198 | 63.55 ab | 652.27 ab | 9 | HS | 96.67 a |
| SHAHEEN BAS | 52.22 a-e | 515.73 def | 7 | S | 76.67 b-d |
| BASMATI 2000 | 64.73 ab | 660.52 ab | 9 | HS | 96.67 a |
| BASMATI SUPER | 65.05 ab | 661.96 ab | 9 | HS | 100.00 a |
| IR-24 | 66.71 a | 681.33 ab | 9 | HS | 100.00 a |
| TN-1 | 68.08 a | 697.56 a | 9 | HS | 100.00 a |

aMean with the same letter are not statistically different at P = 0.05, DF= 798, S.E for comparison = 7.45
bArea under disease progress curve (Mean with the same letter are statistically similar at P=0.05, DF= 39
cRandomized complete block with standard error
dResponse, R= resistant, S= susceptible, MR= moderately resistant, MS= moderately susceptible, HS= highly susceptible, e = Basmati

**Determination of Phenolics Contents**

Total phenolics concentration significantly varied in response to inoculation treatment and negatively correlated with the disease severity. Bacterial leaf blight disease severity showed highly significant negative correlation with the total phenolics concentration. Results revealed a significant increase in phenolics concentration in the leaves of cultivars which showed less amount of infestation to bacterial blight. Significantly high phenolics contents (183.42 mg 100 $g^{-1}$) were recorded in Mehak 2006 followed by Karghni Torh (157.66 mg 100 $g^{-1}$) and Shua 92 with (150.80 mg 100 $g^{-1}$). Resistant varieties showed high concentration of phenolics than the susceptible genotypes. Pearson correlation of disease severity with the phenolics compounds produced in the rice genotypes in response to the inoculation of *X. oryzae* pv. *oryzae* was determined. Nineteen genotypes showed a significant correlation at $P$=0.05 while four genotypes showed highly significant correlation at $P$= 0.01 with the disease severity index and eighteen varieties showed non-significant correlation (Table 3). The overall correlation between the disease severity and phenolics contents produced, showed the value ($r$ = − 0.933) which showed negative correlation (Fig. 2).

## Discussion

Bacterial leaf blight of rice addressed in the present study is now becoming more common in rice growing areas of the Southern Punjab and may cause enormous losses to the crop. Pathogen was isolated from the samples collected from the field and multiplied on artificial media.

**Table 2:** Cluster analysis of various rice genotypes on the basis of genetic resistance

| Clusters | Observations | SS* | AD* | MD* |
|---|---|---|---|---|
| 1 | 14 | 81.1639 | 2.12026 | 4.17224 |
| 2 | 8 | 76.4100 | 2.45783 | 6.05442 |
| 3 | 8 | 65.7282 | 2.41904 | 4.99498 |
| 4 | 6 | 35.9377 | 1.96093 | 4.28732 |
| 5 | 4 | 13.7384 | 1.54277 | 2.79594 |

*SS: Sum of square within cluster; *AD: Average distance from centroid; *MD: Maximum distance from centroid

**Table 3:** Concentration and correlation of total phenolic compounds with disease severity in *Xanthomonas oryzae* pv. *oryzae* inoculated rice genotypes

| Genotype | Phenolic compounds mg/g (dry weight ± S.E.)[a] | Correlation coefficients[b] |
|---|---|---|
| KSK133 | 23.85 ± 0.43 i-n | [c]-0.999* ([d]0.016) |
| KSK-434 | 26.95 ± 4.54 i-m | [d]-0.971 (0.152) |
| KSK-463 | 30.66 ± 4.53 ij | - 0.976 (0.138) |
| KSK-282 | 18.95 ± 0.87 lmn | - 0.999* (0.017) |
| KSK-14 | 63.33 ± 1.24 h | - 0.999* (0.014) |
| IR-6 | 00.95 ± 3.12 p | - 0.192 (0.876) |
| IR-9 | 06.23 ± 1.16 op | - 0.973 (0.146) |
| SHUA-92 | 150.80 ± 2.39 c | - 0.997** (0.004) |
| KARGHNI TORH | 157.66 ± 7.58 bc | - 0.999** (0.001) |
| JAJAI-77 | 106.57 ± 0.33 e | - 0.999* (0.005) |
| PS-2 | 23.80 ± 2.95 i-n | - 0.984 (0.112) |
| SATHRA | 90.47 ± 4.13 f | - 0.998* (0.037) |
| F. MALAKAND | 136.90 ± 1.71 d | - 0.999* (0.016) |
| JP-5 | 29.57 ± 1.64 ijk | - 0.996 (0.053) |
| PK-386 | 28.09 ± 3.32 i-m | - 0.985 (0.107) |
| SARSHAR | 93.00 ± 0.08 f | - 0.998* (0.030) |
| DILROSH-97 | 72.47 ± 3.24 gh | - 0.998* (0.037) |
| MALHAR-346 | 33.04 ± 5.85 i | - 0.970 (0.154) |
| PAKHAL | 22.76 ± 1.28 i-n | - 0.993 (0.071) |
| SHANDAR-2006 | 78.80 ± 1.53 g | - 0.998* (0.040) |
| MEHAK-2006 | 183.42 ± 0.44 a | - 0.999** (0.001) |
| SWAT-1 | 23.66 ± 1.24 i-n | - 0.997* (0.044) |
| SWAT-2 | 13.71 ± 0.72 on | - 0.999* (0.016) |
| DR-82 | 63.42 ± 0.94 h | - 0.997* (0.046) |
| DR-83 | 28.52 ± 4.35 i-l | - 0.971 (0.152) |
| DR-92 | 17.66 ± 5.14 mn | - 0.913 (0.267) |
| BASMATI-385 | 19.61 ± 1.33 k-n | - 0.997* (0.041) |
| RACHNA BAS | 163.28 ± 0.91 b | - 0.998* (0.036) |
| DOKRI BAS | 148.52 ± 0.98 c | - 0.999* (0.014) |
| BASAMTI PAK | 20.23 ± 1.49 j-n | - 0.996 (0.056) |
| BASMATI-370 | 15.09 ± 0.63 on | - 0.998* (0.036) |
| PUSA BASMATI | 65.76 ± 0.13 h | - 0.999** (0.002) |
| BASAMTI 515 | 18.57 ± 0.79 lmn | - 0.998* (0.035) |
| PUNJAB BAS | 14.19 ± 0.76 on | - 0.994 (0.068) |
| BASAMTI 198 | 4.85 ± 0.74 r | -0.999 (0.065) |
| SHAHEEN BAS | 15.38 ± 0.62 on | - 0.998* (0.038) |
| BASMATI 2000 | 36.81 ± 7.84 q | -0.954 (0.191) |
| BASMATI SUPER | 3.23 ± 5.06 s | -0.991 (0.082) |
| IR-24 | 4.66 ± 6.62 sr | -0.098 (0.112) |
| TN-1 | 1.61 ± 3.78 t | -0.997 (0.069) |

[a]Total phenolic concentration (mg/g , dry weight) calculated on the basis of standard concentrations of gallic acid

[b]Correlation (*r*) are for genotypes inoculated with *X. oryzae* pv. o*ryzae,* *Significant at $P \leq 0.05$ and ** Highly Significant at $P \leq 0.01$

cFigures without parenthesis are the pearson correlation coefficient

dFigures in the parenthesis are the probability levels (P)

During the present study evaluation of rice germplasm against the disease showed varying response on the genotypes collected from different research institutes. Each genotype showed different behavior against the pathogen and the virulence reaction involved could be as the behavior of specific genotype. IRRI type varieties showed poor resistance against the pathogen in the field experiment while cold tolerant varieties were relatively better as compared to the other genotypes in response to the disease attack and basmati genotypes possessed less natural resistance against bacterial leaf blight of rice (Ali *et al*., 2004; Shah *et al*., 2009). In the present study significant observations showed that all the basmati genotypes were highly susceptible to the disease with the greater disease severity index. Out of twelve basmati genotypes evaluated, three varieties viz. basmati super, basmati 2000 and basmati 198 responded as highly susceptible, six responded as susceptible, two cold tolerant Rachna basmati and Dokri basmati varieties responded as moderately resistant.

Amna (2008) reported basmati super as a susceptible variety against all strains of the *X. oryzae* pv. *oryzae* while basmati 385 was moderately resistant against the disease. Previous work showed that all the basmati varieties which are cultivated in Punjab were observed to be moderately susceptible to highly susceptible (Cheema *et al*., 1998; Khan *et al*., 2000a, b; Akhtar *et al*., 2008, 2011; Khan *et al*., 2009). Zhang and Mew (1985) tested the resistance of thirteen genotypes against four isolates at three different plant growth stages in the controlled conditions and revealed that there was maximum infestation of disease at the younger stages of plants. Ou *et al*. (1971) showed that preliminary evaluation of the rice cultivars could be done at seedling and flag leaf stages, diversity among the pathogen and genotype performed a significant role in reaction exhibited by the host. Lack of resistance in genotypes having the basmati background has become a serious issue to be exploited otherwise the intensity of disease will increase in coming years. The area under disease progress curve was determined for the disease progress throughout the crop season, which is a complex phenomenon controlled by genes of host and pathogen, it can be significantly exploited as a quantitative measure of disease resistance which requires repeated disease assessments by the regression equation for each variety (Jeger and Rollinson, 2001).

Phenolics compounds are the secondary metabolites having a range of functions and structures generally bearing more than one hydroxyl substituent's (Liu, 2007). The common phenolic compounds found in rice plant are ferulic acid, vanillic acid and syringic acid; flavonoids include flavones and flavonones which retard the microbial activity by accumulating at the point of invasion (Lin and Tang, 2007). In our studies, disease was lesser on varieties producing higher concentration of phenolics compounds and greater on varieties with lower concentration of phenolics contents. These facts have led to speculations that these chemicals might be involved in retarding the pathogen infection and colonization of host (Blodgett and Stanosz, 1997). The pathogen was unable to cause severe infection in

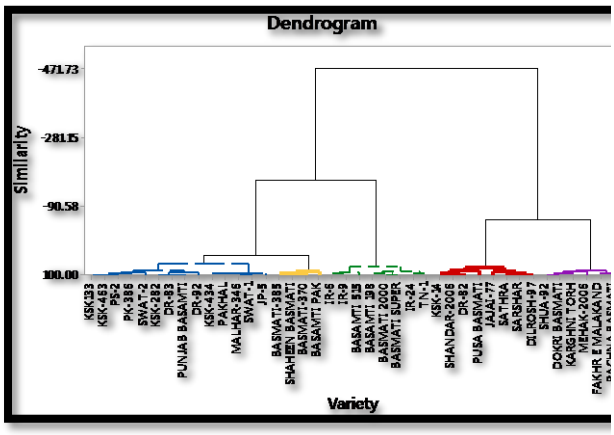


**Fig. 1:** Dendrogram showing genetic similarity among 40 genotypes of rice

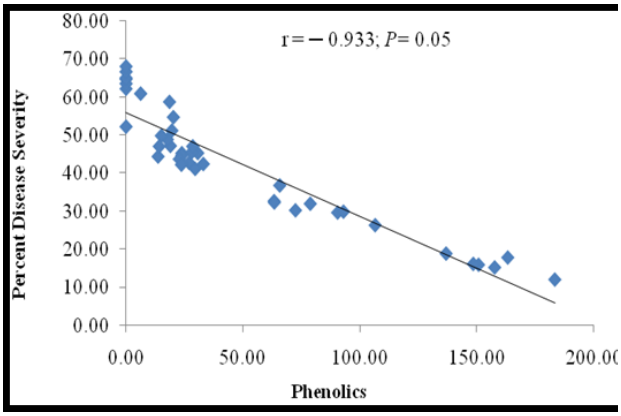


**Fig. 2:** Correlation of Phenolic compound concentration with disease severity of bacterial leaf blight of rice in various genotypes

those varieties with higher phenolics contents as they possessed a strong antimicrobial activity. Plants were able to produce secondary metabolites including phenolics mainly flavinoids, tannins and lignins played crucial role in reduction of disease severity (Tiaz and Zeiger, 2002; Wahid and Ghazanfar, 2006). They possess a strong antimicrobial activity with a wide range of physiological properties (Zhou *et al*., 2004). Our results coincide with Muntana and Prasong (2010) as they evaluated various Thai rice cultivars for the total phenolics content and antioxidant activity using the Folin Ciocalteaue assay and showed high antioxidant efficacy in all the genotypes tested.

In our experiment, few varieties with high disease severity showed the lack of significant effect on the phenolics compounds concentrations. The lack of significant effect on phenoilcs compounds concentration in the present study is however consistent with the previous work as described by Gershezon (1984). These facts may be fruitful for the evaluation of BLB resistant varieties and these resistant genotypes may directly or indirectly used for further breeding programs, genetic engineering and biotechnology for the development of genetically modified crops.

## Conclusion

The screening of available rice germplasm showed that no variety was resistant against the virulence reaction of the pathogen. All the basmati cultivars were susceptible to diseases which are grown commercially in rice growing areas of Pakistan especially in Punjab while the cold tolerant varieties performed relatively well as compared to other cultivars. It is evident from the study that phenolics compounds play an important role in reducing the disease severity due to their antioxidant activity. The response of varieties varied in case of phenolics production which determined the resistance or susceptibility level of the cultivars. The varieties which showed less disease severity were found to be with the highest concentration of phenolics accumulation. The phenolics contents were found to be negatively correlated with resistance of the varieties. Hence, their role in plant defense mechanism is needed to be addressed for future plant breeding programs.

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