Evaluation of Rice Germplasm for Resistance against *Pyricularia oryzae* the Cause of Rice Leaf Blast

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HQ and AR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MA and AR managed the analyses of the study. Authors MA and HQ managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Rice blast caused by *Pyricularia oryzae* is one of the most important diseases in rice growing areas of the world. Fifty two rice genotypes including one susceptible check, Basmati C-622, were evaluated to find out new sources of resistance and assess their diversity based on the reactions against *P. oryzae*. The test genotypes were evaluated against leaf blast after three weeks of inoculation by following the standard evaluation system for rice introduced by the International Rice Research Institute, Philippines. Diversity of the 52 genotypes was also assessed based on blast symptoms. Moderately resistant reactions were observed with genotypes KSK-470, KSK-463, KSK-460, PK 8685-5-1-1-1, KSK-462, KSK-474, PK 3810-30-1, KSK-471 and KSK-472. The 52 genotypes were grouped in 4 clusters. The grouping of some genotypes in same cluster is based on their similar reaction against leaf blast. The results of this study can be useful for selecting suitable genotypes for the development of blast-resistant rice varieties through hybridization.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important crops and staple food of many countries. Rice is rich in carbohydrates, fatty acid and proteins. In Pakistan, rice contributes 0.7% to GDP and it was sown on an area of 2891 thousand hectares with a production of 7005 thousand tones during 2014-15 [1]. Despite the importance of rice in the local consumption and economy of Pakistan, the average production is very low as compared to other rice growing countries of the world [2]. Rice crop is facing many production constraints worldwide. These constraints also include biotic and abiotic disorders. Blast is one of the serious diseases of rice causing higher yield losses. Production of rice in the world is continuously threatened by blast outbreaks [3]. Blast is caused by a fungal pathogen, called *Pyricularia oryzae*, which is an anamorph stage and the teleomorph stage is called as *Magnaporthe grisea* [4]. Fungus attacks all aerial parts of the plant. However, during vegetative growth of plant, most common symptoms appear on leaves causing leaf blast. Neck, nodes and panicle branches are affected by the pathogen during reproductive growth [5]. Leaf lesions reduce the photosynthetic area and neck and node blast lead to significant yield losses [6,7].

Many management practices are being utilized to minimize the effects of rice blast, like fungicides application, cultural practices, resistant cultivars and biotechnological methods [8]. Use of chemicals is now discouraged in the world because of the fact that fungicides are disturbing the ecosystem and causing interruption in the natural processes [9,10]. Environmental issues are directing attention of scientists towards alternative environment friendly methods to manage pathogens. One of these methods could be the use of resistant cultivars. Cultivation of resistant varieties not only reduces the inputs but it is also an environmental friendly method to combat diseases. Different rice varieties can be assessed to find sources of resistance.

Tuhina et al. [11] screened the 52 upland rice accessions including one susceptible check in order to find out new source of resistant genotypes. They evaluated these genotypes under field condition and obtained the cluster on basis of blast lesion type and Disease leaf area.

Few attempt have been made previously to classify the breeding entries in to different clusters based on similarity in their disease resistant reaction against blast and sheath blight [12].

Cluster analysis forms group on basis of similar response of genotypes but it is not necessary all entries in a group have same response or origin. However, it provides useful information that could be used in hybridization program and in selection of suitable parents for development of resistant genotypes. Clusters can identify sources of new resistant genotypes along with high yielding to overcome the changing environment, and study the mechanism of resistance. Therefore, the present study was carried out to screen rice germplasm for resistance against leaf blast.

2. MATERIALS AND METHODS

2.1 Experimental Site and Rice Varieties

The studies were carried out in the years 2013 and 2014 during August to September at Rice Research Institute, Kala Shah Kaku. Fifty two (52) rice varieties procured from the Plant Breeding section and store of the institute and they were screened for resistance against blast.

2.2 Screening of Rice Varieties

Seeds of different varieties were placed on gunny bags and water was sprinkled on them after every 24 hours. The sprouted seeds were then sown on dry prepared raised beds by following the uniform blast nursery method approved by International Rice Research Institute, Philippines. Each line was sown in a single row and the length of the row was about 50 cm. The susceptible variety for rice blast i.e. C-622 was sown as spreader after every two test lines. The distance between the rows was 10 cm. Entire nursery bed was also bordered by two rows of susceptible C-622.

2.3 Isolation and Purification of *Pyricularia oryzae*

Blast infected leaves from the previous year crop were used to isolate the pathogen. Infected parts were cut into small pieces and surface sterilized with 1% Sodium hypochlorite followed by rinsing with distilled water. Sterilized plant pieces were placed in Potato Dextrose Agar (PDA) medium Petri dishes aseptically. These PDA dishes were place in incubator at 25±2℃ for 5-7 days.

The resulting colony was then purified and identified according to its morphological and microscopic characteristics by following the technical bulletin on seed borne disease and seed health testing of rice [13]. Purified cultures of *P. oryzae* were used to prepare the inoculum for blast nursery.

2.4 Preparation and Inoculation of *P. oryzae* Inoculum

The seeds of susceptible variety C-622 were sown in several earthen pots. Aqueous spore suspension was made by using purified isolate of *P. oryzae*. Measured spores (1×10^6/mL) were sprayed on 2-week old seedlings of C-622. After the development of blast disease, the seedlings were cut into small pieces and used as inoculum for the nursery. Inoculation was done after 3 weeks of sowing with the diseased chopped leaves spread over the nursery lines. Water was sprinkled twice a day to maintain the humidity and to facilitate the blast infection. The test lines were evaluated for resistance against blast after three weeks of inoculation by following the Standard Evaluation System for Rice [14].

2.5 Statistical Analysis

Cluster analysis classifies the genotypes into different group on basis of difference. These groups are called clusters. Different techniques are used to group the data in clusters and represented in graphical dendrogram. In this study, we used the complete linkage hierarchical cluster analysis and distances measured by Euclidean distance. It is calculated as follows:

$$\text{Distance } (x, y) = \{\textstyle\sum \mid (x_i - y_i)\, 2\}^{1/2}$$

This analysis was done by using the computer based program STATISICA version 10.0 [15] to find the relationship among the genotypes in response to rice leaf blast.

3. RESULTS AND DISCUSSION

Improvement of crop varieties can only be successful with higher genetic variation and the heritability of desirable traits [16]. Therefore, for establishing relationship among different varieties, assessment of genetic variability has become very important [17,18]. Cluster analysis of the present study classified the rice genotypes in four clusters depending upon the variation present for character under study. Cluster analysis gave dendrogram which distributed the fifty two genotypes into four clusters shown in Table 1 and Fig. 1.

Dendrogram constructed by cluster analysis revealed that the most diversified clusters were 1 and 4. This show for the selected character they had great genetic variation between each other. Most close clusters were 2 and 3 showed the presence of less variation in the genetic material and indicated the presence of same parents in their ancestor. As we move from cluster 1 to 4, the similarity index between cluster increases indicates the presence of some common parents among the genotypes. Genotypes grouped in cluster 1 showed moderately resistant response against leaf blast with disease score of 2 and 3. Genotype KSK-470 included in cluster 1 was most diverse than genotype KSK-472. Cluster 1

Table 1. Distribution of rice genotypes to different clusters

Cluster no.	Disease score	Genotypes
1	2-3	KSK-470, KSK-463, KSK-460, PK 8685-5-1-1-1, KSK-462, KSK-474, PK 3810-30-1, KSK-471, KSK-472
2	4-5	PK 8745-11-1-4-1, Super Basmati, PK 8650-6-1-1-1, KSK-475, PK 8431-1-2-1-2-4, PK 9224, PK 8430-3-1-3-2-1, PK 8431-1-2-1-2-3, KSK-473, KSK-464, PK 8535-15-2, PK 8809-3-1-1-1, PK 8649, PK 8649-5-1-1-2, PK 8647-11-1-1
3	6-7	KSK-133, PK 8677-8-5-1, KSK-469, PK 8644-4-1-3-1-5, PK 8892-4-2-1-1, PK 8892-4-1-3-1, PK 8892-4-1-4-1, PK 8649-4-1-3-13, PK 8660-13-3-1, Basmati-515, KSK-468, PK 8749-2-4-5-1, PK 7274-12-3-2, PK 8431-6-1-1-1-1, PK 7688, PK 8662-20-1-1-1-1, PK 8677-18-1-7-18, PK 8662-2-15-4, PK 7837-11-1-1-1-3-1, PK 8677-18-1-7-14, PK 8677-18-1-7-22, PK 8536-15-1, PK 8662-15-2-1, PK 8662-12-2
4	8-9	KSK-459, PK 8677-18-1-7-16, PK 8677-18-1-7-17, C-622
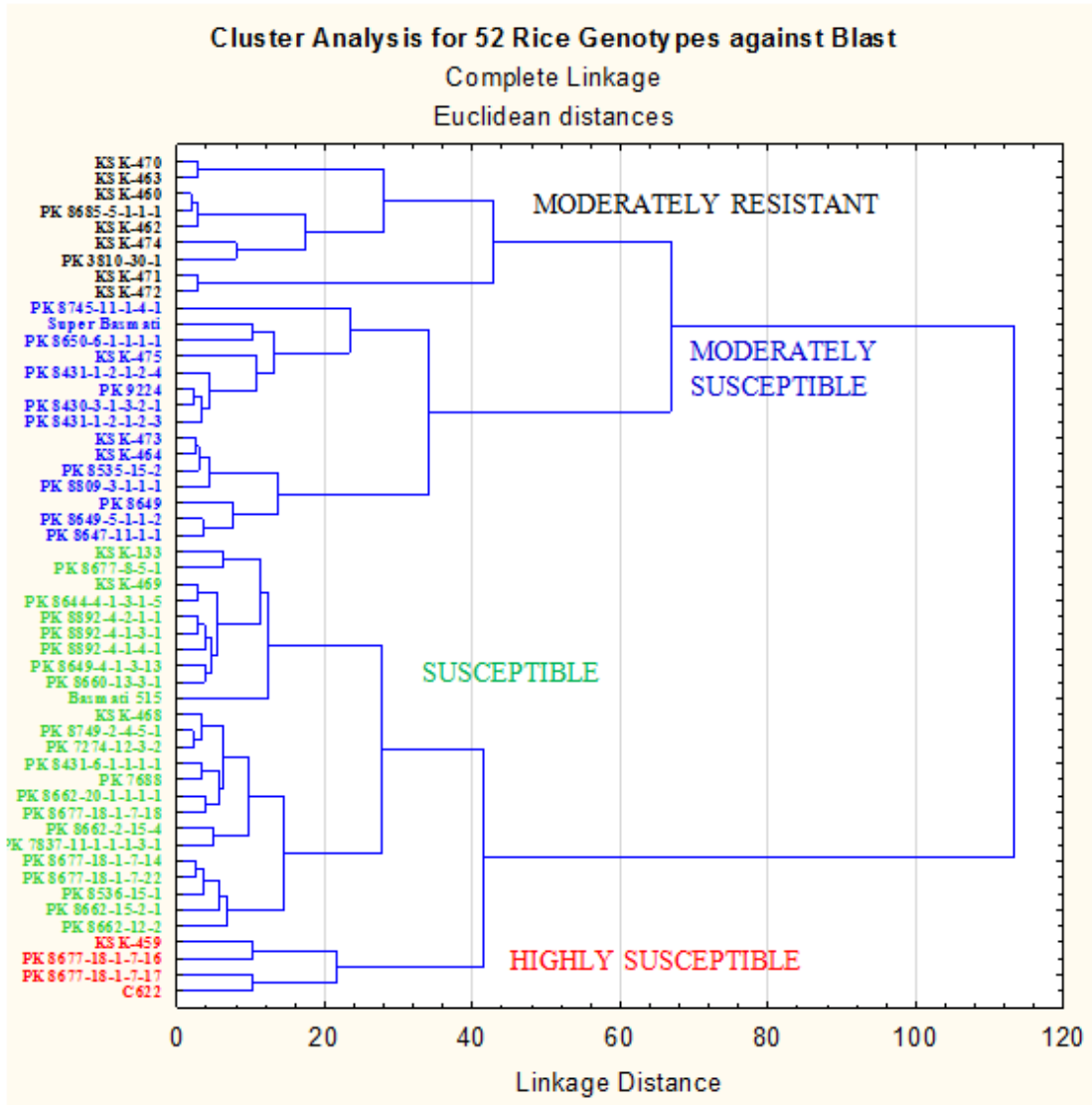
Fig. 1. Dendrogram of rice genotypes response to leaf blast by Ward's method showing linkage and Euclidean distance

included the genotypes showing moderately resistant trait against rice blast among which KSK-470 was superior to other genotypes within the cluster. KSK-471 and KSK-472 were more similar to each other may be because of the similarity in their origin. Similarly, KSK-474 and PK 3810-30-1 showed no genetic diversification within the cluster. Moderately susceptible genotypes of disease score of 4 and 5 were included in cluster 2 and within the cluster PK 8745-11-1-4-1 and PK8647-11-1-1 were most distinct genotypes. This cluster had the average performing genetic material. All the remaining genotypes shared similar genetic material

including Super Basmati and PK 8650-6-1-1-1, PK 9924 and PK 8430-3-1-3-2-1, KSK-473 and KSK-464, PK 8649-5-1-1-2 and PK8647-11-1-1.

Cluster 3, most diverse cluster than cluster 1 and 2, included the genotypes with susceptible response to rice leaf blast disease with 6 and 7 score. The most diverse genotypes within the cluster were KSK-133 and PK 8662-12-2. Some extent of distinctness could also be seen between other genotypes of this cluster, such as, KSK-133 and Basmati 515, KSK-468 and PK 7837-11-1-1-1-3-1, PK 8677-18-1-7-14 and PK 8662-12-2. Diversification between these

genotypes was because of the involvement of other parents in the crosses. Most similar genotypes were PK 8892-4-2-1-1 and PK 8892-4-2-3-1, PK 8649-4-1-3-13 and PK 8660-13-3-1, PK 8749-2-4-5-1 and PK 7274-12-3-2 so on. Highly susceptible genotypes which scored as 8 and 9 were grouped in cluster 4. This cluster included the local check C-622 which showed high susceptibility against rice blast. Within the cluster two distinct genotypes were KSK-459 and C-622. Most similar were KSK-459 and PK 8677-18-1-7-16, PK 8677-18-1-7-17 and C-622. Similar studies were carried out by Telebanco-Yanoria et al. [19], in which 922 rice varieties collected from Asia were evaluated against rice blast. These varieties were grouped in six clusters (A-F) based on the reaction pattern to 20 standard differential blast isolates from the Philippines. Most susceptible varieties in clusters B and C were from Japan, while the varieties form East Asia and Southeast Asia were grouped in the clusters E and F with most resistance. Varieties from South Asia showed the widest variation, occurring in all clusters but less frequently in cluster B.

Cluster analysis is used in many studies regarding resistance of rice genotypes against rice blast. Mukherjee et al. [20] obtained three clusters of rice genotypes according to their response in respect of the various components of resistance. Two clusters had fast-blasting genotypes and one had slow-blasting genotypes. Thirteen genotypes were grouped in slow-blasting cluster. It means these genotypes have characters of rate-reducing resistance and such resistance has the effects of slowing down the development of an epidemic. Jaihom and Parinthawong [21] evaluated different blast pathogen isolates in Thailand for their virulence and tested them on 25 rice varieties. The fugal isolates infecting the rice varieties at similar rate were grouped in same cluster. Some isolates caused severe infection and some showed lowest virulence on the tested varieties, indicating that extent of plant resistance may also depends on the virulence level of the pathogen isolate.

4. CONCLUSION

All the varieties which were evaluated under artificial ephiphytotic condition, only nine varieties exhibited moderate resistance against rice blast. Thus for the development of resistant varieties, genotypes from cluster 1 can be used as parental lines in breeding programmes. The

diversity analysis helps selection of suitable parents for hybridization and development of broad genetic based genotypes which give high yield under disease stress. To our knowledge, this study will be helpful in selection of diverse lines from different clusters as parents for crossing in a hybrid rice breeding programme in Pakistan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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