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SCREENING OF PAKISTANI RICE (*ORYZAE SATIVA L.*) CULTIVARS AGAINST *BURKHOLDERIA GLUMAE*

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Abstract

Burkholderia glumae is a bacterial infectious agent that is carried by seeds and can cause rice disease named Panicle Blight which as a result considerably decreases the yield and quality of rice all over the world. This particular research was carried out in order to isolate bacterial strain from infected rice seeds, characterize the morphology and to screen Pakistani rice varieties against bacterial panicle blight. The samples were isolated and purified by using King's B agar medium. The morphological characters were studied by using Gram staining, Scanning electron microscope and other biochemical tests. The results showed that the isolated bacteria, was Gram negative. On the basis of morphology, bacteria had monotrichous rod shape. Screening of 14 Pakistani rice genotypes revealed that Super Basmati and Super Gold are most susceptible against bacterial panicle blight. This study will indicate and benefit in the selection of high yielding and best resistant Pakistani rice variety/varieties and may also help the plant breeders to select better rice genotypes to have promising rice lines with high yield potential and disease resistance and to enhance the food quality and quantity.

Keywords: Rice, Screening, Panicle Blight, Bacteria, Disease, Rice Lines.

INTRODUCTION

Rice is vulnerable to many diseases amongst which Bacterial Blight is most common and harmful disease of rice throughout the world. In Pakistan it was first recorded in 1977 by Mew and Majid. It is most common in low land irrigated areas especially in rainy season. The occurrence percentage in Punjab is from 5 to 100 percent. It not only reduces the yield but also affect the grain quality (Ibrahim et al. 2016). In severely infected fields BPB can cause seventy-five percent reductions in yield as a result there will be decrease in grain weight, floret sterility, seed germination inhibition, stand reduction etc. BPB is characterized by brown or straw-colored panicle, spikelet abortion, grain rot (Mizobuchi et al. 2018). *Burkholderia glumae* has a wide range of hosts causing bacterial wilt in different plants e.g., *Lycopersicon esculentum*, *Sesamum indicum*, *Perilla frutescens*, *Solanum melongena*. It is a seed borne pathogen due to which infected seeds of rice act as a primary source of inoculum for the following year. This pathogen travels from lower leaves to upper leaves and finally settles in flag leaf which is important for the

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development of disease. This pathogen enters through the opening of stomata in lemma & palea of rice seed. Then it reproduces in spaces present between the cells and for their long-distance travel they use vascular system of the plant (Tsushima, 1996; Jeong et al. 2003). *Burkholderia gluame* has been linked to spikelet infertility and discoloration of bud grains. Pathogens on the leaf sheath play a significant role in initial infection (Tsushima et al. 1991 and Tsushima et al. 1996). It causes considerable economic loss by blocking seed germination along with infertility, sprout failure and grain weight loss (Jeong et al. 2003). Many field experiments have been conducted in the past to better understand the genetic regulation of BPB resistance via inoculation by using syringe at booting stage or by means of spray inoculation at heading stage. Because BPB is highly influenced by environmental factors like moisture and temperature, it is challenging to assess the resistance of cultivars varying in their heading days by using field inoculation method (Tsushima, 1996). The goal of this study was to screen different Pakistani rice varieties for panicle blight resistant on the basis of morphological characterization.

METHODOLOGY

Fourteen different Pakistani rice varieties were chosen to study the yield and its related traits along with effect of Bacterial Panicle Blight caused by *Burkholderia glumae*. These varieties include BAS 198, Punjab Basmati, BAS 370, BAS 385, BAS Pak, Super Gold, Basmati 515, PK 1121 aromatic, PK 386, Kisan Basmati, Chenab Basmati, Super Basmati, Basmati 2019, and Basmati 2000.

Isolation and purification of bacterium:

Bacterial Panicle Blight infected seeds were collected from infected plants, sterilized for one minute with 2% NaClO solution, then they were rinsed with distilled water three times. Seeds were shifted to King's B agar medium under aseptical conditions and were incubated for 48 hours at 29℃ (King *et al*., 1954). The resulting suspension was streaked with an inoculating needle in to and fro motion. Petri dishes were then placed in incubator in inverted position at 29℃ (Murray *et al*., 2003). After two to three days the bacterium started to form colonies.

Bacterial strain identification

Bacterial species were determined using a variety of phenotypic traits including motility, colony color, morphology, and cell shape etc. The pure colonies were examined using gram staining technique, Scanning Electron Microscopy (SEM) and different biochemical tests. Bacteria were recognized by examining the results of all biochemical assays that were performed in this study using Microgen Identification System software.

Staining Technique and Microscopy

Gram Staining

A drop of deionized water along with 24-hour old bacterial culture was used to make a thin smear on a glass slide with the help of a needle. After that it was passed few times over the flame in order to dry it. Then the smear was covered with few drops of crystal

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violet stain (primary dye) for 1 minute. The excess stain was removed by slightly tilting and washing with tap water. Then the smear was inundated with gram's iodine solution for a minute or so, excess stain was removed, washed and dried with blotting paper and then it was dipped in alcohol for few minutes to decolorize it until only faint violet color was remained. After that the slide was further washed with water blot dried and was stained with safranin (secondary dye) stain for 20 to 30 seconds, washed with water, dried and was examined under the microscope for further study.

Scanning Electron Microscopy (SEM)

For fifteen minutes 2ml of bacterial culture was centrifuged in refrigerated centrifuge. The pellet obtained as a result of centrifuge was rinsed with saline and fixated in 1 ml of 3% glutaraldehyde at 4℃ for 2 hours. The pellet was further washed 3 times for 15 minutes each with saline at 4℃ followed by series of acetone treatment with 70-100% ethanol for 15 minute each and was kept in desiccator for whole night. The obtained particles were dispersed on SEM stubs, dried till critical point; platinum covered and was examined under SEM.

Inoculation of bacterium:

Bacterial suspension was made by harvesting the bacterial colonies and mixing 1ml of it with 9ml of sterile water in a flask. The final concentration of suspension was made up to 10^8 cfu per ml. Two methods were used to infect healthy growing rice plants in the field. First method was the rice panicles were sprayed with fresh bacterial inoculum up to 2 ml per panicle. Second method was that at boot leaf stage the rice plants were inoculated with fresh bacterial inoculum at the leaf sheath with clean and sterile syringe at an angle of 45^o. After infecting the plants were covered with polythene bag and were left for few days under natural conditions. For control plants only sterile distilled water was sprayed on them. After few days the polythene bags were removed and plants were observed for disease symptoms and disease percentage was measured (Mizobuchi *et al*., 2018; Gowda *et al*., 2022).

Bacterial Panicle Blight Assessment

The infected genotypes were evaluated for BPB resistance by measuring percent germination, percentage disease incidence, panicle blight severity (PBS), panicle blight susceptibility and resistance and yield loss. Some phenotypic characters were also measured including spikelet fertility, no. of filled grains per panicle, no. of panicles, total thousand grain weight & no. of spikelet per panicle.

RESULTS

Bacterial Identification

The outcomes have been described in table 1 and 2. Five different kinds of bacteria were identified from the selected germplasm which showed various morphological characters that were used to identify them.

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Table 1: Bacterial Species Isolated from Rice Grains on Basis of Morphology

Strain No.	Morphological characters								
	Colony color	Colony texture	Odour	Cell Shape	Gram Stain	Capsule	Motility test	Growth on 5% NaCl	Growth on 5% Glucose
1.	Light Yellow	Smooth	-	Rod	-	+	+	-	+
2.	Yellow	Slimy	-	Rod	-	+	-	-	-
3.	Bright yellow	Smooth	+	Rod	-	-	-	+	-
4.	Bright yellow	Smooth Shiny	-	Rod	-	+	+	+	-
5.	Greenish yellow	Smooth	-	Rod	-	-	+	+	-

Table 2: Bacterial species Isolated from Rice Grains on the Basis of Biochemical Tests

Strain No	Biochemical Tests															Identified Bacterial Species
	Sorbitol Test	Xylose Test	Nitrate Reductase	KOH Solubility Test	Rhamnose Test	Hydrogen Sulfide Production Test	Catalase Test	Indole Test	Urease Test	Mannitol Test	Glucose Test	Lysine Test	Kovacs Hydrolysis	Gelatin Hydrolysis	Arginine Test	
1.	-	-	-	+	-	+	+	-	-	-	+	-	-	-	-	*Xanthomonas oryzae*
2.	-	+	-	-	+	+	-	+	-	-	-	-	-	-	+	*Xanthobacter sp.*
3.	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	*Burkholderia sp.*
4.	-	-	+	+	-	-	-	-	-	-	+	-	-	+	-	*B. glumae*
5.	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	*B. gladioli*

Burkholderia glumae isolation

The bacterium showed best growth on King's B agar medium at 29℃ for 48h at pH 7. *Burkholderia glumae* exhibited yellow colony on King's B agar medium due to yellow-green, water soluble pigment. Yellow coloration in the medium was caused by pigments that were water-soluble produced by *Burkholderia glumae*. The colonies were observed to be circular with smooth margins and convex in shape.

Fig 1: *B. glumae* colony on King's B agar media

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B. glumae Staining and Microscopy

Gram staining results showed that *B. glumae* was gram negative bacteria with pink color as monotrichous rod shaped. The scanning electron microscopy of *B. glumae* showed non spore forming, rod shaped organism having 0.6 to 0.9 x 1.3 to 2.5 µm cell size with rounded ends.

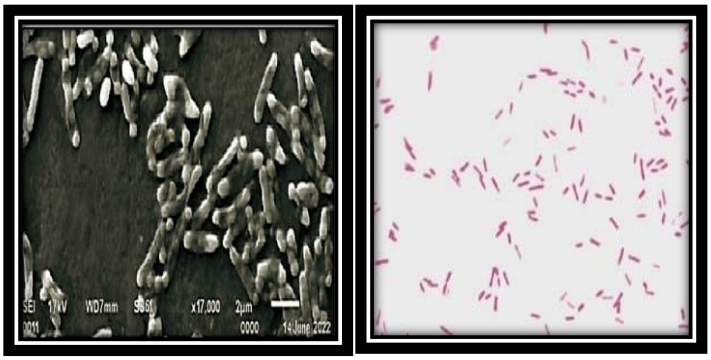

Fig 2: SEM & Gram staining technique showing gram negative bacteria

Symptomatology

The symptoms that were observed on rice cultivars were as follow:

On leaves, lesions were observed that were mostly oval in shape with a reddish-brown border. On leaf sheath, the disease symptoms were as linear lesions with a distinct reddish-brown border with necrotic center. Floret discoloration was also observed that occur usually on the lower half of the growing grain, with a dark brown border.

Fig 3: Symptoms of *B. glumae* on rice genotypes

Correlation Coefficient Analysis

Correlation coefficient analysis between all BPB traits that were studied in this work is presented in figure 4. In this study, the results showed that percentage disease incidence was most positively correlated with panicle blight susceptibility and resistance having the r vale 0.97***. Number of panicles showed strong positive correlation with number of filled grains per panicle & spikelet fertility (r= 0.89***, r= 0.95***). Whereas number of filled grains

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per panicle exhibit strong positive relation with total number of spikelets per panicle (r=0.94***). The weakest significant correlation was observed in total number of spikelets per panicle with panicle blight severity and yield loss (r= -0.59*, r= -0.65*). However thousand grain weight showed overall non-significant relation with all the other traits of following study.

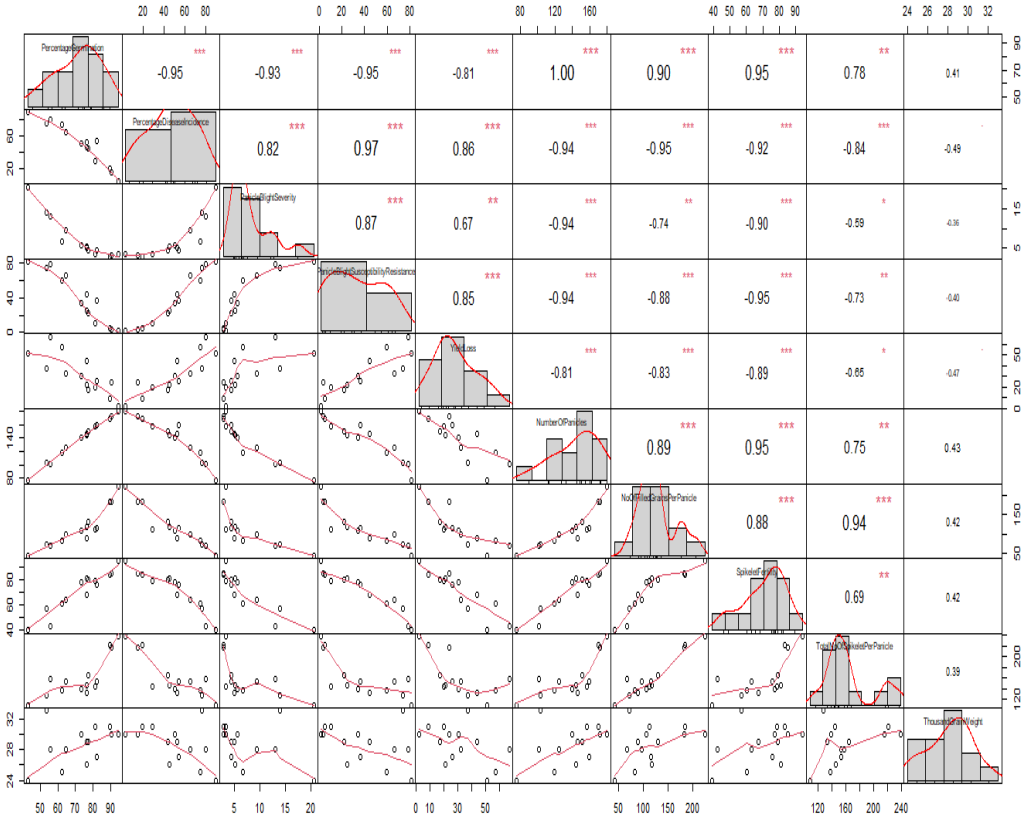

Fig 4: Simple Correlation among different bacterial panicle blight traits of rice genotypes

Principal Component

For all morphological indices of different rice genotypes principal component analysis (PCA) was executed as shown in table 5. Out of ten, only one principal component (PC) showed more than 1 eigen value and exhibited about 81.2% variability between under study traits for each rice genotype. So, for further elucidation this one PC was granted due importance. Eigen value & variance related with each principal, reduced gradually & stopped at 0.0005 & 0.01 respectively (Fig 5).

The following principal component was more associated to no. of filled grains per panicle, no. of panicles, percentage germination, spikelet fertility, thousand grain weight & total no. of spikelets per panicle.

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Table 3: Eigen value, % variance and cumulative eigen values of 10 bacterial panicle blight traits

Traits	PC	Eigen values	% Variance	Cumulative	Cumulative%
Percentage Germination	PC1	8.120	81.2	8.120	0.812
Percentage Disease Incidence	PC2	0.806	8.1	8.926	0.893
Panicle Blight Severity	PC3	0.564	5.64	9.490	0.949
Panicle Blight Susceptibility and Resistance	PC4	0.341	3.41	9.831	0.983
Yield Loss	PC5	0.095	0.95	9.926	0.993
Number of Panicles	PC6	0.039	0.39	9.965	0.997
No of Filled Grains per Panicle	PC7	0.024	0.24	9.989	0.999
Spikelet Fertility	PC8	0.009	0.08	9.998	0.9998
Total no of Spikelet per Panicle	PC9	0.002	0.02	9.999	0.9999
Thousand Grain Weight	PC10	0.0005	0.01	10.00	1.000

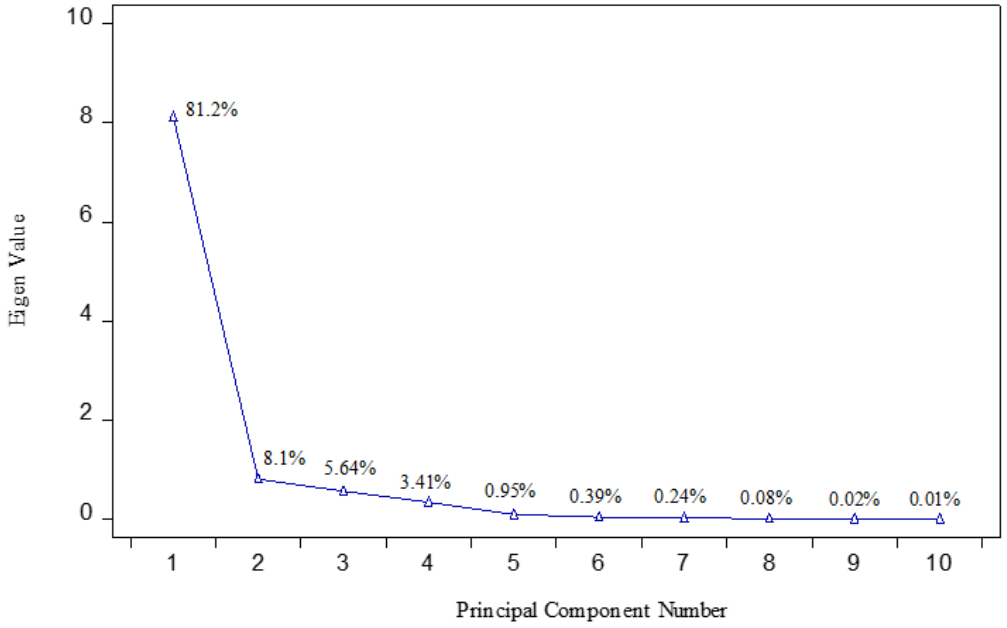

Fig 5: Scree plot of principal component analysis of bacterial panicle blight infected rice genotypes between their eigen values and the number of principal components

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Table 4: PCs for 10 bacterial panicle blight traits of fourteen different rice genotypes

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
No of Filled Grains per Panicle	0.333	-0.006	0.403	-0.019	0.131	-0.271	0.269	0.130	-0.708	-0.212
Number of Panicles	0.343	-0.113	-0.154	0.171	0.127	0.490	0.162	0.170	-0.205	0.681
Panicle Blight Severity	-0.310	0.208	0.433	-0.425	-0.289	0.242	0.423	0.362	0.153	0.130
Panicle Blight Susceptibility and Resistance	-0.339	0.108	0.109	0.115	0.658	0.192	0.422	-0.442	0.042	-0.054
Percentage Disease Incidence	-0.344	-0.023	-0.103	0.033	0.515	-0.034	-0.295	0.712	-0.074	-0.064
Percentage Germination	0.343	-0.133	-0.096	0.182	-0.016	0.487	0.225	0.233	0.238	-0.650
Spikelet Fertility	0.339	-0.093	-0.215	-0.192	0.240	-0.540	0.435	0.179	0.449	0.144
Thousand Grain Weight	0.174	0.945	-0.207	0.164	-0.003	-0.026	0.001	0.075	-0.018	-0.023
Total No of Spikelet per Panicle	0.289	0.065	0.710	0.275	0.186	-0.017	-0.335	0.013	0.409	0.133
Yield Loss	-0.309	-0.090	0.030	0.777	-0.304	-0.246	0.325	0.168	0.022	0.073

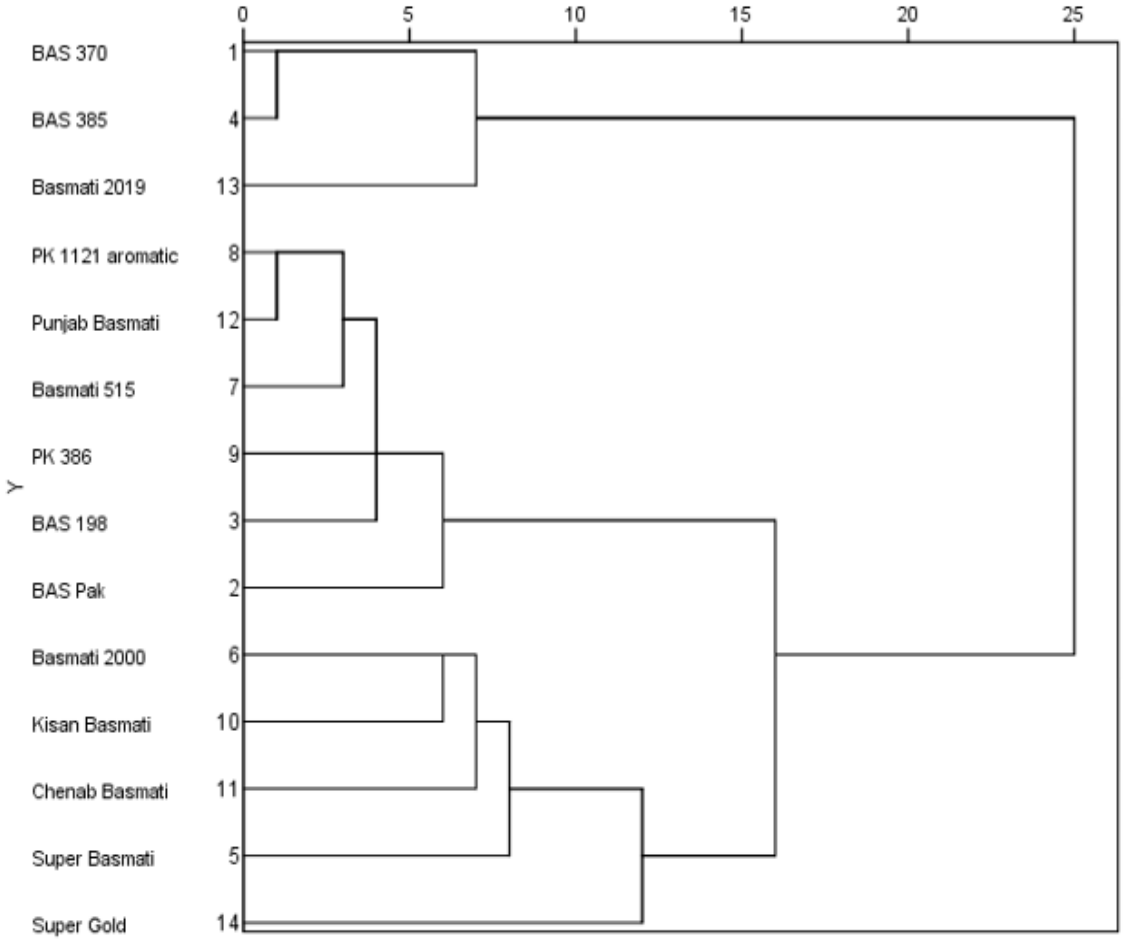

Fig 6: Dendrogram of rice genotypes based on their Bacterial Panicle Blight traits

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Disease Percentage

Out of fourteen rice varieties Super Basmati and Super Gold were found to be most susceptible to bacterial panicle blight as summarized in Table 5.

Table 5: BPB host response

Rice Genotype	Disease Incidence (%)	Score	Host response
BAS 370	3	1	Resistant
BAS Pak	44	5	Moderately Susceptible
BAS 198	10	1	Resistant
BAS 385	4	1	Resistant
Super Basmati	78	9	Highly Susceptible
Basmati 2000	59	7	Susceptible
Basmati 515	36	5	Moderately Susceptible
PK 1121 aromatic	34	5	Moderately Susceptible
PK 386	21	3	Moderately Resistant
Kisan Basmati	66	7	Susceptible
Chenab Basmati	73	7	Susceptible
Punjab Basmati	25	3	Moderately Resistant
Basmati 2019	1	1	Resistant
Super Gold	81	9	Highly Susceptible

DISCUSSION

The current study showed that bacterial panicle blight is very threatening disease. Different isolated bacterial species were characterized on the basis of different phenotypic, biochemical & microscopic characteristics. These traits provide the information of variation among the bacterial strains that further differentiate the bacterial species for their importance depending upon the purification and identification process.

Various isolated identified species i.e. *Xanthomonas oryzae., Xanthobacter* sp., *Burkholderia* sp., *B. glumae and B. gladioli.* The cell shape of all the species were bacilli (rod) and staining were negative respectively (Jungkhun et al. 2022). The colony structure and other characteristics were equally distributed among the bacterial species. A previous study has showed a prevalence of Gram negative bacteria in rice grain tissues (Darmawan et al. 2023). Various pathogen associated with rice crop that causing various diseases of rice grains at different stages i.e. bacterial panicle blight, brown or black spot, grain discoloration etc. Such type of diseases is causing rice yield reduction up to 14-18 % (Ngalimat et al. 2021).

It was observed that the association between genotypes and traits provides the information for screening and selection of resistant rice lines. A positive association is very helpful in selection and identification process and further important to start up a new breeding research. Correlation study provides information on the relationship among variables (dependent & independent). It enables plant breeders to better comprehend the relation among yield related traits, leading to selection of genotypes with desirable characteristics (Nihad et al. 2020; Ara et al. 2022).

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Correlation analysis is one of the best approaches to determine the relationship between different traits and to lead the way of frequency of traits & the screening necessary to be evaluated in enhancing traits.

The Principal Component Analysis was carried out for all the panicle blight traits of rice varieties as shown in the Table 3, Figure 5. Among ten, the PC1 showed more than 1 eigen value & exhibited about 81.2% variability among the variables evaluated for each genotypes. Other principal components exhibited less than one eigen values.

The first PC was more related to percentage germination, percentage disease incidence, panicle blight severity, panicle blight susceptibility and resistance, yield loss, number of panicles, no. of filled grains per panicle, spikelet fertility, total no. of spikelet per panicle & thousand grain weight. In the scree plot the principal component start from the 81.2% and ends at 0.01%. The principal components with eigen value more than one considered to be having more variation than others that displayed more distinction among the rice genotypes for the selection of the diverse parents.

It was also cleared that all the principal components showed maximum variation for all the entire traits. Rice genotypes were also categorized into various clusters on the basis of different panicle blight traits as shown in Fig. 6. The grouping or clustering of genotypes was done on the basis of various characteristics. The genotypes with similar traits/characters were classified in the same group.

The distance among the genotypes present in each cluster determine the genetic variation & each cluster is genetically different from the other. Genetic diversity in the genotypes that based on genetic distance among clusters. The occurrence of 3 groups in under study germplasm indicated that there may be some genetic diversity present which is needed to be identified and yet examined. Rice genotypes were also screened on basis of disease symptoms scoring that determine the genotypes susceptibility and resistivity.

CONCLUSION

The diversity & characterization of various bacterial species isolated from different rice germplasm lines were categorized. This study could be very useful for further breeding of new plant population on the basis of classification of resistant genotypes (Basmati-370, Basmati-198, Basmati-385, and Basmati-2019), tolerant genotypes and susceptible genotypes with respect to their various kinds of pathogens and isolated various bacterial species. This study might also be beneficial to the scientific & agricultural communities for the further investigation in to new insight in scientific field.

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