Screening the effect of *Burkholderiaglumae*on seed germination and seedling growth of rice (*Oryza sativa* L.)

Abstract

The increasing prevalence of diseases like *Burkholderiaglumae*-produced bacterial panicle blight has an influence on rice production worldwide. In rice plants, the symptoms caused by the pathogen include browning of the leaf sheath, grain rot, and seedling rot. It is observed that the pathogen enters the plant through contaminated seeds and environmental sources of the microbe. The present study analysed the effect of *B.glumae* on seed germination and root- shoot growth under control condition, and also the screening of different varieties against BPB under glasshouse condition.In all the studied varieties growth responses were varied whereas healthy seeds showed maximum responses followed by artificially infected and naturally infected seeds respectively for root and shoot growth.

Keywords: shoot growth, Burkholderiaglumae, seed germination, pathogen

Introduction:

Bacterial Panicle Blight (BPB) is a dangerous bacterial disease that affects rice seedlings. In the 1950s, Japan released its first reports on it. The main cause of this condition is the polar, flagellated, rod-shaped, Gram-negative bacterium *Burkholderiaglumae***(K 1956)**.A staple meal enjoyed by most people worldwide, rice (*Oryza sativa* L.) belongs to the Gramineae family. A staple food for half of the world's population, rice is one of the three most significant crops in the world. Since over 90% of the world's rice is produced and consumed in Asia, its production is crucial to the region's food security **(Kumar et al. 2018).** Over half of the world's population depends on it as their main source of nutrition **(Leser 2013),**representing almost 30% of the world's and South Asia's total nutritional consumption**(Lobell et al. 2008).** Furthermore, a significant portion(38%) of South Asia's impoverished population reside in regions where rice is the main crop**(Young et al. 2012).**India, a major user and producer of rice in South Asia, nevertheless has severe problems with hunger, malnutrition, poverty, and food insecurity even though the green revolution has significantly increased the output of food crops, including rice**(Kumar et al. 2013).** Ninety percent of the total production comes from Asia alone. Since it accounts for 60% of household caloric intake and 90% of global rice production and consumption occurs in Asia, it is the most significant food crop in the region **(FAOSTAT, 2012).**

Diseases brought on by bacteria, fungus, and nematodes are among the many obstacles to rice production that generate significant financial losses for India [25]. Major diseases of rice are Bakanae, Brown Spot, Sheath Blight, False Smut, Sheath Rot, Stem Rot, Bacterial Leaf Blight, Bacterial Leaf Streak, Blast and Khaira Disease **(Mew and Rosales 1992).** Due to factors like decreased grain weight, floret sterility, inhibition of seed germination, decreased stands, and year-to-year transmission due to the pathogen's seed-borne nature, bacterial panicle blight can potentially reduce rice yield by up to 75% in areas that are severely affected **(Trung 1993).***Burkholderiaglumae* is linked to rice grain discolouration, seedling sheath rot, and bacterial panicle blight (BPB).This disease is exacerbated by high nighttime temperatures and humidity **(Cottyn 1996)**. This bacteria is spread *via* contaminated seeds**(Sayler, Cartwright, and Yang 2006).**First identified as the cause of seedling blight and grain rotting in Japan **(Goto and Ohata, 1956 and (Uematsu et al. 1976)**Burkholderiaglumae is regarded as one of the country's most significant rice diseases inJapan **(Azegami et al. 1987).**Astounding high nighttime temperatures in 1995 and 1998 coincided with panicle blight epidemics, which resulted in yield losses in certain areas of up to 40%. Louisiana also suffered significant losses in the year 2000 **(Nandakumar et al. 2008; Shahjahan et al. 2000).**

This study aimed to observe the effect of *B. glumae* on seed germination as well as root and shoot formation under controlled conditions after considering the literature review.

Methodology:

Material: The bacterium (*Burkholderiaglumae)* in Paddy (*Oryza sativa* L.) seed. For seed germination investigations, three groups of seeds, healthy seeds (C1), artificially inoculated seeds (C2), and naturally infected seeds (C3) were used. The five varieties—PR19, PR16, PR113, NDR 359, and MTU 7029 were tested to determine how the bacteria affected the length of the roots and shoots.

Isolation of Bacteria: Paddy seeds were harvested from plants displaying the characteristic symptoms of bacterial panicle blight. After being sterilised for two minutes with a 1% sodium hypochlorite solution, the tainted seed samples were washed three times in sterilised distilled water. Twenty-five seeds per Petri plate were aseptically placed in King's B agar medium, and the plates were incubated for 48 hours at 28±1˚C**(ISTA, 2000).**

Purification of bacterium: Each sterile Petri plate (9 cm in diameter) is filled with 25 millilitres of strelized King's B agar medium after it has been refrigerated to around 45°C. These plates were inverted as they solidified. By pushing the infected needle back and forth, a streak was made across the medium's surface on plates using a loopful of the bacteria. Two more plates were streaked without adding further bacterial suspension to the wire loop. After labelling, petri plates were inverted and incubated at 28±1°C. Bacteria develop colonies in two days. On King's B agar medium, single colonies were often replicated. In order to maintain the culture, it was moved into culture tubes on King's B agar medium and kept cold until additional research could be done.

Experimental site: Pantnagar is located in the Tarai belt, 343.84 meters above mean sea level, and is next to the foothills of the Shivalik range in the Himalayas.It is located at 29^0N latitude and 79.3^0E longitude.Pantnagar has a humid subtropical climate. The current investigation's studies were all carried out at the departments of veterinary anatomy, microbiology, and plant pathology. The field test was carried out during the kharif season at the G. B. Pant University of Agriculture and Technology's Crop Research Centre in Pantnagar.

Rolled Paper Towel Method: The creator of this technique was **(Warham 1990)**. In a lab setting, the seed germination was assessed using towel paper techniques. Overnight, three towel sheets were soaked in flowing tap water. A second water-soaked towel paper and butter paper were laid on top of the rows of seeds, which were spaced equally apart and contained 100 seeds each. For a week, these rolled towel sheets were kept in an incubator set at 28±1˚C in an angled orientation. Normal seedlings, aberrant seedlings, seed rot, and ungerminated seeds were counted after a week. In accordance with the International Rules for Seed Testing, ten seeds were chosen at random for each characteristic as well as for average root and shoot length**(ISTA, 2002).**

RESULTS:

Effect of bacterial infection on seed germination

The seeds of three categories apparently healthy seeds (C1), artificially inoculated seeds (C2) and naturally infected seeds (C3) were subjected for seed germination parameters. It was found that the maximum percentage of normal seedling 85.00 % was in (C1). In C2, the percentage of normal seedling was 76.66% while the percentage of normal seedling was 64.00 % in C3 indicating the adverse effect of bacterium on seed germination. The percent abnormal seedlings, ungerminated seed and seed rot were 6%, 4%, 5% respectively which were minimum in C1 as compare to rest two categories of seeds C2 and C3 (fig.1).

The maximum percentage of abnormal, unterminated seed and seed rot of 20%, 7% and 9% respectively were in naturally infected seed (C3). In C1 and C2, the seed rot was at par but was significantly different from the seed rot in naturally infected seed (9.0%) (Table 1).

Table 1: Effect of *Burkholderaglumae* on seed germination in different categories of seed

Categories of seeds	**Percent seed germination**			
	Normal seedling	**Abnormal seedling**	**Ungerminated seed**	**Seed rot**
C1	85.00	6.00	4.00	5.00
C2	76.66	12.00	6.00	6.00
C3	64.00	20.00	7.00	9.00

C1= Apparently healthy seeds

C2= Artificially inoculated seeds

C3= Naturally infected seeds

Effect of bacterium on root and shoot growth in different varieties

In all the varieties, subjected for examination, the maximum root and shoot length was in seedlings raised from C1 of all the varieties. In C2 andC3, the root and shoot length decreased in all the varieties used. The maximum root and shoot length of 10.70cm and 6.60cm, respectively was in seedlings raised from PR 19 while the minimum of root and shoot length of 5.33cm and 3.63cm respectively was in seedlings raised from C3 of variety PR 16. In variety PR 19, C1 exhibited maximum root length of 10.70cm. However, the root length decreased in seedlings raised from C2 (8.86cm) and it was minimum in seedlings of C3 (7.33cm) of same variety. Similarly, the shoot length was again maximum in seedlings raised from C1 (6.60cm) which went on decreasing in C2 (5.86cm) and was minimum in C3 (4.80cm) in the same variety.

C1 exhibited maximum root length of 6.80cm in variety PR 16. However, the root length decreased in seedlings raised from C2 (6.20cm) and it was the minimum in seedlings of C3 (5.33cm) of same variety. Similarly, the shoot length was again maximum in seedlings raised from C1 (5.20cm) which went on decreasing in C2 (4.63cm) and was minimum in C3 (3.63cm) in the same variety. In variety PR 113, C1 exhibited maximum root length of 7.53 cm. However, the root length decreased in seedlings raised from C2 (6.83cm) and it was minimum in seedlings of C3 category (5.73cm) of same variety. In the same way the shoot length was again maximum in seedlings raised from C1 (5.70cm) which went on decreasing in C2 (4.96cm) and was minimum in C3 (4.00cm) in the same variety.

In variety NDR 359 C1 showed maximum root length of 9.50cm. However, the root length deduced in seedlings raised from C2 (8.36cm) and it was minimum in seedlings of C3 (6.66cm) of same variety. In the same way the shoot length was again maximum in seedlings raised from C1 (6.10cm) which went on decreasing in C2 (5.76cm) and was minimum in C3 (4.36cm) in the same variety. In variety MTU 7029 C1 showed maximum root length of 8.20cm. However, the root length deduced in seedlings raised from C2 (7.63cm) and it was minimum in seedlings of C3 (6.16cm) of same variety. Similarly, the shoot length was again maximum in seedlings raised from C1 (5.93cm) which went on decreasing in C2 (5.30cm) and was minimum in C3 (4.37cm) in the same variety (fig.2).

Table 2: Effect of *Burkholderiaglumae* on root length and shoot length of seedlings in different categories of seeds in different varieties.

Variety	C1		C2		C3	
	Root length(cm)	Shoot length(cm)	Root length(cm)	Shoot length(cm)	Root length(cm)	Shoot length(cm)
PR 19	10.70	6.60	8.86	5.86	7.33	4.80

PR 16	6.80	5.20	6.20	4.63	5.33	3.63
PR 113	7.53	5.70	6.83	4.96	5.73	4.00
NDR 359	9.50	6.10	8.36	5.76	6.66	4.36
MTU 7029	8.20	5.93	7.63	5.30	6.16	4.37
CD at 5%	0.40	0.26	0.37	0.21	0.41	0.27

Discussion:

A serious bacterial disease of rice seeds is called Bacterial Panicle Blight (BPB)**(Zhou-qi et al. 2016)**. In nations that cultivate rice, such as those in Asia, South and Central America, and Africa, BPB has been often seen suffering with this disease**(Mondal, Mani, and Verma 2015; Safni and Lubis 2019)**. B. glumae-infected rice plants exhibit a number of disease signs, including as panicle blighting, leaf-sheath browning, and seedling rot**(Zhou-qi et al. 2016).** In comparison to the control, the BSB1 bacterial solution impacted the growth of seedlings in F-67, causing a 26.49% reduction in shoot length. Likewise, in F-2000, the BCB11 strain resulted in a 15.21% reduction in root length**(Peñaloza Atuesta et al. 2020)**. BPB disease reduces the seed germination rate and root and shoot growth, whereas *Streptomyces*-treatments improved the GR, shoot and root growth of rice**(Ngalimat et al. 2021)**.**Pedraza et al., 2018** found that *B. glumae* can colonise seedlings of rice plants that were formed from infected seeds or from the substrate. Over time, this bacterial population can be established and maintained, and the rice plants can be used as a habitat for the bacteria until symptoms of bacterial panicle blight appear. The reduction of shoot and root growth was also observed in a study by **Singh, 2015.**It appears from indentations that B. glumae is found in the sheath, seed, and stem rather than the leaf and root **(Mulaw et al., 2018).** In a study by**Noor et al., 2006** demonstrated that, Since the age of the host plant affects the development of bacterial blight, all five kinds were injected at three distinct growth stages: the seedling stage, the maximum tillering stage, and the leaf flag stage, or after 30, 60, and 90 days of germination, respectively.

Conclusion:

The seeds of three categories apparently healthy seeds (C1), artificially inoculated seeds (C2) and naturally infected seeds (C3) were subjected for seed germination studies. It was found that the maximum percentage of normal seedling 85.00 was in (C1). In C2, the percentage of normal seedling was 76.66 while the percentage of normal seedling was 64.00 in C3 indicating the adverse effect of bacterium on seed germination. The maximum percentage of abnormal seedling, ungerminated seed and seed rot was 20, 7, and 9, respectively in naturally infected seed (C3). In C1 and C2, the seed rot was at par but was significantly different from the per cent seed rot in naturally infected seed (9.0).

Five varieties viz., PR19, PR16, PR113, NDR 359 and MTU 7029, were subjected to evaluate the effect of bacterium on root and shoot length. The maximum root and shoot length was in seedlings raised from apparently healthy seeds (C1) in all the varieties. In artificially inoculated seed (C2) and in naturally infected seed (C3), the root and shoot length reduced in all the varieties. The maximum root and shoot length of 10.70 cm and 6.60 cm, respectively was in seedlings raised from apparently healthy seeds of variety PR 19 while the root and shoot length was minimum 5.33 cm and 3.63 cm respectively was in seedlings raised from C3 of variety PR 16. Similar observation were found in rest of the varieties.

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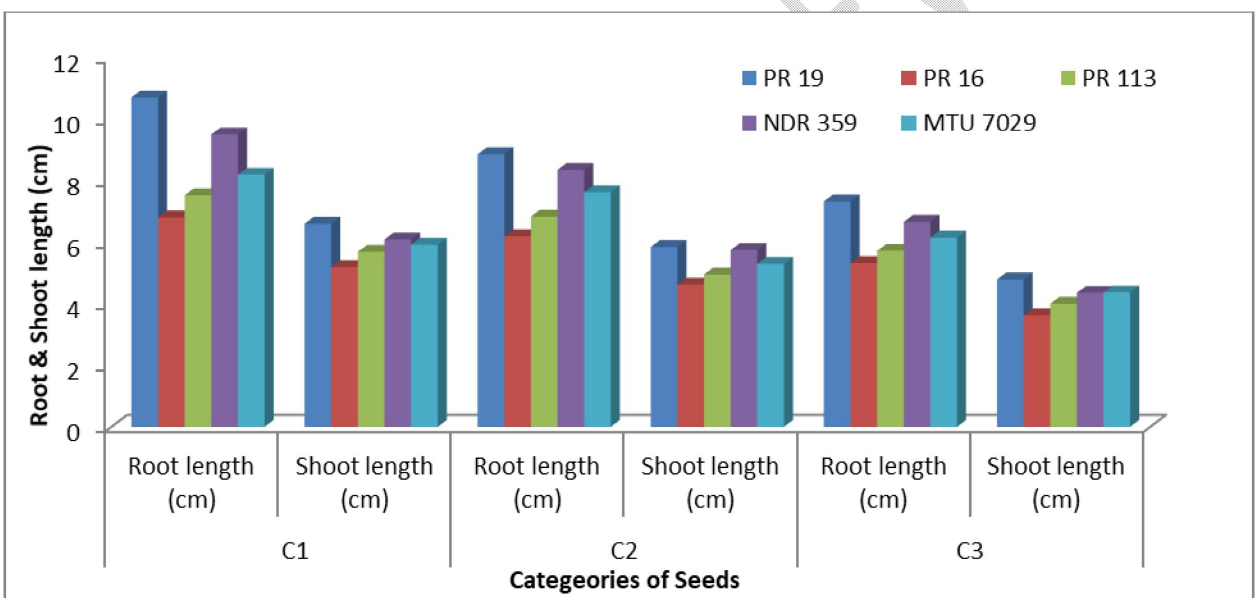

Fig. 1: Effect of *Burkholderiaglumae* on root length and shoot length of seedlings under control conditions

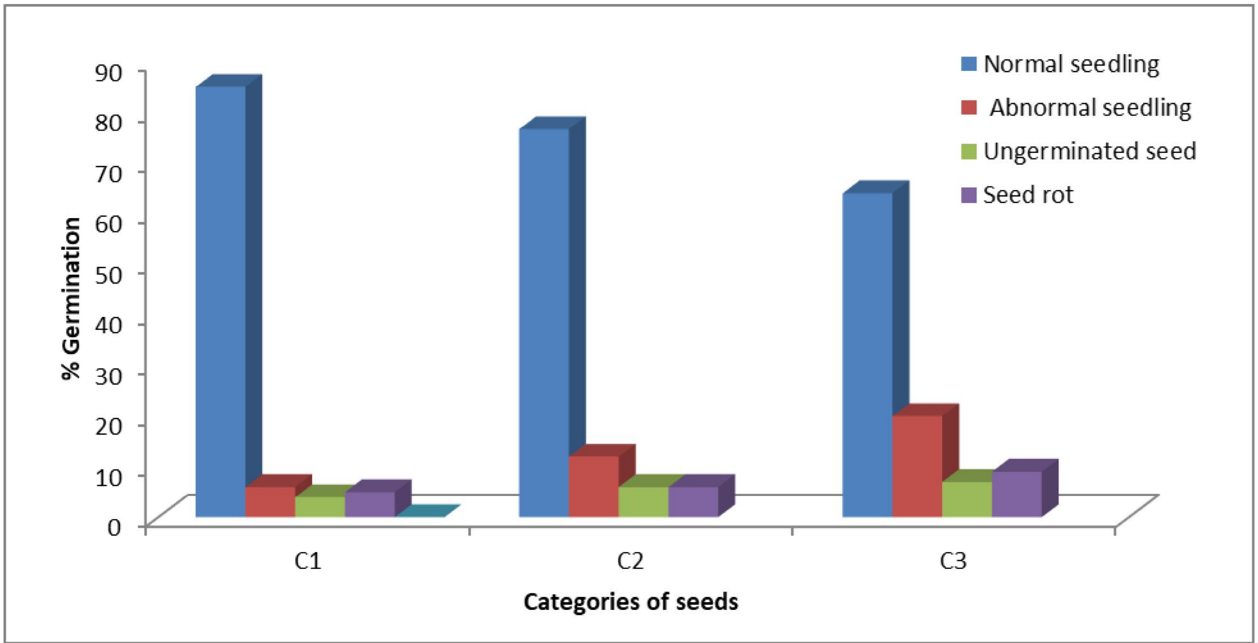

Fig. 2: Effect of seed infection on normal seedling, abnormal seedling, seed rot and ungerminated seeds in different categories of seeds