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Screening of aromatic rice genotypes for resistance against bacterial blight and neck blast diseases

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Abstract
A total of 30 aromatic rice genotypes were screened against bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and neck blast (*Pyricularia oryzae*) diseases under artificial inoculation conditions, with screening against neck blast under natural hotspot location. Among these test genotypes, two genotypes *viz*., Bony Cay and Kalikhasa were found to be resistant and only one genotype namely IR 841-85-1-1-2 was moderately resistant against bacterial blight and thus can be used as donors in basmati breeding programme. However, most of the genotypes showed highly susceptible reaction to neck blast disease except Pusa Basmati 1637 and Tetep which was observed as moderately resistant and two genotypes *viz*., Kalikhasa and UPR-2828-7-1 were found as susceptible.

Keywords: Aromatic rice, bacterial blight, neck blast, screening, resistance

Introduction
Rice (*Oryza sativa* L.), a cereal grain is the most important staple food for a large part of world's population. Among rices, aromatic rice is well known for its aroma, premium quality and high palatability. But the yield of aromatic rice is poor due to its increasing susceptibility to foliar diseases and more than 10 per cent of rice yield is lost because of diseases in tropical Asia (Savary *et al* 2000; Willocquet *et al* 2004) [15]. Among these, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* and neck blast caused by *Pyricularia oryzae* are the most destructive diseases of rice. Both of these diseases are known to occur worldwide and are widespread in Asia causing significant yield losses (Swings *et al* 1990).
The typical symptoms of bacterial blight ((*Xanthomonas oryzae* pv. *oryzae*) are expressed as leaf wilting and rolling in rice seedlings during kresek phase followed by change in leaf color from grayish-green to yellow and death of seedling. In blight phase, lesion begins as water-soaked stripes on leaf blades that increase in size and color turns to yellow or grayish-white until whole leaf dries up (Fig. 2). In Punjab, during epiphytotic years the yield losses of 60-70 % have been reported to be caused by bacterial blight (Raina *et al* 1981) [14].
Aromatic rice cultivars are particularly highly susceptible to neck blast disease and now a days it is prevalent throughout the Punjab state (Singh *et al* 2018). The fungus *Pyricularia oryzae* infects the crop from vegetative to reproductive stage as it also infects the nodes, neck and panicle branches (Fig. 3). Among these, neck blast is the most destructive phase of the disease as it causes two times the losses as caused by vegetative phase (Puri *et al* 2009, Khan *et al* 2014 and Titone *et al* 2015) [12, 8]. The disease is known to be highly adaptable to variable environmental conditions.
The use of chemicals to control these diseases is effective but still expensive and overuse of pesticides causes severe environmental hazards and pesticide residues in the produce. Thus, the use of resistant varieties is one of the most effective and economic ways to minimize the losses from these diseases. Though, Basmati has limited germplasm base but the aromatic rice possesses a wide array of diversity which needs to be mined out for this exploitation in Basmati improvement programs. So, in the present study aromatic rice germplasm was screened against bacterial blight and neck blast with an aim of identifying some genotypes which can be used as donors for incorporating resistance against the above said devastating diseases

Materials and Methods
The experimental material for this study comprised of 30 aromatic rice genotypes including susceptible and resistant checks. For screening against bacterial blight (BB) disease, these

genotypes were grown during *Kharif* 2017 at Rice Experimental Fields, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. For evaluation against neck blast disease the germplasm lines were grown at two locations *viz.*, Rice Experimental Fields, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *Kharif* 2017 and at Rice and Wheat Research Centre, Malan, Himachal Pradesh (hotspot location) during *Kharif* 2018. Two checks Pusa Basmati 1637 and Tetep also included in the material when these lines were screened against neck blast to compare the material against these checks, because these had shown moderate resistant.

A) Screening against Bacterial Blight (BB): For screening against BB, all the test genotypes (5 plants/genotype) were artificially inoculated with pure cultures of *Xanthomonas oryzae* pv. *oryzae* pathotype (*PbXo-7*) by using clip inoculation technique at maximum tillering stage (Kauffman *et al* 1973) [7]. Observations were recorded 15 days after inoculation using SES scale (0-9) IRRI (Anonymous 1996) [1] as shown in Table 1.

Table 1: Standard evaluation scale for bacterial blight (Anonymous 1996) [1]

Score	Plant response
0	Highly resistant
1	Resistant
3	Moderately resistant
5	Moderately Susceptible
7	Susceptible
9	Highly susceptible

B) Screening against neck blast

i) Under Artificial inoculation conditions: For evaluation against neck blast disease, five plants (5 necks per plant) of each entry were artificially inoculated with most virulent isolate *NB- 7* of *Pyricularia oryzae* at 50% flowering stage using bit wrap technique (Jain *et al* 2017) [6], (Figure 1). The inoculations were done during late evening hours to prevent desiccation of fungal conidia. The data was recorded for lesion length (mm) with the help of ruler after 15 days of inoculation using scale devised by Jain *et al* (2017) [6] Table 2.

Fig 1: Artificial inoculation of neck blast fungas by bit wrap technique

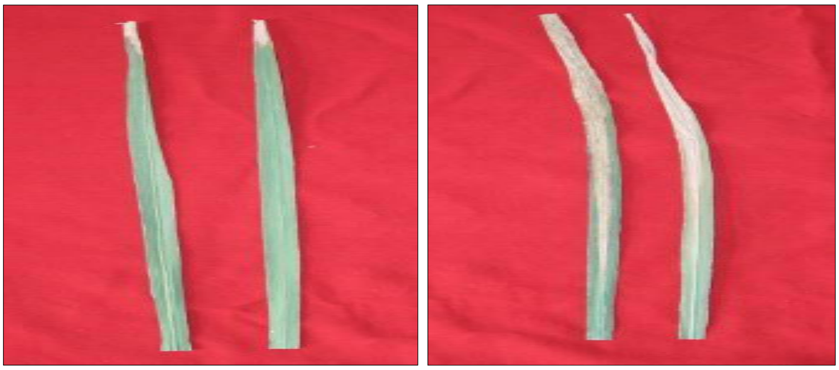
Table 2: Disease scoring based on lesion length (Jain *et al* 2017) [6]

Score	Symptoms (Lesion Length)
0	No visible lesion
1	Lesion size< 2 mm
3	Lesion size (2.1- 5mm)
5	Lesion size (5.1-10 mm)
7	Lesion size (10.1-20 mm)
9	Lesion size > 20 mm

ii) Under Natural Epiphytotic conditions: All the test entries were observed for their reaction towards neck blast disease under natural epiphytotic conditions. Data were recorded for lesion length (mm) with the help of ruler and disease score was recorded using 0-9 SES Scale (IRRI 2013).

Results and Discussion

Disease reaction to bacterial blight: At present there are ten prevalent pathotypes of *Xanthomonas oryzae* pv. *oryzae* under Punjab conditions. Among these pathotypes *PbXo-7* is the most prevalent pathotype in Punjab (Lore *et al* 2011) [11]. So, the screening of germplasm was done against pathotype (*PbXo-7*) and the results are presented in Table 3. Two genotypes, Kalikhasa and Bony Cay were found to be resistant. One genotype, IR 841-85-1-1-2 showed moderately resistant reaction to the disease whereas all other genotypes were found susceptible to bacterial blight disease. Among the check varieties, Punjab Basmati 3, Punjab Basmati 4 and Punjab Basmati 5 were found to be resistant as they are already reported to carry resistance genes (*Xa* 21 and *xa* 13) whereas Punjab Basmati 2, Pusa Basmati 1509, Pusa Basmati 1121 and Basmati 370 gave susceptible reaction to bacterial blight. Several studies aiming at the identification of resistance germplasm against BB had been made by researchers and have been used as donors and sources of germplasm base broadening. Jain and Lore (2016) [5] evaluated 886 entries against the prevalent pathotypes *viz*; *PbXo-7*, *PbXo-8* and *PbXo-10* under artificial inoculation conditions. Out of these, 53 genotypes revealed resistant reaction to all the three pathotypes.



(a) Bacterial blight resistant reaction (b) Bacterial blight susceptible reaction

Fig 2: Pictorial representation of resistant and susceptible reaction of Bacterial Blight (a- b)

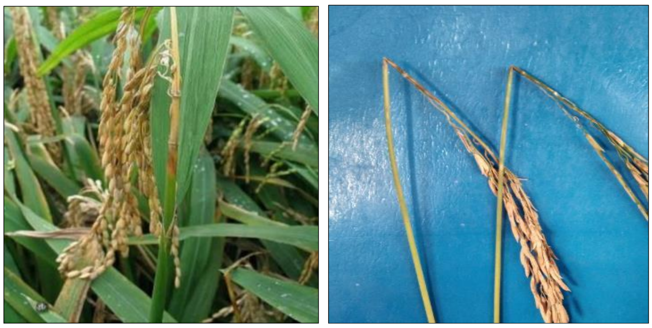
The resistant reaction of genotypes against bacterial blight was also reported by many other scientists, Khan *et al* (2009) [9]; Banito *et al* (2012) [3]; Shehzad *et al* (2012) and Lore and Jain (2014) [10].

Table 3: Reaction of aromatic rice genotypes against bacterial blight (*Xanthomonas oryzae* pv. *oryzae*)

Sr. No.	Designation	BB Score	Disease reaction
1	Laldhan	7	Susceptible
2	Sathi	9	Susceptible
3	Chimbalate Basmati	7	Susceptible
4	Mahisugandha	9	Susceptible
5	Yamini	9	Susceptible
6	Basmati Bahar	9	Susceptible
7	Basmati 106-12	7	Susceptible
8	Kalikhasa	3	Resistant
9	Muskan	7	Susceptible
10	Basmati-6141	7	Susceptible
11	Basmati 5884	9	Susceptible
12	Bony Cay	3	Resistant
13	Hung-mi-hriang-matsan	7	Susceptible
14	Longku Labat	7	Susceptible
15	IR 841-85-1-1-2	5	Moderately resistant
16	IR 628773-227-1-16	7	Susceptible
17	IR 62873-238-2-3	7	Susceptible
18	Hasan Sarai	7	Susceptible
19	UPR 35565-10-1-1	7	Susceptible
20	UPR 2828-7-2-1	7	Susceptible
21	IET 18033(RP3644-9-5-3-2)	7	Susceptible
22	IET 22187 (RP4594-121-148-24-11)	7	Susceptible
23	Basmati 867	9	Susceptible
24	Basmati 370	7	Susceptible
25	Punjab Basmati 2	7	Susceptible
26	Punjab Basmati 3	3	Resistant
27	Punjab Basmati 4	3	Resistant
28	Punjab Basmati 5	3	Resistant
29	Pusa Basmati 1509	9	Susceptible
30	Pusa Basmati 1121	9	Susceptible

However, most of the reports are for the non-aromatic type of germplasm and reports on availability of diverse sources of BB resistance in aromatic germplasm are limited. The resistant genotypes *viz;* Kalikhasa and Bony Cay can be used for transfer of bacterial blight resistance in Basmati breeding programme *viz;* broadening the Basmati germplasm base.

b) Disease reaction to neck blast: Neck blast is emerging as one of the severe problems in Basmati rice cultivation and the excessive use of pesticides for the control of the disease followed by residue problems is threatening the Basmati trade these days. More than 100 genes have been identified for blast disease but major focus has been on leaf blast. So, an attempt was made in this study to screen the germplasm lines against neck blast. The results of the genotypes screened for neck blast under artificial inoculation conditions as well as natural epiphytotic conditions are presented in Table 4. Among the test entries, none was found to possess resistance against neck blast under both artificial inoculation conditions as well as natural epiphytotic conditions. Only two genotypes Pusa Basmati 1637 and Tetep were found to be moderately resistant with average lesion length of 5.4 mm and 4.2 mm respectively.



(c) Neck blast resistant reaction (d) Neck blast susceptible reaction

Fig 3: Pictorial representation of resistant and susceptible reaction of neck blast (c-d)

Two genotypes, Kalikhasa and UPR 2828-7-2-1 were found to be susceptible with lesion length of 15 mm and 18 mm respectively but, these entries did not flower under natural field conditions at Rice and Wheat Research Centre, Malan (H.P). The lesion length (mm) produced under artificial inoculation conditions ranged between 15-33 mm with minimum in Kalikhasa (15 mm)

Table 4: Reaction of aromatic rice genotypes against neck blast (*Pyricularia oryzae*)

Sr. No.	Designation	Artificial inoculation		Natural conditions (hotspot)	
		Average Lesion Length (mm)	Score	Average Lesion Length (mm)	Score
1	Laldhan	29	9	40	9
2	Sathi	28	9	32	9
3	Chimbalate Basmati	33	9	46	9
4	Mahisugandha	24	9	39	9
5	Yamini	26	9	37	9
6	Basmati Bahar	28	9	36	9
7	Basmati 106-12	24	9	34	9
8	Kalikhasa	15	7	-	-
9	Muskan	32	9	48	9
10	Basmati-6141	32	9	48	9
11	Basmati 5884	22	9	38	9
12	Bony Cay	27	9	35	9
13	Hung-mi-hriang-matsan	26	9	38	9
14	Longku Labat	20	9	43	9
15	IR 841-85-1-1-2	23	9	40	9
16	IR 628773-227-1-16	24	9	50	9
17	IR 62873-238-2-3	23	9	37	9
18	Hasan Sarai	29	9	40	9
19	UPR 35565-10-1-1	25	9	35	9
20	UPR 2828-7-2-1	18	7	-	-
21	IET 18033(RP3644-9-5-3-2)	25	9	38	9
22	IET 22187 (RP4594-121-148-24-11)	27	9	38	9
23	Basmati 867	32	9	43	9
24	Basmati 370	37	9	45	9
25	Punjab Basmati 2	31	9	49	9
26	Punjab Basmati 3	31	9	46	9
27	Punjab Basmati 4	34	9	42	9
28	Punjab Basmati 5	32	9	45	9
29	Pusa Basmati 1509	29	9	43	9
30	Pusa Basmati 1121	31	9	46	9
31	Pusa Basmati 1637	5.4	5	4.6	5
32	Tetep (Resistant Check)	4.2	5	4.0	5

And maximum in Chimbalate Basmati (33 mm). The lesion length ranged between 32mm (Sathi) to 50 mm (IR 628773-227-1-16) under natural conditions at hot spot location. Reports on differential behavior of germplasm resources have been published by many researchers. These were all aromatic or combination of aromatic and non-aromatic. Puri *et al* (2006) [13] screened 30 different tropical rice lines for neck blast resistance. Out of these, 9 were resistant, 13 were moderately resistant, 7 were moderately susceptible and one was susceptible. Singh *et al* (2018) evaluated 69 rice germplasm lines under both artificial inoculation and natural epiphytotic conditions and reported that four genotypes Pusa Basmati 1637, INGR 15001, INGR15002 and Tetep showed moderate resistance against neck blast while moderate susceptibility was reported in one genotype, RYT 3672.

Conclusion

The non-availability of diverse resistant/tolerant resources against neck blast in present study warrants need to screen more diverse germplasm for identification of donors possessing resistance/tolerance against the disease. Narrow germplasm base is always reported as one the major drawbacks in Basmati improvement programmes. Identification of such donors in aromatic/Basmati background may prove valuable sources for incorporating disease resistant and maintaining eliteness of this speciality rices possessing resistance/tolerance against neck blast disease. The resistance genes in Kalikhasa and Bony Cay need further confirmation for presence of novel genes with the aid of molecular markers. Pyramiding of such new resistance genes into rice genotypes would be thus an efficient strategy to ensure durable resistance against BB pathogen.

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