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Screening of M₂ and M₃ mutants of rice against bacterial leaf blight

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Abstract

A study was held at N. E. Borlaug Crop Research Centre during the kharif season of 2018-19, 2019-2020 and 2020-21. The seeds of various basmati and non-basmati lines were irradiated with γ rays at NBRI, Lucknow in 2018-19. The seeds were grown in the same year, the observed viability was less than 99.7 % (M₁). The seeds were very meagre and so they were collected and grown in the kharif season of 2019-20. In this generation a screening test for examining BLB resistance was conducted. Resistant mutants were identified in the M₂ generation. Similarly, the seeds obtained from M₂ were grown as the M₃ generation and again screening was done for the BLB. Some mutants were identified which revealed resistance and could be used in the future breeding program for the alleviation of disease susceptibility and catering resistance.

Keywords: Xoo, *Xanthomonas oryzae* pv. *oryzae*, M₁, M₂, M₃, γ -irradiation

1. Introduction

Rice (*Oryza sativa* L.) is the prime food for about 1/3 of the population of the world which occupies almost 1/5 of the total land covered by cereals. Rice belongs to the family Poaceae having diploid chromosome number of 24. (Chakravarthi and Naravaneni, 2006) [4]. About 90 % of the world rice is eaten up as staple food in Asia (Gumma, *et al.* 2011) [6]. A decreasing trend has been noticed in the genetic diversity owing to genetic erosion but it was balanced by the equal pace of domestication. Rice crop is an epitome of better-quality genome having a small size which makes it easier to study the impact of mutations (Rafi *et al.*, 2019) [5]. The impact of biotic stress is considerable and it led to the decrease in the productivity of the crop even when all the conditions of nutrition and irrigation are met. Speaking of rice there are multitude of diseases which have an adverse impact on production and its productivity. Bacterial leaf blight is one of the most dreadful diseases of rice and it is very frequently occurring in the terai regions of our nation. BLB was first observed by the farmers in Kyushu island at a place called Fukuoka in the last decade of 19th century (Mannam *et al.*, 2013) [10]. When the plants are infested at early stage the incurred losses in yield may be as high as 50% and infection at seedling stage cause approx. 20 to 40 % reduction in yield (Yasmeen *et al.*, 2017). White *et al.* (2009) [15, 13] studied the virulence pattern of Xoo and the complex interaction pertaining to their mechanism. In various parts of the world research work is concurrently carried out to know more about the virulence (Ryan *et al.*, 2011) [11]. To control the disease, its identification and assessment of the magnitude is very crucial. Now a days a number of approaches have been prevalent to produce and increase the resistance whilst gene pyramiding and marker assisted breeding. Creating variability through mutations has therefore grown to be among the most important tools to improve rice (Viana *et al.*, 2019) [12]. Among the modes of generation of variability mutations is one of most pioneering methods to create variation. However, mutations may cause even cause loss function along with the gain (Mohapatra *et al.*, 2014) [16]. The nature and quantity of genetic variability are suitable for selecting characters which contribute to higher yields (Jana and Roy, 1973) [7]. Variation could be produced in a number of traits. Here screening of the population was done based on the impact of BLB on the mutant generation both in M₂ and M₃ generations during the year 2019-20. In the irrigated and rainfed environments BLB is the most frequently occurring disease. Its causal organism is *Xanthomonas oryzae* pv. *oryzae*. yield could be decreased by up to 20-50%. The research work by scientists revealed that there are approximately twenty-six resistant genes for BLB which are identified viz., Xa1 to Xa 26. *Xanthomonas oryzae* pv. *oryzae* has 12 strains and 10 races.

2. Material and Methods

Common symptoms are leaves becoming yellow and dry whilst seedlings becoming wilted (irri.org). BLB is most likely to occur in the region which are abundant in weed and diseased plant stubbles. It can easily occur in any climate be it tropical or temperate. When the humidity reaches beyond 70 % and the temperature ranges between 25-34 °C then there can be preponderance in its occurrence. Blowing of strong winds causes the bacteria to spread more and more. The bacteria are in the droplets that ooze out from the infected leaves. The infected leaves have water-soaked lesions which may later become yellow orange. These leaves were cut into tiny pieces (5mm infected tissue and 5mm of adjacent healthy tissue) and placed in 70% ethanol for 10 seconds, washed twice with sterilized distilled water and dipped in 300ul sterilized distilled water for 15 minutes in microcentrifuge tubes. A loopful of the water containing the bacterial ooze was streaked on PSA (peptone 1.2%, sucrose 1.2%, agar agar 2%) plates and left in incubator at 30 °C for 3 to 4 days for bacterial growth. The yellow colonies were picked up with sterilized wire loop and purified on fresh PSA plates. As per Agrawal *et al.* (1989) [2] the identification and characterization of the pathogen was done with the aid of the journal on seed-borne diseases and health testing of rice and IARI manual for

trainees.

2.1 Procedure of Screening

Twenty-six mutants from M3 generation were grown in 2019-2020 and 2020-2021. twenty-one mutants from M2 generation and 26 mutants from M3 generation were screened for BLB resistance along with their originating parent varieties in a nursery at Norman E. Borlaug Crop Research Centre, Pantnagar. Seeds were broadcasted on dry land (raised beds, 1ft.x 1 ft. for each entry with 1 ft interval), making it more aerated by rubbing it with bare hands and a thin layer of well decayed farmyard manure (FYM) was spread over them, and covered with straw, then watered with a hand sprinkler three times a day. After 4th day of sprouting the nursery was flooded for first time. At the age of 40 days plants were transplanted having 9 inches plant to plant and row to row distance. Xoo inoculum was obtained in nutrient broth by keeping at 37 °C for 48 hours in shaker incubator and thereby suspending in distilled water. Plants were inoculated just 4-5 days before panicle emergence. The scissors were dipped in the inoculum and one-fourth of top 3- 4 leaves were cut with the help of the scissors. Data were collected after three weeks of inoculation under the following scale (Anonymous, 1996). Scale for BLB (for field test, lesion area).

Table 1: The following table will be used for scoring

Percentage of Infection	Score values	Behavior of the host
0-3%	1	Highly resistant
4-6%	2	Resistant
7-12%	3	Resistant
13-25%	4	Moderately resistant
26-50%	5	Moderately susceptible
51-75%	6	Susceptible
76-87%	7	Susceptible
88-94%	8	Highly susceptible
95-100%	9	Highly susceptible

Table 2: Effect of artificial inoculation on the mutants in M2 generation during the year 2019-2020

Sr. No.	Genotypes	Disease scale	Response of host
1.	PR-121	5.5	Moderately susceptible
2.	PR-121- 10kR	4.3	Moderately resistant
3.	PR-121- 20 kR	4.7	Moderately susceptible
4.	UPR-7029	4.8	Moderately susceptible
5.	UPR-7029-11-10 kR	2.7	Resistant
6.	UPR-7029-11- 20kR	4.5	Moderately susceptible
7.	PD-19	5.7	Susceptible
8.	PD-19-10 kR	5.1	Moderately susceptible
9.	PD-19-20 kR	5.5	Susceptible
10.	PB-2	5.8	Susceptible
11.	PB-2- 10 kR	5.0	Moderately susceptible
12.	PB-2- 20 kR	5.6	Moderately susceptible
13.	Jhumri Selection-3	4.5	Moderately resistant
14.	Jhumri Selection-3- 10 kR	3.2	Resistant
15.	Jhumri Selection-3-20 kR	3.4	Resistant
16.	Jhumri Selection-7	3.9	Moderately resistant
17.	Jhumri Selection 7- 10 kR	2.1	Resistant
18.	Jhumri Selection 7- 20 kR	3.3	Resistant
19.	Taraori	5.3	Moderately Susceptible
20.	Taraori- 10 kR	4.3	Moderately resistant
21.	Taraori- 20 kR	5.0	Moderately susceptible

Table 3: Effect of inoculation on mutants in M3 generation in the year 2020-2021

Sr. No.	Genotypes	Disease scale	Response of host
1.	PR-121	5.8	Moderately susceptible
2.	PR-121- 10kR	4.4	Moderately resistant
3.	PR-121- 20 kR	4.8	Moderately susceptible
4.	UPR-7029	5.1	Moderately susceptible
5.	UPR-7029-11-10 kR-12	3.1	Resistant
6.	UPR-7029-11- 20kR-11	4.5	Moderately susceptible
7.	UPR-7029-11- 20kR-4	3.1	Resistant
8.	UPR-7029-11- 20kR-2	3.4	Resistant
9.	UPR-7029-11- 20kR-3	3	Resistant
10.	UPR-7029-11- 20kR-5	5.5	Susceptible
11.	UPR-7029-11-20 kR-12	4.8	Moderately susceptible
12.	PD-19	5.6	Susceptible
13.	PD-19-10 kR	5.3	Moderately susceptible
14.	PD-19-20 kR	5.8	Susceptible
15.	PB-2	5.6	Susceptible
16.	PB-2- 10 kR	4.3	Moderately resistant
17.	PB-2- 20 kR	5.3	Moderately susceptible
18.	Jhumri Selection-3	4.8	Moderately resistant
19.	Jhumri Selection-3- 10 kR	3.4	Resistant
20.	Jhumri Selection-3-20 kR	3.2	Resistant
21.	Jhumri Selection-7	4.2	Moderately resistant
22.	Jhumri Selection 7- 10 kR	2.8	Resistant
23.	Jhumri Selection 7- 20 kR	3.2	Resistant
24.	Taraori	5.1	Moderately Susceptible
25.	Taraori- 10 kR	3.8	Moderately resistant
26.	Taraori- 20 kR	4.5	Moderately susceptible

3. Results

BLB is observed with variable intensities in whole of Pantnagar during the kharif season as it is a hotspot for BLB. The diseased material was identified and used for the isolation of the pathogen. On PSA plates, *Xanthomonas oryzae* pv. *oryzae* having circular, entire, smooth, convex, opaque, whitish yellow at first and straw yellow later was identified. One trial was held in year 2019-20 comprising of 21 lines including mutants and the parental varieties. In this trial some mutants were found to be resistant, some had moderate resistance whilst their parents were susceptible. This could be due to the effect of mutation in producing new gene combinations. The resistant types are UPR-7029 irradiated at 10 kR dose, Jhumri selection 3 at 10 kR and 20 kR dose and Jhumri selection 7 at 10 kR and 20 kR dose. Second trial was conducted during 2020-21 in which M3 generation was screened. In M3 there were a total of 26 lines including mutants and parents out of which some of the mutants showed resistance namely, UPR-7029-10 kR-12, UPR-7029-10 kR-4, UPR-7029-10 kR-2 and UPR-7029-10 kR-3, Jhumri selection 3 at 10 kR and 20 kR dose and Jhumri selection 7 at 10 kR and 20 kR dose. A few mutants also exhibited moderate resistance in M2 namely, PR-121 at 10 kR dose and Taraori basmati at 10 kR dose. Parental lines Jhumri selection 3 and Jhumri selection 7 also showed moderate resistance. In M3 generation some lines showed moderate resistance namely, PR-121 at 10 kR dose and Taraori basmati at 10 kR dose. Remaining lines were showing moderately susceptible to susceptible reaction. (Cheema *et al.*, 1998; Khan *et al.*, 2000a; Khan *et al.*, 2000b) ^[1, 8, 9]. So, it is clear from the findings that the 10 kR dose is producing a greater number of resistant and moderately resistant types. Jhumri selection 3 & 7 parental lines were already having moderate resistance to the pathogen and their mutants at 10 kR and 20 kR doses showed complete resistance too. The resistant mutants can be used in the disease improvement programs for imparting resistance to the hybrids or to improve a specific trait of a particular variety.

4. Conclusion

Bacterial leaf blight is one of the most fatal diseases of rice which causes loss in the yield to the tune of 70% (irri.org). This disease is a night mare for the farmers all over the world especially the ones who reside in the terai region of India. Terai region is a major rice growing belt in India and is a major hub for growing basmati varieties. The rice varieties involved in this study are subjected to γ - irradiation and then two mutant generations were grown viz., M2 and M3. Screening of resistance against BLB was studied after artificially infecting the plants with the Xoo. Most of the parents were susceptible but their mutants showed a range of disease reaction right from the moderate resistance to resistance reaction. The dose which was most potent to produce the resistant types was 10 kR dose of γ irradiation in both M2 and M3. However, Jhumri selection 3 & 7 showed resistance at both the doses in M2 as well as M3 generations.

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