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Phenotypic screening of segregating population for bacterial leaf blight resistance in rice variety 'Aiswarya' (*Oryza sativa*)

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Abstract

Bacterial leaf blight is one of the most devastating diseases of rice across the world. Most of the existing varieties are getting susceptible to this deadly disease now a days. Since the host plant resistance is the one and only strategy to mitigate this disease, identification of the new sources of resistance is a necessity. The present study is focussed on evaluation of 20 segregants of Aiswarya to study the response of the lines against bacterial leaf blight. A single line among the 20 was identified as resistant and it can be incorporated in the resistance breeding program as donor parent.

Keywords: Rice germplasm, bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*

Introduction

Rice is life for thousands of millions of people. More than half of the world population depends upon this crop for their daily required calories (Shehzad *et al.* 2012)^[12]. The rice crop is susceptible to a number of diseases among which bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama)^[16] Swings *et al.*, (1990) is one of the most destructive disease of rice throughout the world and widespread in Asia and it is the most serious disease of rice in South East Asia, particularly in Japan, Philippines, Indonesia and India (Ou, 1985; Srivastava, 1967; Ahmed & Singh, 1975; Singh *et al.*, 1977; Rangasawami, 1975)^[7, 14, 1, 13, 10]. The disease was first noted by farmers of Fukuoka areas in Japan during 1884. However, during 1910 -11, it was prevailing from central to southwestern Japan (Tagami and Mizukami, 1962)^[16]. In India losses were 6-60% or even upto 81% in some cultivars (Srivastava, 1967; Ahmad and Singh, 1975)^[14, 1]. However, during 1960's, the disease became common in Asian rice growing regions due to use of TN1 and IR8 which proved to be susceptible to the disease (Amna, 2008)^[2]. The only feasible and economical way of controlling diseases is the use of resistant rice cultivars. In view of the importance of genetic resistance for disease control, studies were undertaken to evaluate the rice genotypes against BLB disease.

Material And Methods

Isolation of Bacteria

Infected samples were collected and chopped into small pieces using a sterile scissors. The chopped leaflets were dipped in sterile water after sterilization and washing. The bacterial ooze containing water is used to inoculate the PSA (Peptone sucrose agar) plated petri plates using a sterile loop. The streaked plates were incubated at 30°C for at least 3 days till the appearance of yellow colonies. Single purified colonies were obtained using sub-culturing.

Confirmation of Bacterial Isolates by Koch Postulate

Isolates were inoculated using susceptible variety s i.e., Aiswarya. Net house grown healthy plants were selected at three leaves stage. After 14 days of inoculation, data were recorded for lesion length. Diseased leaves were detached and again bacteria were successfully isolated on plates using modified PSA media.

Plant Materials

20 segregants of Aiswarya along with susceptible check Aiswarya and resistant check were used for screening against bacterial leaf blight isolates to reconfirm resistance in field conditions.

Inoculum Preparation and Inoculation of Plants

The inoculum was prepared using 20g of Nutrient agar in powdered form added to distilled water. Pure culture of bacteria was taken from plate with the help of wire loop and transferred to volumetric flask having nutrient agar media (Fig 1). Inoculation was clipping method (Kauffman *et al.* 1973)^[6] by dipping a clean sharp scissor in the prepared broth and leaf tip of around 2 to 3 cm were clipped (Fig 2). The inoculation was done during seedling and maximum tillering stage and done either at early morning or in the late evening.



Fig 1: The prepared inoculum for clipping



Fig 2: Clip inoculated plants at maximum tillering

Disease Scoring

Lesion length on each line was noted after 14 days of inoculation. The percentage of the incidence of the disease was calculated by the following formula (Gnanamanickam *et al.* 1999)^[5].

$$\text{Diseased leaf area percentage (\%)} = \frac{\text{Lesion length}}{\text{Total leaf area}} \times 100$$

Segregants were classified into different categories according to their diseased leaf area percentage using standard IRR procedure (Anonymous, 2013)^[3]. The score chart is given in Table 1.

Table 1: IRR score chart for bacterial leaf blight

| Disease score | Lesion area percentage (%) | Disease reaction |
|---------------|----------------------------|-----------------------------|
| 0 | 0% | Highly resistant (HR) |
| 1 | >1-10% | Resistant (R) |
| 3 | >10-30% | Moderately resistant (MR) |
| 5 | >30-50% | Moderately susceptible (MS) |
| 7 | >50-75% | Susceptible (S) |
| 9 | >75-100% | Highly susceptible (HS) |

Results and Discussion

Among the 20 segregants, single line A-19 showed highly resistance response against the pathogen with zero symptoms. The donor parent ISM was also did not exhibited any symptoms of the disease. A-5 and A-12 were identified as the resistant lines with diseased leaf area percentage of 6.20 and 8.70%. The diseased leaf area percentage of the lines along with the parents are given in Table 2. The moderately resistant class was made up of 5 individuals such as A-1, A-6, A-7, A-9 and A-20 with diseased leaf area percentage 14%, 11.20%, 13.40%, 11% and 12%. Four, three, and five lines were classified as moderately susceptible, susceptible and highly susceptible classes. The susceptible parent Aiswarya was included in the highly susceptible class with 80% diseased leaf area percentage.

The morphological data on each segregants were taken prior to harvest and presented in table 3. The highly resistant line A-19 matured within 122 days and around 109.2 cm tall. The 10 number of productive tillers and cm 20.9cm long panicles were produced A-19. The 1000 grain weight was 22.18g and grains were red kernelled like Aiswarya. A considerable reduction in the yield contributing traits was noticed in the susceptible and highly susceptible classes.

Similar results were also presented by Samiullah and coworkers (2015)^[11]. The highly susceptible varieties among the 23 indigenous germplasm screened showed considerable reduction in panicle length and other quantitative traits. Zhang and Mew (1985) also tested 13 cultivars for resistance to four races at three different growth stages. Arya Gopinath *et al.* (2022)^[4] screened 25 segregants of the popular rice variety Prathyasa and identified two lines as resistant against BLB. Ou *et al.* (1971)^[8] reported that the testing at seedlings and flag leaf stage would be sufficient for preliminary screening of a large number of rice cultivars against BB disease.

Table 2: Diseased leaf area percentage, score and host response of segregants after inoculation with pathogen

| SL number | Sample number | Diseased leaf area percentage (%) | Score | Host response |
|-----------|---------------|-----------------------------------|-------|---------------|
| 1 | A-1 | 14.00 | 3 | MR |
| 2 | A-2 | 75.00 | 9 | HS |
| 3 | A-3 | 32.10 | 5 | MS |
| 4 | A-4 | 31.20 | 5 | MS |
| 5 | A-5 | 6.20 | 1 | R |
| 6 | A-6 | 11.20 | 3 | MR |
| 7 | A-7 | 13.40 | 3 | MR |
| 8 | A-8 | 76.00 | 9 | HS |
| 9 | A-9 | 11.00 | 3 | MR |
| 10 | A-10 | 41.20 | 5 | MS |
| 11 | A-11 | 43.00 | 5 | MS |
| 12 | A-12 | 8.60 | 1 | R |
| 13 | A-13 | 78.00 | 9 | HS |
| 14 | A-14 | 65.20 | 7 | S |
| 15 | A-15 | 77.20 | 9 | HS |
| 16 | A-16 | 65.40 | 7 | S |
| 17 | A-17 | 70.30 | 7 | S |
| 18 | A-18 | 76.00 | 9 | HS |
| 19 | A-19 | 0.00 | 0 | HR |
| 20 | A-20 | 12.00 | 3 | MR |
| A | Aiswarya | 80.00 | 9 | HS |
| B | ISM | 0.00 | 0 | HR |

Table 3: Morphological data of segregants of Aiswarya

| SL number | Sample number | Days to Maturity | Plant Height (cm) | Number of productive tillers per plant | Panicle length (cm) | 1000 grain weight (g) | Kernel colour |
|-----------|---------------|------------------|-------------------|--|---------------------|-----------------------|---------------|
| 1 | A-1 | 122.00 | 108.50 | 10.00 | 20.30 | 22.21 | Red |
| 2 | A-2 | 123.00 | 115.00 | 6.00 | 15.00 | 19.14 | Red |
| 3 | A-3 | 122.00 | 108.00 | 11.00 | 19.10 | 22.08 | Red |
| 4 | A-4 | 126.00 | 115.00 | 10.00 | 18.81 | 22.01 | Red |
| 5 | A-5 | 123.00 | 108.00 | 8.00 | 19.90 | 22.91 | Red |
| 6 | A-6 | 125.00 | 111.00 | 9.00 | 20.10 | 23.01 | Red |
| 7 | A-7 | 121.00 | 112.60 | 10.00 | 20.00 | 22.05 | Red |
| 8 | A-8 | 123.00 | 109.00 | 4.00 | 15.30 | 19.04 | Red |
| 9 | A-9 | 122.00 | 109.30 | 11.00 | 20.00 | 21.50 | Red |
| 10 | A-10 | 123.00 | 109.00 | 9.00 | 18.20 | 20.91 | Red |
| 11 | A-11 | 123.00 | 110.00 | 9.00 | 17.20 | 22.10 | Red |
| 12 | A-12 | 125.00 | 115.00 | 9.00 | 21.00 | 21.91 | Red |
| 13 | A-13 | 129.00 | 108.00 | 5.00 | 15.00 | 18.45 | Red |
| 14 | A-14 | 120.00 | 108.00 | 7.00 | 16.70 | 21.91 | Red |
| 15 | A-15 | 122.00 | 106.00 | 5.00 | 16.20 | 18.91 | Red |
| 16 | A-16 | 128.00 | 113.00 | 6.00 | 18.80 | 19.51 | Red |
| 17 | A-17 | 120.00 | 109.00 | 5.00 | 18.20 | 19.61 | Red |
| 18 | A-18 | 125.00 | 108.00 | 5.00 | 16.00 | 19.01 | Red |
| 19 | A-19 | 122.00 | 109.20 | 10.00 | 20.90 | 22.18 | Red |
| 20 | A-20 | 124.00 | 111.30 | 11.00 | 18.30 | 21.14 | Red |
| A | Aiswarya | 120.00 | 108.00 | 13.00 | 23.00 | 26.10 | Red |
| B | ISM | 146.00 | 115.00 | 15.00 | 21.00 | 15.20 | White |

Conclusion

Bacterial leaf blight is one of the most devastating diseases of rice and causes the severe yield losses during epidemics. Utilization of the host plant resistance is the most appropriate strategy to mitigate this disease. In the present study out of the 20 lines screened one line A-19 was identified as resistant. These lines can be used as the resistant source in further breeding programmes for the development of resistant varieties against BLB. Moreover, incorporation of these varieties may reduce the linkage drag which is common with the land races and wild relatives.

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