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**Indra Kumar**
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

**Ashok Kosariya Krishna Kumar Maravi**
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

**Leelesh Kumar Sahu**
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India, India

**AS Kotasthane**
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India, India

**Correspondence**
**Indra Kumar**
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

# Evaluation of near Isogenic lines and Pyramids against *Xoo* Isolates Collected from three different geographical location of Chhattisgarh

**Indra Kumar, Ashok Kosariya Krishna Kumar Maravi, Leelesh Kumar Sahu and AS Kotasthane**

**Abstract**
The reactions of 10 near isogenic lines and 10 pyramiding lines possessed single or combination of *Xa* genes for resistance to *Xoo*. Obtained from International Rice Research Institute Hyderabad and seventeen local variety were evaluated for the resistance to three different isolates in Chhattisgarh Under *in vivo* condition among the NIL's IRBB-10 carrying *Xa10* resistant gene for *Xanthomonas oryzay pv. Oryzae* expressed resistant to Raipur and Kanker isolate while moderate resistant to DRR and Dhamtari isolate and IRBB-8 and IRBB-11 expressed resistant to only Raipur isolate. The rest NILs showed susceptible to moderately susceptible reaction to all the isolates In the present investigation the host specific nature / incompatible interactions of the isolates on the near isogenic lines indicated that responses are clearly the result of the molecular cross talk between avirulence gene product(s) and corresponding R gene product(s) the pyramiding line containing two to three resistance gene combination IRBB-52 (*Xa 4 + Xa 21 )*, IRBB-54 (*xa 5 + Xa* 21 ), IRBB-57 (*Xa 4 + xa 5 + Xa21 )* and IRBB-58 (*Xa 4 +xa 13 + Xa 21 )* were expressed highly resistant reaction to Raipur isolate and moderate resistant to resistant reaction to the most of isolates collected from different geographical location of Chhattisgarh. Local varieties were more susceptible than improved varieties to leaf blight disease. Among Local varieties, H.M.T., Pant-4 and Safri-17 was the most susceptible and Maheshwari was resistant to *Xoo isolate.* The overall results indicated that resistance gene(s) behave differentially against different isolates. The combination of genes some time exhibited additive resistance while non-additive in some combination and against different *Xoo* cultures.

**Keywords:** Bacterial Leaf Blight; Isogenic and pyramids Lines; Xanthomonas oryzae

## 1. 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. Rice cultivation is the principal activity and source of income for millions of households around the globe, and several countries of Asia and Africa are highly dependent on rice as a source of foreign exchange earnings and government revenue.The rice crop is susceptible to a number of diseases among which bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* (Ishiyama) Swings *et al*., (1990) is one of the most destructive disease of rice throughout the world and widespread in irrigated and rainfed environments of 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).. The disease has become a major rice disease in last three decades because of the introduction of modern cultivars, which is highly responsive to nitrogen fertilizer. There are no effective ways of protecting rice from the disease other than by the development of resistant cultivars. Host Plant resistance is an important component of an integrated management program for this disease. To minimize the risk of attack by bacterial blight, evolving resistant cultivars against the pathogen is the best non chemical method for management of the disease. To develop high yielding varieties with durable resistance to bacterial blight, it is necessary to understand the population structure of this pathogen. The existing population of the bacterium was classified into virulence groups/races based on their interaction with differential cultivars.
The pyramiding of several resistance genes through marker assisted selection also has been a quite effective strategy for combating the disease. Resistant rice cultivars mainly based on a single resistance gene were developed, however, large-scale and long-term cultivation of those varieties and the rapid adaptation of the pathogen race cause the breakdown of disease resistance trait in those cultivars. One of the important strategies to prolong the useful life of major gene resistance is to pyramid many major resistance genes in a single gene resistance

cultivar. The gene pyramiding technique provided a broad-spectrum of resistant cultivar which is economical and effective method for BB management. However, pyramiding major genes using conventional breeding method based on phenotype alone is perhaps inappropriate because of the difficulty caused by the interference of expression among major genes and it is time consuming. Therefore, molecular breeding dealing with genotypes provides more advantages to detect the transfer of target resistant gene during the breeding program precisely and easily. 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. The purpose of this study is to identify resistance sources for controlling the rice bacterial blight in Chhattisgarh

## 2. Materials and Methods

### 2.1 Test varieties/ genotypes and preparation of plant

The field studies were conducted to study the plant resistance and its nature of response to *Xanthomonas oryzae* pv. *oryzae (Xoo*). Twenty near isogenic (IRBB lines) and pyramided lines namely, IRBB-1, IRBB-3, IRBB-4, IRBB-5, IRBB-7, IRBB-8, IRBB-10, IRBB-11, IRBB-14, IRBB-50, IRBB-51, IRBB-52, IRBB-53, IRBB-54, IRBB-55, IRBB- 56, IRBB-57, IRBB-58, IRBB-59, IRBB-60, possessed single or combination of *Xa* genes for resistance to *Xoo*. Obtained from the rice gene bank international rice research institute, (Table 1) and other seventeen local varieties namely; HMT, pant- 4, Bamleshwari, IR 64, Durgeshwari, Tapaswani, Chepti, Jawaful, Maheshwari, Safri-17, HR-12, Mahamaya, Kranti, MTU 1010, Swarna, Syamla and TN-1. collected from IGKV Raipur (C.G.).(Table 2) were used in the present study. This study was conducted during *kharif* 2014 and *Rabi* 2015 seasons under *in vivo* condition. The Healthy seedlings of the genotypes were raised under seed beds and 30 day-old seedlings were transplanted in well puddled field with in two rows, each of two meter length adopting spacing of 20 x 15 cm between plants and between rows. Fertilizer in the form of urea was applied in three equal split doses at basal, active tillering and boot leaf stages to provide a total of 120 kg N/ha. And second applied 60: 40 and 25 kg /ha.

The following listed varieties/genotypes were used as per the planned studies for evaluation of bacterial leaf blight disease:

**Table 1:** List of rice genotypes (IRBB lines)

| S. N | Rice lines | Cross | Resistance gene (s) |
|---|---|---|---|
| 1 | IRBB -1 | IR24 × 5/KOGYOKU | *Xa 1* |
| 2 | IRBB -3 | IR24 × 5/CHUGOKU 45 | *Xa 3* |
| 3 | IRBB-4 | IR24 × 5/IR20 | *Xa 4* |
| 4 | IRBB-5 | IR24 × 5/IR1545-339 | *xa 5* |
| 5 | IRBB-7 | IR24 × 5/DV85 | *Xa 7* |
| 6 | IRBB-8 | IR24 × 5/P1231129 | *Xa 8* |
| 7 | IRBB-10 | IR24 × 5/CAS209 | *Xa 10* |
| 8 | IRBB-11 | IR24 × 5/IR8 | *Xa 11* |
| 9 | IRBB-13 | BJ1/5 × IR24 | *xa 13* |
| 10 | IRBB-14 | TAICHUNG NATIVE 1/5 × IR24 | *Xa 14* |
| 11 | IRBB-50 | | *Xa 4 + xa 13* |
| 12 | IRBB-52 | IRBB4/66700-3-3-3-4-2 | *Xa 4 + Xa 21* |
| 13 | IRBB-53 | IRB4/IR66699-9-1-1-5-2 | *xa 5 + xa 13* |
| 14 | IRBB-54 | | *xa 5 + Xa 21* |
| 15 | IRBB-55 | | *xa 13 + Xa 21* |
| 16 | IRBB-56 | AY4+5/IR68311-13-3-42 | *Xa 4 + xa 5 + xa 13* |
| 17 | IRBB-57 | AY4+5/IR66700-4-2-9-5-2 | *Xa 4 + xa 5 + Xa 21* |
| 18 | IRBB-58 | NH11-35/NH9-53 | *Xa 4 +xa 13 + Xa 21* |
| 19 | IRBB-59 | NH11-35/NH9-53 | *xa 5 +xa 13 + Xa 21* |
| 20 | IRBB-60 | NH11-35/NH9-53 | *Xa4+xa5+xa13+Xa21* |
| 21 | DV-85 | | *xa5 +Xa7* |
| 22 | TN-1 | | |
| 23 | AJAYA | | |

**Table 2:** list of common rice cultivars

| | | | | | |
|---|---|---|---|---|---|
| 1 | HMT | Indigenous | 9 | Jawaful | |
| 2 | Pant-4 | Indigenous | 10 | Maheshwari | |
| 3 | Bamleshwari | RP2151-40-1 × IR9828-23 | 11 | Safri-17 | Selection from safri |
| 4 | IR-64 | | 12 | Mahamaya | Asha × kranti |
| 5 | Durgeshwari | | 13 | Kranti | Cross116 × IR8 |
| 6 | Tapaswani | | 14 | MTU 1010 | Krishnaveni × IR-64 |
| 7 | Chepti | | 15 | Syamla | R60-2713×R238-6 |
| 8 | HR-12 | | 16 | Swarna | Vaisishtha × mashuri |
| | | | 17 | TN1 | |

### 2.2 Isolation and purification of the pathogen *(Xanthomonas oryzae* pv. *oryzae)*

During the *Kharif* season of 2014 bacterial blight diseased samples were frequently collected from the three geographical area of Chhattisgarh (Raipur, Dhamtari, and Kanker) and carried to the laboratory for isolation of the pathogen. The entire work of isolation was done under isolation chamber and laminar flow, which were sterilized by ethyl alcohol or formaldehyde and UV radiation, prior to use. The bacterial causal organism was isolated from naturally infected rice plant following procedure described by (Kotasthane, 2003). The technique takes the advantage of the natural phenomenon of oozing from the hydathodes/ cut end of the rice leaves. The procedure involves the use of moist chamber prepared by lining the petri-plates with moderately thick layer of wet blotter paper discs (lid and bottom). Rice plant showing natural bacterial blight infection (with advancing lesion) was collected. Small pieces (3-4 cm.) from the infected region were cut using a sharp scalpel blade and were placed in the moist chamber and incubated at 22 ± 1 ºC for 5-6 hours. The cut ends oozed out mass of bacterial cell, which were observed as viscous yellow and opaque against transmitted light under a dissecting stereo binocular microscope in the laminar flow. Using a fine point sterilized inoculation needle the bacterial mass from the cut end was then lifted, and streaked on to the medium (wakimoto's) slants and were incubated at 27± 1 ºC. Bacterial colonies were observed the next day or the day after as smooth, convex, butyrous, whitish yellow to straw yellow colour, and opaque against transmitted light. The organism was then confirmed by challenge

inoculation on susceptible rice cultivar TN (1). Culture of *Xanthomonas oryzae* pv. *oryzae* isolate thus obtained was purified by repeated isolations on the medium and maintained on the PSA slants and incubated at 27 ± 2ºc till further use. The isolate was identified on the basis of the colony colour and colony characters of the bacterium by confirming with standard reports Ishiyama (1922); Wakimoto (1955, 1967); Isaka (1970).

**2.3 Culturing of the test pathogen**

The Wakimoto's medium/potato sucrose agar medium (PSA) was used for culturing the test pathogen. Sterilization of media was done by autoclaving at 1.41 kg $cm^{-2}$ pressure for 20 minutes. After repeated sub-culturing on petriplates poured with PSA**,** the bacterium was sub-cultured to slants. The inoculated slants were kept in an incubator at 27 ± 2 ºc for the growth. In all the studies 72 hours old culture was used. For all the studies, 1.0 optical density of the bacterial concentration was uniformly used.

**2.4 Pathogenicity test of the pathogen**

The experiment was conducted in January 2014 under complete randomized block design (CRD) with three replications. The rice cultivar was tested under pot condition in glass house (Fig. 1) and the seeds were sown in pots with a specified manner and at proper distance. The sizes of the pots were 60 cm length, 35 cm width and 27 cm height and filled with 10 kg soil on each pot. Three replications were maintained to test the development of bacterial blight. For each replication three pots were maintained. Basal fertilizers were incorporated at the rate of 60 kg N/ha and 50 kg P/ ha Two top dressings at the rate of 30 kg N/ha were given at tillering and panicle initiation stage of the crop.

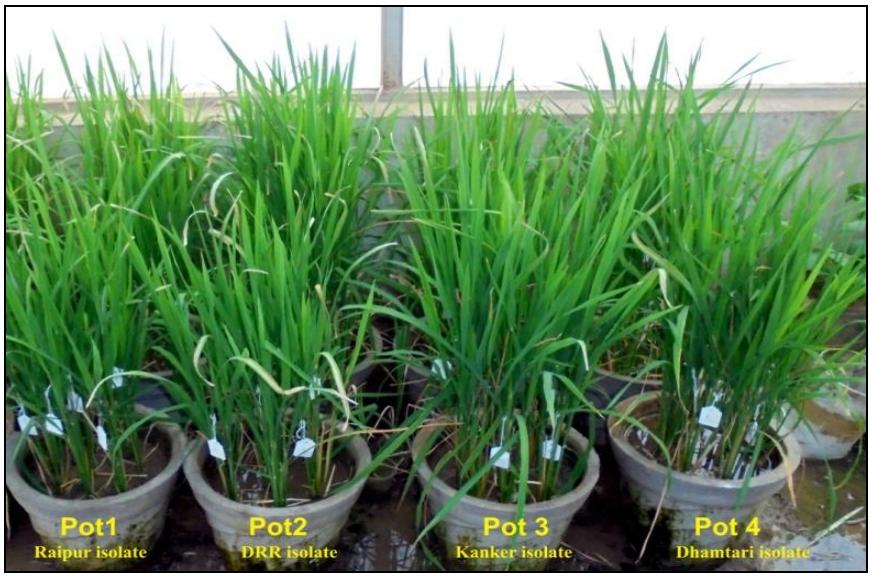


**Fig 1:** Pathogenicity test of the pathogen

Four bacterial isolates from infected leaf of Rice were tested for pathogenicity by inoculation of four-week-old seedlings of cultivar TN1. Bacteria were grown for 72 h on PSA. And the resulting suspension was adjusted turbid metrically to approximately $10^6$ CFU/ml. For each isolate, the top five leaves were inoculated by clip inoculated method each plant 30 days after sowing with 30 ml of bacterial suspension containing $5 \times 10^6$ cfu /ml. After inoculation, plants were kept in a mist chamber with 30 ºC day temperature and 85% RH. Plants inoculated with sterile water served as negative control. The per cent disease developed was recorded after 10 and 21 day of inoculation.

$$\text{Per cent Disease severity (\% DS)} = \frac{\text{Total lesion length}}{\text{Total length of leaf}} \times 100$$

**2.5 Inoculation procedure (Kauffman *et al.* 1973)**

The virulent isolates of *Xanthomonas oryzae* pv. *oryza**e*** was maintained under *in vitro* condition at 15 ºC was utilized for further study of inoculation. The pure culture of the pathogen was sub cultured and used for inoculation by clip inoculation method (Kauffman *et al.* 1973). the seventy two hours old culture of *Xoo* were used for inoculation of plants at seedling, tillering and booting stage. The selected plants each hill were tagged and inoculated gently by clip inoculation method. The plants leaves were clipped (approximately 2 cm from the tip) with scissors predipped in the bacterial inoculums. This procedure was followed in all the artificial inoculation tests, unless otherwise mentioned separately

**2.6 Disease estimation**

The per cent infected area was recorded in study. This was converted to grade as per Standard Evaluation System (SES) (IRRI, 1996) as follows:

**2.7 Disease estimation**

The per cent infected area was recorded in study. This was converted to grade as per Standard Evaluation System (SES) (IRRI, 1996) as follows:

**Table 3:** Disease score for BLB under Standard Evaluation System (IRRI, 1996)

| Score | Percentage of infected leaf area | Reaction |
|---|---|---|
| 1 | 1-5 | Highly Resistant |
| 3 | 6-12 | Resistant (R) |
| 5 | 13-25 | Moderately Resistant (MR) |
| 7 | 26-50 | Susceptible (S) |
| 9 | >50 | Highly Susceptible (HS) |

## 3. Results and Discussion

### 3.1 Testing of the pathogenicity

The pathogenicity of isolated bacterium was tested on the susceptible rice plants by clip inoculation technique. The inoculated rice plants produced water soaked lesion with light pale green to grayish green colour after three days of inoculation. Infected lesions on rice leaves were later became yellow to grey after five days, then it turned to whitish-straw colour from its initial water soaked grayish or yellowish hue in 1-2 weeks. The same pathogen *Xanthomonas oryzae* pv. *oryzae* was re-isolated from these infected rice leaves. The pathogenicity of *Xanthomonas oryzae* pv. *oryzae* on rice plants was also confirmed by Koch's postulates.

The Disease severity varied with the different isolates. Significant variation existed among isolates (Table no 4). Isolates of kanker, Directorate of rice research (Hyderabad) and Raipur were proved the most pathogenic isolates as indicated by the disease severity index of bacterial leaf blight disease among the all isolates the highest disease severity index (80.22%) was provided by the isolate of DRR, followed by the disease severity of Raipur isolate (74.09) and Kanker (66.25%) after two weeks of post inoculation, while Dhamtari isolates showed lowest disease severity (62.63) as the weakest pathogenic ones. One of four bacterial isolates (DRR) was selected for further investigations. Re-isolation from the artificially inoculated plants revealed isolates similar to the original ones. In this study, four isolates of *X. oryzae* pv. *oryzae*, originally isolated from diseased rice plants grown in mist chamber.

**Table 4:** Comparision of the disease severity of bacterial leaf blight isolates on 21 days of inoculation

| Isolates | leaf length | lesion length | % Disease severity |
|---|---|---|---|
| DRR | 42.93 | 34.44 | **80.22** |
| Raipur | 38.60 | 28.59 | **74.09** |
| Kanker | 42.53 | 28.17 | 66.25 |
| Dhamtari | 37.20 | 23.30 | 62.63 |

**Table 5:** Percent Disease severity of four bacterial isolates of *Xanthomonas oryzae pv. oryzae* collected from different geographical area on NIL's and pyramid lines after inoculation

| Designation | Raipur Isolates | | | | DRR Isolates | | | | Dhamtari isolate | | Kanker isolates | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Kharif 2014 | | Rabi 2015 | | Kharif 2014 | | Rabi 2015 | | Rabi 2015 | | Rabi 2015 | |
| | 10 DAI | 20 DAI | 10 DAI | 20 DAI | 10 DAI | 20 DAI | 10 DAI | 20 DAI | 10 DAI | 20 DAI | 10 DAI | 20 DAI |
| IRBB-1 | 12.02 | 48.06 | 52.52 | 94.20 | 34.4 | **57.8** | 49.21 | 100 | 12.59 | 40.45 | 23.64 | 68.21 |
| IRBB-3 | 9.12 | 56.08 | 38.97 | 94.90 | 21.2 | 50.6 | 38.16 | 86.26 | 7.0 | 28.02 | 30.49 | 49.64 |
| IRBB-4 | 11.89 | 54.55 | 23.53 | 70.60 | 29.6 | 49.7 | 35.60 | 87.12 | 4.60 | 21.27 | 23.43 | 88.28 |
| IRBB-5 | 19.54 | 49.67 | 36.67 | 81.30 | 27.4 | 45.2 | 34.26 | 83.22 | 5.84 | 20.77 | 22.06 | 97.93 |
| IRBB-7 | 5.19 | 46.75 | 27.95 | 82.60 | 19.5 | 58 | 30.86 | 54.32 | 6.25 | 23.1 | 13.81 | 31.57 |
| IRBB-8 | 4.69 | 11.72 | 25.19 | 48.90 | 7.3 | 26.3 | 26.57 | 51.75 | 8.95 | 20.89 | 31.49 | 65.35 |
| IRBB-10 | 5.14 | 5.87 | 8.70 | 22.40 | 8.28 | 15.9 | 37.41 | 64.75 | 6.41 | 14.86 | 5.13 | 12.32 |
| IRBB-11 | 4.09 | 11.01 | 28.68 | 61.20 | 20.8 | 40.3 | 26.47 | 56.62 | 9.05 | 18.84 | 22.95 | 41.80 |
| IRBB-13 | 5.02 | 35.91 | 45.38 | 76.90 | 8.55 | 24.3 | 39.2 | 76 | 7.46 | 15.67 | 29.68 | 53.90 |
| IRBB-14 | 23.08 | 66.43 | 42.44 | 90.10 | 18.2 | 42.7 | 41.13 | 82.91 | 7.23 | 18.23 | 18.60 | 40.69 |
| IRBB-50 | 11.03 | 73.53 | 39.26 | 74.80 | 21.3 | 55.1 | 31.97 | 59.86 | 3.79 | 10.34 | 27.51 | 64.42 |
| IRBB-52 | 1.23 | 4.73 | 2.33 | 7.67 | 14.4 | 42.8 | 4.06 | 20.63 | 2.73 | 11.64 | 3.44 | 15.17 |
| IRBB-53 | 1.64 | 5.29 | 5.43 | 14.10 | 11.8 | 22.5 | 4.28 | 16.79 | 1.87 | 4.66 | 3.26 | 5.79 |
| IRBB-54 | 2.44 | 4.38 | 20.80 | 39.40 | 4.69 | 8.44 | 5.06 | 17.91 | 5.03 | 20.46 | 5.03 | 11.40 |
| IRBB-55 | 4.90 | 60.14 | 25.00 | 48.70 | 9.44 | 43.4 | 23.82 | 40.27 | 3.43 | 11.06 | 28.05 | 62.58 |
| IRBB-56 | 2.87 | 8.91 | 15.49 | 34.20 | 8.14 | 14.7 | 6.53 | 18.08 | 2.53 | 6.52 | 8.27 | 18.70 |
| IRBB-57 | 1.36 | 3.23 | 12.50 | 27.20 | 4.66 | 12.2 | 8.59 | 19.14 | 4.63 | 8.33 | 4.68 | 13.28 |
| IRBB-58 | 1.09 | 2.02 | 5.07 | 22.10 | 5.81 | 11.2 | 4.88 | 15.79 | 2.31 | 4.96 | 3.70 | 9.62 |
| IRBB- 59 | 10.97 | 36.13 | 26.97 | 73.00 | 33.5 | 74.7 | 32.28 | 81.1 | 10.71 | 27.85 | 26.95 | 67.37 |
| IRBB-60 | 5.04 | 23.53 | 29.32 | 66.90 | 7.14 | 18.1 | 29.28 | 58.57 | 5.82 | 10.95 | 28.82 | 72.97 |
| DB- 85 | 10.64 | 21.78 | 25.66 | 49.30 | 24.3 | 41 | 27.09 | 52.26 | 7.69 | 23.37 | 9.49 | 25.13 |
| TN-1 | 33.33 | 48.52 | 44.76 | 69.40 | 23.0 | 51.7 | 23.40 | 50.61 | 24.46 | 59.46 | 44.28 | 68.92 |
| AJYA | 3.65 | 9.47 | 6.15 | 14.30 | 4.56 | 9.47 | 2.41 | 5.83 | 2.37 | 6.47 | 23.80 | 57.14 |

### 3.2 Reactions of Near-Isogenic Lines to Isolates of *X. oryzae pv. oryzae*.

Interactions between near-isogenic lines and isolates of *X. oryzae pv. oryzae* are shown in (Table 6.) The degree of disease reaction in this study showed different relationship between combination of rice varieties and bacterial isolates. Among the near isogenic lines containing single gene for resistance, IRBB-4, IRBB-5, IRBB-7, IRBB-8, IRBB-10, IRBB-11, IRBB-13, and IRBB-14 carrying resistant genes *Xa4 xa 5, Xa7, Xa8, Xa10, Xa11, Xa 13* and *Xa14* showed moderately resistant to Dhamtari isolate with Range from 14 to 20 % disease severity after twenty days of inoculation. IRBB-10 carrying *Xa10* resistant gene for *Xanthomonas oryzay pv. Oryzae* express resistant to Raipur and Kanker isolate while moderate resistant to DRR and Dhamtari isolate and IRBB-8 and IRBB-11 expressed resistant to only Raipur isolate after twenty days of inoculation with percent disease severity 11 %, TN1 Cultivar (may be has not major functional gene for resistance to Chhattisgarh isolates) was used as susceptible check. However, None of NILs were showing highly resistant to isolates of *Xoo* in Chhattisgarh.

**Table 6:** Reaction of near-isogenic lines to Isolates of *X. oryzae pv. oryzae*.collected from different geographical area after twenty days of inoculation'

| S.N. | Designation | Resistance gene (s) | Raipur Isolate | | DRR Isolate | | Dhamtari Isolate | Kanker Isolate |
|---|---|---|---|---|---|---|---|---|
| | | | kharif 2014 | Rabi 2015 | kharif 2014 | Rabi 2015 | Rabi 2015 | Rabi 2015 |
| 1 | IRBB-1 | *Xa 1* | S | HS | HS | HS | S | HS |
| 2 | IRBB-3 | *Xa 3* | HS | HS | S | HS | S | HS |
| 3 | IRBB-4 | *Xa 4* | HS | HS | S | HS | MR | HS |
| 4 | IRBB-5 | *xa 5* | S | HS | S | HS | MR | HS |
| 5 | IRBB-7 | *Xa 7* | S | HS | HS | HS | MR | S |
| 6 | IRBB-8 | *Xa 8* | R | HS | S | HS | MR | HS |
| 7 | IRBB-10 | *Xa 10* | R | S | MR | HS | MR | R |
| 8 | IRBB-11 | *Xa 11* | R | HS | S | HS | MR | S |
| 9 | IRBB-13 | *xa 13* | S | HS | MR | HS | MR | HS |
| 10 | IRBB-14 | *Xa 14* | HS | HS | S | HS | MR | S |
| 11 | TN-1 | | S | HS | HS | HS | HS | HS |
| 12 | AJYA | | R | MR | R | R | R | HS |

### 3.3 Reaction of Pyramiding Lines to Isolates of *X. oryzae pv. oryzae*.

Interactions of Bacterial isolates and pyramiding lines expressed in (Table 7 )Rice Pyramids lines expressing highly resistant reaction against more than one isolates collected from different geographical locations The isolates (derived from Raipur Dhamtari) based on their interaction with the Pyramids containing different gene(s) in combination ( *Two gene: Xa4 +Xa21, xa5 + Xa 21, xa5 + xa13, Three gene: Xa4 + Xa13 +Xa21, Xa4 + xa5 + Xa21)* was speculated to contain *avrXa4, avrxa13, avrXa 21* genes in Dhamtari isolate where as *avrXa4, avrxa5, avrxa13, avrXa 21* genes in Raipur isolate..

**Table 7:** Reaction on pyramiding lines to Isolates of *X. oryzae pv. Oryzae* collected from different geographical area of chhattisgarh

| S.N. | Designation | Resistant genes | Raipur Isolate | | DRR Isolate | | DhamtariIsolate | Kanker Isolate |
|---|---|---|---|---|---|---|---|---|
| | | | kharif 2014 | Rabi 2015 | kharif 2014 | Rabi 2015 | Rabi 2015 | Rabi 2015 |
| 1 | IRBB-50 | *Xa 4 + xa 13* | HS | HS | HS | HS | R | HS |
| 2 | IRBB-52 | *Xa 4 + Xa 21* | HR | R | S | MR | R | R |
| 3 | IRBB-53 | *xa 5 + xa 13* | R | MR | MR | MR | HR | R |
| 4 | IRBB-54 | *xa 5 + Xa 21* | HR | S | R | MR | MR | R |
| 5 | IRBB-55 | *xa 13 + Xa 21* | HS | S | S | S | R | HS |
| 6 | IRBB-56 | *Xa 4 + xa 5 + xa 13* | R | S | MR | MR | R | MR |
| 7 | IRBB-57 | *Xa 4 + xa 5 + Xa 21* | HR | S | R | MR | R | MR |
| 8 | IRBB-58 | *Xa 4 +xa 13 + Xa 21* | HR | MR | R | MR | HR | R |
| 9 | IRBB- 59 | *xa 5 +xa 13 + Xa 21* | S | HS | HS | HS | S | HS |
| 10 | IRBB-60 | *Xa4+xa5+xa13+Xa21* | S | HS | MR | HS | R | HS |
| 11 | DB- 85 | *xa5 +Xa7* | S | HS | S | HS | MR | MR |

Among the pyramiding line containing two to four resistance gene IRBB-52 and IRBB-54 having two gene combination *Xa 4 + Xa 21 and xa 5 + Xa* 21 and IRBB-57 and IRBB-58 having three gene combination *Xa 4 + xa 5 + xa 13 and Xa 4 +xa 13 + Xa 21* express highly resistant reaction to Raipur isolate Kharif 2014 with 4.73, 4.38 3.23 and 2.02 percent disease severity respectively and express moderate resistant to resistant reaction to the most of isolates collected from different geographical location of Chhattisgarh. Maheshwari Cultivar (maybe has major functional gene for resistance to Chhattisgarh isolate) was used as resistant check. High level of resistance was observed in combination of three and four dominant resistance genes *Xa4+Xa21, xa5 +xa13, xa5 + Xa21, Xa4 + xa5 + Xa21 and Xa4 + xa13 +Xa 21,* (IRBB52, IRBB53, and IRBB54, IRBB57, and IRBB58 respectively ). Rice NILs and Pyramids expressing resistant reaction against more than one isolates collected from different geographical locations **(**Table 7). Rice pyramid lines containing two gene combinations IRBB-52 (*Xa4 + Xa21),* IRBB-54*(xa5 + Xa21)* and three gene combinations IRBB-56 (*Xa4 + xa5 + xa13),* IRBB-58 *(Xa4 + xa13 +Xa 21),* expressed resistance on challenge inoculation with different isolates collected from different geographical location of Chhattisgarh. The overall results indicated that resistance gene(s) behave differentially against different isolates. The combination of genes some time exhibited additive resistance while non-additive in some combination and against different *Xoo* cultures

Bacterial leaf blight (BB), caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*), is a devastating disease in the rice growing countries of Asia. Infection at maximum tillering stage results in blighting of leaves, which eventually causes significant yield losses in severely infected fields ranging from 20 to 30%, but this can reach as high as 80% (Mew *et al.* 1992; Noh *et al.* 2007; Shin *et al.* 1992). Korean BB isolates have been grouped into five races (K1 to K5) by using five rice cultivars as the *Xoo* differential system (Yun *et al.* 1985). Recent pathotyping results indicated that the Korean race K1 has shown a decreasing trend in infection by the spread of rice cultivars with Xa1 and Xa3 genes, whereas races K2 and K3 have increased their pathogenicity in Korea (Kim *et al.* 2009; Noh *et al.* 2007; Shin *et al.* 1992). Most of the japonica cultivars possess Xa1 or Xa3 or Xa4 genes for BB resistance, but these genes are showing susceptibility to the new BB strains of Korea (Jeung *et al.* 2006; Kim *et al.* 2009; Shin *et al.* 2011). A new BB race, K3a, that evolved recently caused serious damage to rice production in the southwestern coastal areas of Korea in 2003 (Noh *et al.* 2003). Moreover, BB disease is spreading to all regions of Korea because of the effect of climate change and it is causing genetic vulnerability in modern cultivars. Therefore, rice yield has declined and grain quality has decreased by the infection of bacterial blight (Noh *et al.* 2007; Shin *et al.* 1992). Breeding and the

development of resistant cultivars carrying major resistance (R) genes have been the most effective and economical strategy to control BB disease to have a neutral effect on the environment (Huang *et al.* 1997; Jena and Mackill, 2008; Singh *et al.* 2001). Qualitative resistance, which confers major gene-specific resistance against some pathogen races, is the easiest to incorporate into breeding programs and is usually considered a gene-for-gene type of resistance. For many pathogens and insects, this type of qualitative resistance is not often durable because of rapid changes in the virulence in the pathogen or biotype of the population (Leach *et al.* 2007). As a result, increasing attention has focused on the accumulation of major disease resistance genes in crop plants. Pyramided lines carrying two, three or four bacterial blight resistance genes showed broad spectrum and higher resistance than the lines with a single resistance gene (Gu *et al.* 2005; Jeung *et al.* 2006; Kim *et al.* 2009; Singh *et al.* 2001; Suh *et al.* 2009a). However, conventional breeding methods to improve rice cultivars for BB resistance have not found much success (Shin *et al.* 2011). To date, at least 38 BB resistance genes conferring host resistance against various strains of *Xoo* have been identified (Bhasin *et al.* 2012; Natrajkumar *et al.* 2012). All these resistance genes follow a Mendelian pattern of major gene inheritance and express resistance to a diverse group of *Xoo* pathogens (Cheema *et al.* 2008; Gu *et al.* 2005; Korinsak *et al.* 2009; Lee *et al.* 2003; Several of these genes have already been incorporated into rice cultivars, which are now widely cultivated in many countries (Huang *et al.* 1997; Singh *et al.* 2001; Sundaram *et al.* 2008). Of the 38 R genes, six are physically mapped (*Xa2, Xa4, Xa7, Xa30, Xa33* and *Xa38*) and six are cloned (*Xa1, xa5, Xa13, Xa21, Xa26 + Xa3* and *Xa27*) (Bhasin *et al.* 2012; Cheema *et al.* 2008; Gu *et al.* 2005; Liu *et al.* 2006; Natrajkumar *et al.* 2012; Song *et al.* 1997; Yang *et al.* 1998). BB resistance gene *Xa4* is one of the most widely exploited resistance genes in many rice breeding programs and it confers durable resistance in many commercial rice cultivars (Mew *et al.* 1992; Sun *et al.* 2003). The *Xa21* gene was identified in the wild species *Oryza longistaminata* and is highly effective against BB races of South and Southeast Asia (Khush *et al.* 1990). The *xa5* gene, which is naturally found only within the Aus subpopulation of rice (Garris *et al.* 2003), provides recessive resistance to several *Xoo* races of the Philippines.

### 3.4 Performance of common cultivars under Chhattisgarh condition

The field performance of resistant / tolerant varieties reported in one or other place were included in the evaluation under artificial inoculated condition during 2014 in *Rainy* season (Table 8).

The field performance of resistant/ tolerant varieties reported in one or other place were included in the evaluation under artificial inoculation condition during 2014 in *Rainy* season years. Among all the varieties only one variety were recorded resistant i.e. maheshwari, with percent disease severity 10.0%, three varieties i.e. Bamleshwari, Tapaswani and Swarna were recorded moderately resistant with (23.8%, 22.0%, 21.9% ) respectively. eight variety susceptible Pant -4, Durgeshwari, Chepti, HR-12, Jawaphool, Mahamaya, MTU 1010, and shyamala with above 26% and five varieties were found highly susceptible, after 21 days of inoculation.

Agrawal and Philip (1982); Kotasthane and Agrawal (1991) and Agrawal (2000) also evaluated the rice entries/cultivars against the bacterial blight disease variation in disease severity was also reported during the last 20 years on some popularly grown varieties in this state.

**Table 8:** Bacterial blight score in cultivars grown

| S.N. | Varities | % DS | Score | Reaction | S.N | Varities | % DS | Score | Reaction |
|---|---|---|---|---|---|---|---|---|---|
| 1 | TN-1 | 75.3 | 9 | HS | 10 | Jawaphool | 38.5 | 7 | S |
| 2 | H.M.T | 73.6 | 9 | HS | 11 | Maheshwari | 10.0 | 1 | R |
| 3 | Pant -4 | 34.7 | 7 | S | 12 | Safri- 17 | 54.4 | 9 | HS |
| 4 | Bamleshwari | 23.8 | 3 | MR | 13 | Mahamaya | 32.5 | 7 | S |
| 5 | IR-64 | 50.5 | 9 | HS | 14 | Karanti | 55.7 | 9 | HS |
| 6 | Durgeshwari | 46.1 | 7 | S | 15 | shyamala | 26.7 | 7 | S |
| 7 | Tapaswani | 22.0 | 3 | MR | 16 | MTU 1010 | 32.2 | 7 | S |
| 8 | Chepti | 29.2 | 7 | S | 17 | Swarna | 21.9 | 3 | MR |
| 9 | HR-12 | 38.2 | 7 | S | | | | | |

The major aims of this study was to develop rice cultivars with effective resistance genes to *Xanthomonas oryzae pv. oryzae*. In this study, we evaluated the level of resistance to the bacterial blight pathogen conferred by a single gene individually and multiple gene combinations. Previous results on the basis of host-pathogen interaction showed that there were four bacterial leaf blight isolates in Chhattisgarh. These Isolates were tested on 10 international differentials of near-isogenic lines and 10 varieties of pyramided lines and 17 local and improved varieties to support a gene deployment approach to managing the disease using resistant cultivars. The phenotype reactions between the single-gene lines and isolates were clear and easily classified into virulence or avirulence patterns. Among 10 near-isogenic lines with single gene, IRBB1 (Xa1) was low resistance and IRBB10 (Xa10) was high resistance on the basis of percentages of the compatible strains, also IRBB10 (Xa10) was not resistance to all tested isolates. Among common varieties were tested in this study. Local varieties were more susceptible than improved varieties to leaf blight disease. these results indicate that IRBB8 (Xa8), IRBB11 (Xa11) and especially IRBB10 (Xa10) was resistant to the most isolates. Among local varieties, H.M.T., IR 64, Kranti,Safri- 17 was the most susceptible. Hence it was concluded that, Xa8, Xa10 and Xa11 were the durable mono genes that expressed under this agro-climatic region, to combat the disease. the pyramided lines showed high and broad spectra of resistance against most of the Xoo isolates (Le *et al*., 2006). A quantitative complementation among the genes might also have taken place. Therefore, these genes should be induced for bacterial blight resistance breeding programme against all the pathotypes. Adhikari *et al*. (1999) showed that, pyramided lines have displayed higher levels and or wider spectra of resistance to bacterial blight than parental NILs with single resistance genes, suggesting synergism and complementation among resistant genes. Our results also confirmed with the findings of Adhikari *et al*. (1999). The rest NILs showed susceptible to moderately susceptible reaction to all the isolates, therefore these genes were unable to prevent the attack of different pathotypes in the present case. NILs were

the best material to study pathogenecity and value of resistance genes. Pyramided lines were found to be most effective to combat bacterial blight here. So, for breeders' perspective, gene pyramiding was proved to be novel way to achieve a solution against the occurrence of the disease. Besides, some single gene resistance was also found to be effective for agro-climatic region of Chhattisgarh.

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