

# Measurement of Phenotypic Variation for Control and Bacterial Leaf Blight Inoculated Rice Lines and Varieties

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**Abstract:** Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is a devastating disease of rice, the staple food crop of Bangladesh. Under field condition the disease significantly damages the phenotypic characteristics of the plants affecting the yield. An assessment of the phenotypic variations due to the disease in 10 rice lines and 17 rice varieties were studied at the maximum tillering stage in both natural and induced epiphytotic conditions. Along with the indigenous cultivars the test materials including the check resistant and susceptible cultivars were collected from IRRI. Significant differences in both plant height and the number of effective tillers were recorded in the check and inoculated lines and varieties. Among the rice lines, RC222 resulted the maximum reduction in plant height (3.18%) in the inoculated plants whereas RC217 yielded the minimum reduction of 1.27%. Among the rice varieties, Binadhan-8 and Binadhan-10 showed the maximum reduction in plant height (11.43% and 18.22% respectively) for the inoculated plants where IRBB21, IRBB65 and Kumragur resulted 0% reduction. The number of effective tiller among the rice lines, RC251 yielded the maximum reduction of 26.19% in the inoculated plants whereas RC192 showed as minimum as 4.16% reduction. For rice varieties, Binadhan-8, Binadhan-10 and BR-11 yielded the maximum reduction of 52.62%, 50.07% and 50.06% while IRBB21, IRBB65 and Kumragur showed 0 % reduction. The findings of such phenotypic variations may contribute significantly to the selection of best lines and further research to develop the disease free commercial varieties of rice against the disease.

**Keywords:** Bacterial Leaf Blight, Phenotype, Resistance, Plant Height, Tiller Number

## 1. Introduction

Bacterial leaf blight (BLB) is a destructive disease of rice which is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo.) [1]. This disease is mostly common in rice growing countries in Asia specially where there is irrigated and rainfed lowland [2]. BLB can reduce grain production by 20-50% [3] by affecting the panicle emergence and grain filling. The yield loss can go up to 80% if severely affected which depends on the crop stage, environmental condition and susceptibility [4-7]. Besides different control measures, development of

resistant cultivar by breeding technique is the best cost-effective method to approach the disease [8, 9].

At the early period of the infection, at the leaf tips lesions are observed. These lesions become yellowish and turn into irregular lesions with wavy margins covering the whole leaf blade with wavy margin at the later stage [4]. As a result of the infection immature and sterile and poor quality grains are produced [10].

During vegetative stage by the phenotypic characteristics of the plant yield loss can be predicted. If the phenotypic characteristics like plant height, effective tiller number etc. of

the plant shows abnormality from the ideal plant characteristics it can be assumed that the plant will not give expected yield. Also, percent infected leaf area and growth stage directly affect the yield production of rice [7]. Moreover, BLB disease clearly shows this kind of phenotypic symptoms where all the plant become diseased and burned and show less effective tiller number with less grain production [11, 12].

In this study, an experiment was conducted to ten rice lines and seventeen rice varieties by inoculating with bacterial strain BXO-09 (*Xoo.*) responsible for BLB disease with an objective to assess the reduction of plant height and tiller number in these rice lines and varieties. For the selection of best rice lines to develop as commercial variety this result can contribute

significantly. Moreover, this result may also contribute towards understanding the effect of BLB on the phenotype of the rice plant in terms of plant height and tiller number.

## 2. Materials and Methods

### 2.1. Experimental Setup

In the experimental field of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA) the study was conducted. Different rice lines and varieties were taken for the study (Table 1) where the variety IRBB21 and BR-11 were considered as the resistant and susceptible cultivars for check [13-15] respectively.

**Table 1.** List of lines and varieties of rice used for the study.

| Type    | Name of the test material  | Source of Collection |
|---------|--|----------------------|
| Line    | RC191, RC192, RC193, RC217, RC221, RC222, RC225, RC229, RC251, RC249                           | BINA                 |
| Variety | BR-10, BR-11, BR-14, BR-16, BR-26, BRRI Dhan31, BRRI Dhan32, IRBB 5, IRBB 21, IRBB 60, IRBB 65 | BRRI                 |
| Variety | Binadhan-7, Binadhan-8, Binadhan-10, Binadhan-11, Binadhan-12                                  | BINA                 |
| Variety | Kumragur   | Gazipur              |

BINA= Bangladesh Institute of Nuclear Agriculture; BRRI = Bangladesh Rice Research Institute

### 2.2. Preparation of Pre-germinated Seeds for Sowing

Collected seeds were heat-treated in an oven set for 5 days at 50°C to break the seed dormancy. The seeds were then soaked in tap water for 24 hours at room temperature, washed and rinsed properly with tap water, placed on petri dishes with moistened filter papers and incubated for 48 hours at 30°C to germinate.

### 2.3. Preparation of Soil Tray

Soil trays were prepared for raising the germinated seeds successfully. The trays were first filled up 1/3 portion with soil and then these were puddle carefully until the soil was soft enough to grow a germinated seed successfully. The required amount of fertilizers and manures were applied time to time.

### 2.4. Sowing of Seeds and Maintenance of Seedlings

Twenty germinated seeds for each lines and varieties were sown in trays following line sowing method. The young seedlings were nursed carefully by following agronomic management practices time to time.

### 2.5. Preparation of Bacterial Inoculum and Inoculation of Rice Plant

BXO-09, the most virulent strain in Bangladesh [16] was collected from Bangladesh Rice Research Institute. This isolate was maintained and grown on peptone sucrose agar (PSA) medium for 72 hours at 30°C. Then, by mixing the cultured bacteria with 10 ml sterile distilled water, the inoculum was prepared. Prior to inoculation using sterile distilled water, the concentration of bacterial suspension was adjusted to 10<sup>8</sup> CFU/ml. After preparing the bacterial inoculum, the inoculation of rice seedlings was done

following clipping method [17]. After 21 days of inoculation, data was taken for the measurement of phenotypic variation.

### 2.6. Measurement of Phenotypic Variation

Two types of data, plant height and total tillers and effective tillers/hill, were taken in case of measuring the phenotypic variation of rice plant caused due to BLB infection. All these data were taken both from the infected rice plants and uninfected/control plants. Plant height was taken from the ground level to the tip of the main stem at maximum tillering stage of the plant and was expressed in cm. For the measurement of total tillers and effective tillers/hill, total tiller numbers in a hill were counted then total effective tiller numbers in a hill were checked at maximum tillering stage.

## 3. Results and Discussion

### 3.1. Plant Height

At maximum tillering stage, significant difference in plant height between control and BLB inoculated rice lines and varieties were observed. Among the rice lines RC222 showed maximum reduction in plant height for BLB inoculated plants although it was only 3.18 % whereas RC217 showed the minimum reduction in plant height (Table 2). In case of RC192, RC193 and RC251 average reduction in plant height were observed (Fig. 1). It indicates that RC222 may have the maximum effect on bacterial leaf blight disease and maximum reduction in yield may happen in case of RC222. This result is consistent with our previous work of screening of BLB resistant rice lines which showed that RC222 is moderately susceptible line [18] which may be the reason of showing much effect on plant height in this study.

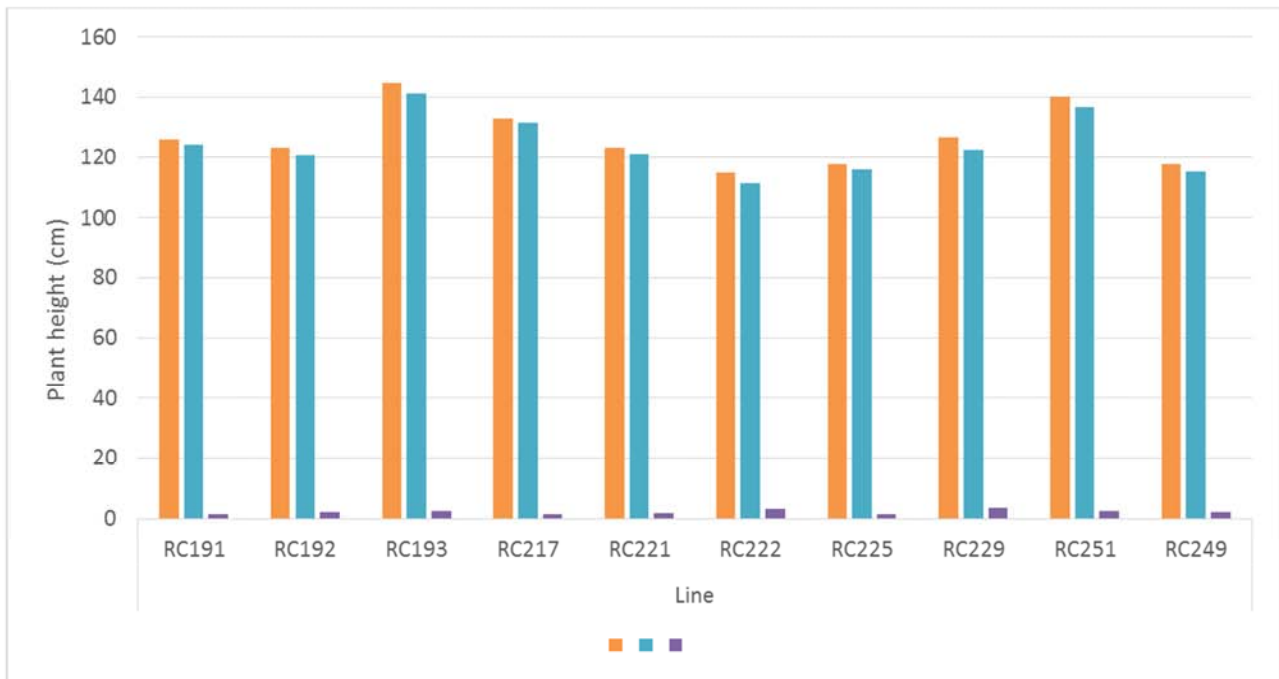


Figure 1. Graphical view of plant height variation between control and BLB inoculated rice lines.

Table 2. Measurement of plant height for control and BLB inoculated rice lines.

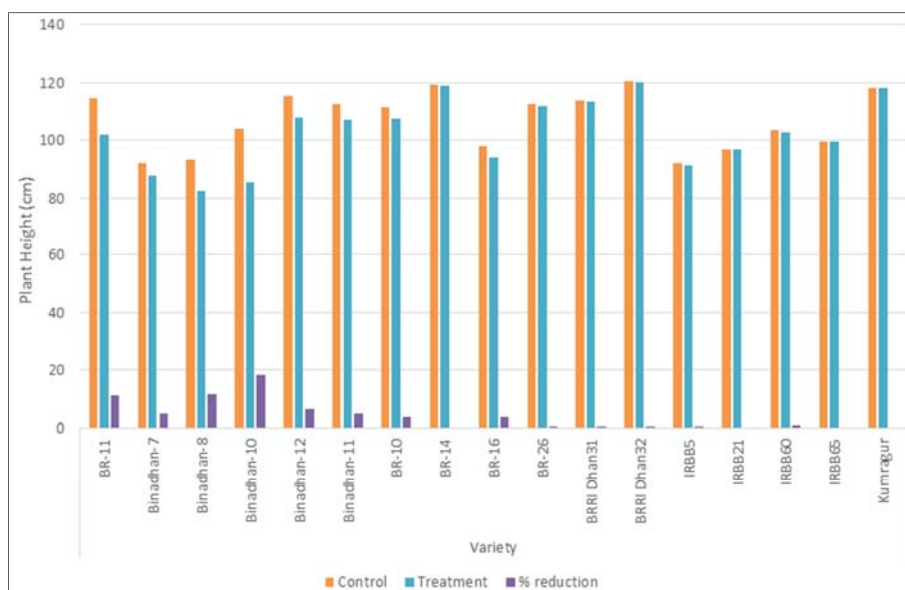
| Line                  | Plant height (cm) |           |             |
|-----------------------|-------------------|-----------|-------------|
|                       | Control           | Treatment | % reduction |
| RC191                 | 126.0 cd          | 124.3 bcd | 1.293 e     |
| RC192                 | 123.3 cd          | 120.7 cd  | 2.220 bc    |
| RC193                 | 144.7 a           | 141.3 a   | 2.303 bc    |
| RC217                 | 133.0 abc         | 131.3 abc | 1.277 e     |
| RC221                 | 123.0 cd          | 121.0 cd  | 1.637 de    |
| RC222                 | 115.0 d           | 111.3 d   | 3.180 a     |
| RC225                 | 117.7 d           | 116.0 d   | 1.283 e     |
| RC229                 | 126.7 bcd         | 122.3 cd  | 3.407 a     |
| RC251                 | 140.2 ab          | 136.7 ab  | 2.507 b     |
| RC249                 | 117.7 d           | 115.3 d   | 1.913 cd    |
| LSD (0.05)            | 13.06             | 12.84     | 0.539       |
| Level of significance | **                | **        | **          |

At maximum tillering stage, significant difference in plant height between control and BLB inoculated varieties were also observed. Binadhan-8, BR11 and Binadhan-10 showed maximum reduction in plant height (11.43%, 11.47 and 18.22% subsequently) for BLB inoculated plants where IRBB21, IRBB65 and Kumragur showed 0% reduction from control condition (Table 3). In case of Binadhan-7, Binadhan-11, BR-10 and BR-16, they performed moderate reduction for BLB inoculated rice plants compared to its control condition (Fig. 2). This result is consistent with our previous study which showed that Binadhan-8 and BR11 are the highly susceptible variety [18] and thus may have ultimate

effect on plant height and yield generation. Similar findings were observed in another study of Jeyalakshmi *et al.* (2010) where it was observed that plant height significant varies due different treatments and BLB resistance for that which has direct impact on plant height [12].

Table 3. Measurement of plant height for control and BLB inoculated rice varieties.

| Variety               | Plant height (cm) |            |             |
|-----------------------|-------------------|------------|-------------|
|                       | Control           | Treatment  | % reduction |
| BR-11                 | 114.7 ab          | 101.7 de   | 11.47 b     |
| Binadhan-7            | 92.00 f           | 87.33 ghi  | 5.070 cd    |
| Binadhan-8            | 93.00 ef          | 82.33 i    | 11.43 b     |
| Binadhan-10           | 104.0 bcd         | 85.00 hi   | 18.22 a     |
| Binadhan-12           | 115.3 a           | 108.0 bcd  | 5.783 c     |
| Binadhan-11           | 112.7 abc         | 107.3 bcd  | 4.713 cd    |
| BR-10                 | 111.7 abc         | 107.7 bcd  | 3.567 cd    |
| BR-14                 | 119.3 a           | 119.0 a    | 0.2767 d    |
| BR-16                 | 97.67 def         | 94.00 efgh | 3.707 cd    |
| BR-26                 | 112.7 abc         | 112.0 abc  | 0.590 d     |
| BRR1 Dhan31           | 114.0 abc         | 113.3 ab   | 0.5967 d    |
| BRR1 Dhan32           | 120.7 a           | 120.0 a    | 0.5467 d    |
| IRBB5                 | 91.67 f           | 91.00 fghi | 0.703 cd    |
| IRBB21                | 96.67 def         | 96.67 efg  | 0.0000 d    |
| IRBB60                | 103.3 cde         | 102.3 cde  | 0.9533 cd   |
| IRBB65                | 99.33 def         | 99.33 def  | 0.0000 d    |
| Kumragur              | 118.3 a           | 118.3 a    | 0.0000 d    |
| LSD (0.05)            | 9.57              | 8.79       | 4.46        |
| Level of significance | **                | **         | **          |



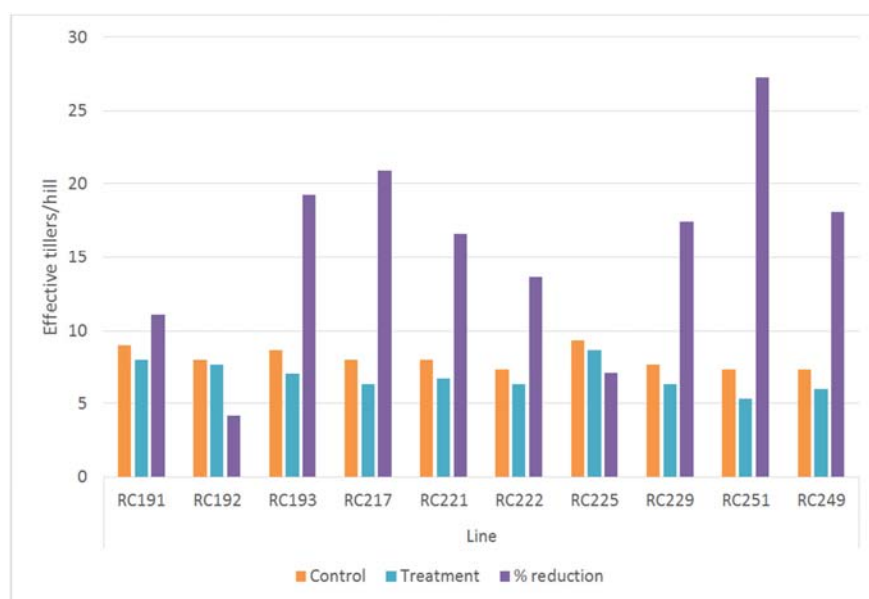
**Figure 2.** Graphical view of plant height variation between control and BLB inoculated rice varieties.

### 3.2. Effective Tillers/Hill

At maximum tillering stage, significant difference in effective tiller number between control and BLB inoculated rice lines/varieties was observed. Among the rice lines RC251 showed maximum reduction (26.19%) (Table 4) in effective tiller number for BLB inoculated plants whereas RC192 showed minimum reduction (4.16%) in effective tiller number (Fig. 3). It indicates that RC222 may have the maximum effect on bacterial leaf blight disease and maximum reduction in yield may happen in case of RC222. This result shows consistency with our previous work of screening of BLB resistant rice lines which described RC222 as a moderately susceptible rice line [18] and may be due to this, line RC222 has much effect on plant effective tiller number for BLB in this study.

**Table 4.** Measurement of tiller number for control and BLB inoculated rice lines.

| Line                  | Effective tillers/hill |           |             |
|-----------------------|------------------------|-----------|-------------|
|                       | Control                | Treatment | % reduction |
| RC191                 | 9.000 ab               | 8.000 ab  | 11.20 d     |
| RC192                 | 8.000 bcd              | 7.667 bc  | 4.167 f     |
| RC193                 | 8.667 abc              | 7.000 cd  | 19.16 b     |
| RC217                 | 8.000 bcd              | 6.333 de  | 20.50 b     |
| RC221                 | 8.000 bcd              | 6.667 de  | 16.80 c     |
| RC222                 | 7.333 d                | 6.333 de  | 11.57 d     |
| RC225                 | 9.333 a                | 8.667 a   | 7.037 e     |
| RC229                 | 7.667 cd               | 6.333 de  | 17.26 c     |
| RC251                 | 7.333 d                | 5.333 f   | 26.19 a     |
| RC249                 | 7.333 d                | 6.000 ef  | 20.37 b     |
| LSD (0.05)            | 1.13                   | 0.871     | 1.7         |
| Level of significance | **                     | **        | **          |



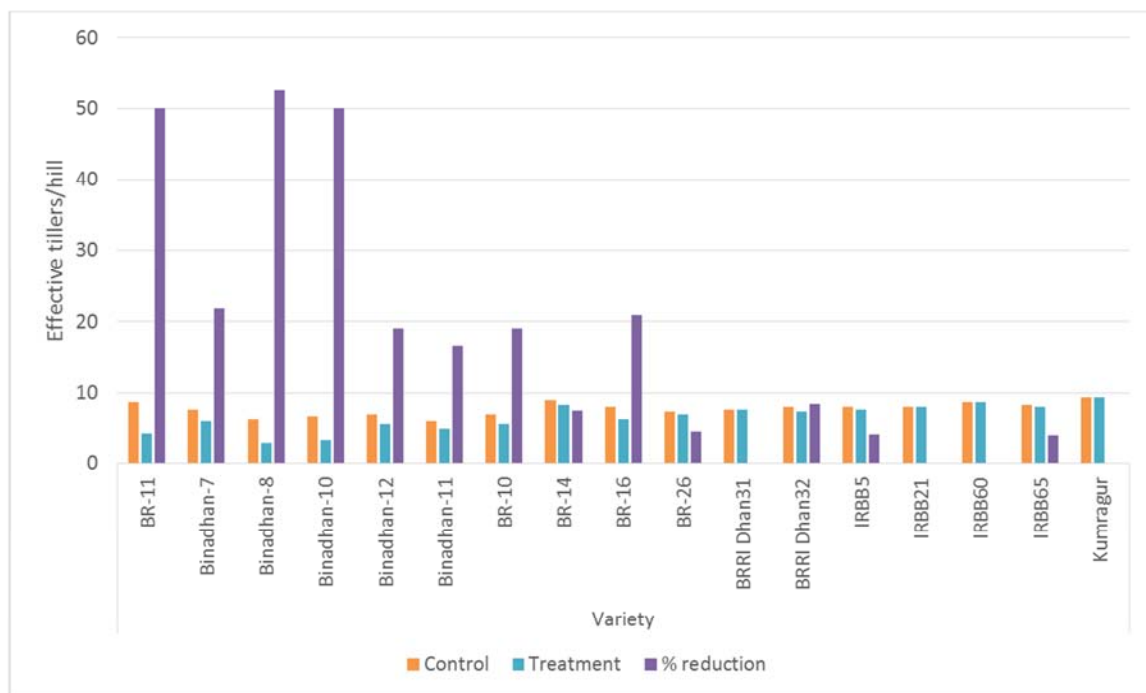
**Figure 3.** Graphical view of effective tiller variation between control and BLB inoculated rice lines.

For the rice varieties, Binadhan-8, Binadhan-10 and BR-11 showed maximum reduction in effective tiller number (52.62%, 50.07% and 50.06% subsequently) for BLB inoculated plants (Table 5) where IRBB21, IRBB65 and Kumragur showed 0% reduction from control condition (Fig. 4). This result is consistent with our previous study which showed that Binadhan-8 and BR11 are the highly susceptible

variety [18] and thus may have ultimate effect on plant effective tiller number and yield generation. Similar finding was resulted by Jeyalakshmi *et al.* (2010) where it was observed that plant effective tiller number varies due different treatments and BLB resistance level of the plant [12].

**Table 5.** Measurement of tiller number for control and BLB inoculated rice varieties.

| Variety               | Effective tillers/hill |            |             |
|-----------------------|------------------------|------------|-------------|
|                       | Control                | Treatment  | % reduction |
| BR-11                 | 8.667 abc              | 4.330 hi   | 50.000 a    |
| Binadhan-7            | 7.667 abcdef           | 6.000 efg  | 21.300 b    |
| Binadhan-8            | 6.333 ef               | 3.000 i    | 51.590 a    |
| Binadhan-10           | 6.667 def              | 3.333 i    | 49.200 a    |
| Binadhan-12           | 7.000 cdef             | 5.667 fgh  | 18.650 bc   |
| Binadhan-11           | 6.000 f                | 5.000 gh   | 15.080 bcd  |
| BR-10                 | 7.000 cdef             | 5.670 fgh  | 17.860 bc   |
| BR-14                 | 9.000 ab               | 8.333 abc  | 7.4070 bcd  |
| BR-16                 | 8.000 abcde            | 6.333 defg | 20.500 b    |
| BR-26                 | 7.330 bcdef            | 7.000 cdef | 4.167 cd    |
| BRR1 Dhan31           | 7.670 abcdef           | 7.667 bcd  | 0.000 d     |
| BRR1 Dhan32           | 8.000 abcde            | 7.333 bcde | 7.870 bcd   |
| IRBB5                 | 8.000 abcde            | 7.667 bcd  | 4.763 cd    |
| IRBB21                | 8.000 abcde            | 8.000 abc  | 0.000 d     |
| IRBB60                | 8.667 abc              | 8.667 ab   | 0.000 d     |
| IRBB65                | 8.333 abcd             | 8.000 abc  | 4.167 cd    |
| Kumragur              | 9.333 a                | 9.333 a    | 0.000 d     |
| LSD (0.05)            | 1.49                   | 1.31       | 13.79       |
| Level of significance | **                     | **         | **          |



**Figure 4.** Graphical view of effective tiller variation between control and BLB inoculated rice varieties.

## 4. Conclusion

Ten IRRI advanced rice lines and seventeen rice varieties were analyzed to assess the reductions in plant height and

effective tiller number due to bacterial leaf blight disease. Rice line RC222 showed maximum reduction in plant height (3.18%) and RC217 showed minimum (1.27%). Also rice variety Binadhan-8 and Binadhan-10 showed maximum reduction (11.43 % and 18.22%) for BLB inoculated plants

where IRBB21, IRBB65 and Kumragur showed 0 % reduction. For effective tiller number rice line RC251 showed maximum reduction (26.19%) and RC192 minimum (4.16%). Rice variety Binadhan-8, Binadhan-10 and BR-11 showed maximum reduction in (52.62%, 50.07% and 50.06% subsequently), where IRBB21, IRBB65 and Kumragur showed 0 % reduction among the BLB inoculated rice plants. This result may have significant impact on yield as the plants lost their photosynthetic area to produce grain. Also as the lines are not released as variety yet, this result also contributes to the selection of best lines to develop as commercial variety.

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