Identification of Blast Disease Resistant Finger Millet \([Eleusine coracana \, (L.) \, Gaertn.]\) RILs Screened Under Natural Hot Spot

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**A B S T R A C T**

Finger millet \([Eleusine coracana \, (L.) \, Gaertn.],\) sub-species coracana, belongs to the family poaceae. Among the several production constraints, blast (neck and finger blast) caused by the fungus \(Pyricularia grisea\) (Cooke) affects different aerial parts of the plant at all plant growth stages. The 360 \(F_4, F_5\) and \(F_6\) RILs derived from the cross PR 202 × GPU 48 were screened for blast disease incidence. Analysis of variance revealed highly significant mean squares attributable to ‘RILs’ and ‘check varieties’ for both neck blast and finger blast incidence among \(F_4, F_5\) and \(F_6\) generations. The observed range was higher for both neck and finger blast in \(F_4, F_5\) and \(F_6\) generations. Low and moderate GCV were observed for neck and finger blast respectively, in \(F_5\) generation, however high GCV and PCV were observed for neck and finger blast in \(F_5\) and \(F_6\) generation. Few RILs were identified which are moderately resistant to neck and finger blast disease.

**Keywords**

Finger millet, Blast, Hotspot, RILs.

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**Introduction**

Finger millet \([Eleusine coracana \, (L.) \, Gaertn.],\) sub-species coracana, belongs to the family poaceae. The cultivated \(E. \, coracana\) is a tetraploids \((2n=4X=36)\) and exhibits morphological similarity to both \(E. \, indica\) \((2n=18)\) and \(E. \, africana\) \((2n=36)\). It is the third most widely cultivated millet after pearl millet \((Pennisetum glaucum)\) and foxtail millet \((Setaria italica)\) in the semi-arid tropical and subtropical regions of the world (Reddy \textit{et al.}, 2009). Finger millet represents one of the critical plant genetic resources for the agriculture and food security of farmers inhabiting arid, infertile and marginal lands (Barbeau and Hilu, 1993). It has excellent nutritional value as its seeds contain 7 – 14\% protein (Barbeau and Hilu, 1993) and is rich in calcium, iron, methionine, phosphorus, carbohydrate and other nutrients (Leung \textit{et al.}, 1968).

Finger millet production is affected by a number of diseases like blast, foot rot, smut, streak and mottling virus (Govindu \textit{et al.}, 1970). Among these, blast disease caused by the fungus \(Pyricularia grisea\) (Cooke) Sacc. (formerly \(Pyricularia oryzae\) Cavara.), an anamorph of \(Magnaporthe grisea\) (Hulbert et
Blast disease in finger millet affects different aerial parts of the plant at all stages of its growth starting from seedling to grain formation with yield losses up to 28 per cent (Viswanath et al., 1986). The blast disease that occur on neck, the region below the ears (neck blast) and fingers of ears (finger blast) are considered as economically important in terms of the magnitude of production losses.

Appearance of brown and subsequent blackening of the area immediately below the ear is an indication of neck blast. An olive grey growth of the fungus is also observed at advanced stages (Patro and Madhuri, 2014). Appearance of brown and chaffy fingers on the ear is an indication of finger blast. Finger blast usually begins from the apical portion and runs toward the base of the finger (Patro and Madhuri, 2014).

Frequent and prolonged dry periods with cool temperature in day time are more favourable for disease infection and development. Several physical and micro-climatic factors are reported to influence the life cycle of the pathogen (Hashimoto, 1981), including spore liberation, transport, deposition, infection, latency and sporulation. Low temperature and high relative humidity (>80%) and high rainfall are conducive for blast disease development (Nagaraja et al., 2010).

Therefore, development of pure-line varieties resistant to blast disease without compromising grain yield is the major objective of finger millet breeding programmes. The objective of the present study was to identify blast disease resistant finger millet recombinant inbred lines (RILs) derived from the cross involving blast susceptible widely adapted variety PR 202 (selection from Peddapuram local) and blast resistant pure-line variety GPU 48 (Indaf 5 × IE 1012).

Materials and Methods

Material

Material for the study consisted 360 F₄, F₅ and F₆ RILs derived from PR 202 × GPU 48. The checks used are PR 202 (susceptible parent), GPU 48 (resistant parent) and Uduru mallige (susceptible check). The seeds of 360 F₄, F₅ and F₆ RILs along with two parents and uduru mallige as checks were sown in 18 compact blocks following augmented design (Federer, 1956) during 2013, 2014 and 2015 rainy seasons respectively. Each block consisted of 20 RILs, three checks and two border rows. The RILs were unreplicated while the three checks were repeated twice in each block. Each entry was sown in a single row of 3 meters length with a spacing of 0.3 m between rows. Ten days after sowing, seedlings were thinned by maintaining 0.1 m between plants within a row. The mean temperature and relative humidity that prevailed at the experimental location during crop growth period is provided in Table 1.

Infector-row method

For ensuring availability of sufficient inoculum load to facilitate uniform disease spread, after every five rows of entries uduru mallige, a local variety with medium duration and highly susceptible for blast disease was sown as infector row.

Disease scoring

Data were recorded for neck and finger blast disease incidences in each of 360 F₄, F₅ and F₆ RILs. Finger blast and neck blast disease were recorded at dough stage. The disease incidences of RILs and checks for neck and finger blast were scored and expressed in per cent using the following formulae.
The response of F_4, F_5 and F_6 RILs and checks to neck and finger blast disease infection under natural conditions were assessed using 1-6 rating scale (Table 2).

**Statistical analysis**

**Analysis of variance (ANOVA)**

Analysis of variance was performed to partition the total variance of entries (RILs+parents+check) into those attributable to ‘RILs’, ‘checks’ and ‘RILs vs. checks’ as per augmented design. The mean NBI and FBI of each of the 360 F_4, F_5 and F_6 RILs were adjusted for block effect. The effect of each block (B_j) was estimated as, B_j = X_j - X..

Where,

\[ \overline{X}_j = \text{The mean NBI and FBI of check entries in j^{th} block} \]

\[ \overline{X}.. = \text{The mean NBI and FBI of all the checks in all the blocks.} \]

The estimate of B_j was used to adjust the NBI and FBI of the RILs relevant to the block. Thus, the mean NBI and FBI of each RIL evaluated in j^{th} block was adjusted by subtracting the block effect ‘B_j’ of the j^{th} block from actual NBI and FBI of the RILs.

Adjusted mean NBI and FBI values were used for estimating descriptive statistics such as mean NBI and FBI, standardised range, phenotypic (PCV) and genotypic co-efficient of variation (GCV) (Burton and De Vane, 1953). Standardised range was estimated as,

\[ \text{Standardised range} = \frac{\text{Highest mean NBI or FBI - Lowest mean NBI or FBI}}{\text{Mean NBI or FBI}} \]

PCV was estimated as phenotypic standardised deviation of NBI or FBI. GCV was estimated as genotypic standardised deviation of NBI or FBI/mean NBI or FBI. Heritability in broad-sense (h^2) was estimated as h^2 = (Vg/Vp) ×100 where, Vg = Genotypic variance, Vp = Phenotypic variance. The mean scores of responses of RIL to neck and finger blast disease infection were computed. Based on 1-6 rating scale F_4, F_5 and F_6 RILs were classified into different response groups (Table 2). The mean score of responses of F_4, F_5 and F_6 RILs classified into different response groups was computed.

**Parent-offspring regression**

Inter-generation correlation between F_4 to F_5 and F_5 to F_6 were estimated. Narrow-sense heritability (h^2) was calculated using regression (b) as (8/15)*b for F_4 to F_5 and (16/31)*b for F_5 to F_6 (Burton and Fortson, 1969). The significance of differences in response of the RILs to neck and finger blast disease classified into different groups was tested using one way ANOVA to assess the efficiency of classification of RILs.

**Coefficients of skewness and kurtosis**

Skewness the third degree statistics and kurtosis the fourth degree statistics were estimated (Snedecor and Cochran, 1994) to infer the nature of distribution of mean scores of the RILs. Genetic expectations of skewness (-3/4 d^2 h) reveal the nature of genetic control...
of the traits (Fisher *et al.*, 1932). The parameters ‘d’ represents additive gene effects and ‘h’ represents dominance gene effects. Kurtosis indicates the relative number of genes controlling the traits (Robson, 1956). The adjusted mean scores of response of each RIL to neck and finger blast disease were used to estimate coefficients of skewness and kurtosis using ‘SPSS’ software program.

**Results and Discussion**

**Analysis of variance**

Analysis of variance revealed highly significant mean squares attributable to ‘RILs’ and ‘check varieties’ for both neck and finger blast incidence among F$_4$, F$_5$ and F$_6$ generations (Table 3). Mean squares attributable to 'RILs vs check varieties' were significant for neck blast and finger blast incidence among F$_4$, F$_5$ and F$_6$ generations.

These results suggested significant differences among the RILs and they differed from the checks for neck blast and finger blast incidence. Lule *et al.*, (2012) also reported significant variations among finger millet genotypes for blast disease infection. The scores of responses of F$_4$, F$_5$ and F$_6$ RILs to neck and finger blast disease were normally distributed (Fig. 1 and 2).

**Descriptive statistics for responses of RILs to blast disease incidence**

The estimates of means of F$_4$, F$_5$ and F$_6$ RILs were comparable for neck blast incidence and finger blast incidence indicating average response of population across the generations. The estimates of standardised range were comparable in F$_4$, F$_5$ and F$_6$ RILs for neck blast and finger blast incidence suggesting occurrence of extreme RILs for blast response in all generations (Table 4). Wide range of response of RILs from moderately resistant to susceptible reaction for blast disease was observed. The variance of scores of response of RILs to neck and finger blast disease was lower in F$_4$ generation compared to those of F$_5$ and F$_6$generations (Table 4) indicating possible fixation of alleles controlling response of RILs to blast disease. This is expected, as selfing generation advances, variance between lines increases and variance within the lines decreases.

GCV for neck blast incidence was low in F$_4$ RILs (8.69%) and high in F$_5$ (34.71%) and F$_6$ RILs (38.21%). GCV for finger blast incidence was moderate in F$_4$ (17.47%) and high in F$_5$ (34.92%) and F$_6$ RILs (37.47%). Whereas, PCV was moderate for neck and finger blast incidence (19.78% and 18.63%) in F$_4$ RILs.

**Table 1** Meteorological data prevailed during crop growth period at Vizianagaram, AP

| Season           | Weather variables | Average rainfall (mm) |
|------------------|-------------------|-----------------------|
|                  | Temperature (˚C)  | Relative humidity (%) |                       |
|                  | Min       | Max       | Max       |                       |
| 2013 rainy season| 27.87     | 30.91     | 82.37     | 798.50                |
| 2014 rainy season| 24.88     | 29.50     | 80.65     | 786.40                |
| 2015 rainy season| 28.35     | 32.30     | 85.82     | 974.10                |
Table 2: Classification of RILs based on neck/finger blast disease incidence

| Sl. No. | Per cent Disease Incidence (PDI) | Reaction group       |
|---------|----------------------------------|----------------------|
| 1       | 0.00                             | Highly resistant     |
| 2       | <5.00                            | Resistant            |
| 3       | 5.01-10.00                       | Moderately Resistant |
| 4       | 10.01-25.00                      | Moderately susceptible |
| 5       | 25.01-50.00                      | Susceptible          |
| 6       | >50.00                           | Highly susceptible   |

Table 3: Analysis of variance for response to blast disease incidence among F_{4}, F_{5} and F_{6} RILs in finger millet

| Source of variation | Degrees of freedom | Neck blast incidence (%) | Finger blast incidence (%) |
|---------------------|--------------------|--------------------------|----------------------------|
|                     |                    | F_{4}                     | F_{5}                     | F_{6}                     | F_{4}                     | F_{5}                     | F_{6}                     |
| Blocks              | 17                 | 2.92                      | 13.92                     | 5.70                      | 3.88                      | 13.35                     | 9.65                      |
| Entries (RILs + checks) | 362             | 73.70**                   | 188.85**                 | 198.95**                 | 70.71**                   | 182.89**                 | 177.70**                 |
| Checks             | 02                 | 6200.65**                 | 14711.88**               | 15456.89**               | 6276.29**                 | 13838.58**               | 12711.27**               |
| RILs               | 359                | 32.42**                   | 80.20**                  | 88.12**                  | 29.83**                   | 85.75**                  | 94.48 **                 |
| Checks vs. RILs     | 01                 | 2637.82**                 | 10146.20**               | 9468.07**                | 2332.43**                 | 7744.02**                | 4986.36**                |
| Error              | 34                 | 3.20                      | 11.43                     | 5.45                      | 3.29                      | 7.38                      | 6.16                      |

** Significance @ P=0.01

Table 4: Descriptive statistics among F_{4}, F_{5} and F_{6} finger millet RILs for response to neck and finger blast disease infection under natural hotspot (Vizianagaram, AP)

| Traits                  | Neck blast incidence (%) | Finger blast incidence (%) |
|-------------------------|--------------------------|---------------------------|
|                         | F_{4}                    | F_{5}                     | F_{6}                     | F_{4}                    | F_{5}                     | F_{6}                     |
| Mean ± SE               | 21.94±0.42               | 22.75±0.49                | 22.66±0.50                | 22.64±0.41               | 24.14±0.50                | 23.88±0.52                |
| Skewness                | 0.06                     | 0.10                      | 0.06                      | 0.11                     | 0.23                      | 0.15                      |
| Kurtosis                | -0.93                    | -1.04                     | -1.17                     | -0.83                    | -0.92                     | -1.14                     |
| Minimum                 | 7.35                     | 5.71                      | 7.15                      | 8.75                     | 7.16                      | 7.68                      |
| Maximum                 | 38.00                    | 41.84                     | 41.85                     | 45.20                    | 45.86                     | 43.98                     |
| Standardised Range      | 1.40                     | 1.59                      | 1.53                      | 1.61                     | 1.60                      | 1.52                      |
| GCV (%)                 | 8.69                     | 34.71                     | 38.21                     | 17.47                    | 34.92                     | 37.47                     |
| PCV (%)                 | 19.78                    | 37.76                     | 39.58                     | 18.63                    | 36.69                     | 38.89                     |
| h^2_{bs}                | 0.89                     | 0.85                      | 0.93                      | 0.88                     | 0.91                      | 0.93                      |
| Expected GAM (%)        | 36.36                    | 65.74                     | 76.00                     | 33.75                    | 68.47                     | 74.38                     |

Table 5: Parent-offspring correlation (r) and regression (b_{y,x}) of the F_{4}:F_{5} and F_{5}:F_{6} generations, additive genetic variance (\sigma^2_A) and narrow-sense heritability (h^2_{ns}) for neck and finger blast disease incidence

| Trait                    | F_{4}:F_{5} | F_{5}:F_{6} |
|--------------------------|------------|------------|
|                         | r          | b_{y,x}    | \sigma^2_A | h^2_{ns} | r          | b_{y,x}    | \sigma^2_A | h^2_{ns} |
| Neck blast incidence (%) | 0.52       | 0.58**     | 1.16       | 0.31     | 0.45       | 0.46**     | 0.92       | 0.24     |
| Finger blast incidence (%) | 0.48      | 0.56**     | 1.12       | 0.30     | 0.49       | 0.51**     | 1.02       | 0.26     |

** Significance @ P=0.01
Table 6. Number of F₄, F₅ and F₆ RILs corresponding to different disease response groups in finger millet

| Disease response groups | Number of RILs in F₄ | Number of RILs in F₅ | Number of RILs in F₆ |
|-------------------------|-----------------------|-----------------------|-----------------------|
|                         | Neck blast incidence (%) | Finger blast incidence (%) | Neck blast incidence (%) | Finger blast incidence (%) | Neck blast incidence (%) | Finger blast incidence (%) |
| Highly resistant (0)    | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| Resistant (<5.00)       | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| Moderately Resistant (5.01-10.00) | 22             | 15                    | 23                    | 8                     | 35                    | 17                    |
| Moderately susceptible (10.01-25.00) | 221            | 211                   | 193                   | 183                   | 179                   | 181                   |
| Susceptible (25.01-50.00) | 117            | 134                   | 144                   | 169                   | 146                   | 162                   |
| Highly susceptible (> 50.00) | 0               | 0                     | 0                     | 0                     | 0                     | 0                     |

Table 7. Estimates of mean blast disease response of F₄, F₅ and F₆ RILs classified into different disease response groups in finger millet

| Response groups | Generations | Highly resistant (0) | Resistant (<5.00) | Moderately Resistant (5.01-10.00) | Moderately susceptible (10.01-25.00) | Susceptible (25.01-50.00) | Highly susceptible (> 50.00) | Pr>F |
|-----------------|-------------|----------------------|-------------------|-----------------------------------|-------------------------------------|---------------------------|-----------------------------|------|
| Neck blast incidence (%) | F₄          | -                    | -                 | 8.66                              | 18.30                               | 31.30                      | -                           | 0.00 |
|                 | F₅          | -                    | -                 | 8.31                              | 17.59                               | 32.08                      | -                           | 0.00 |
|                 | F₆          | -                    | -                 | 8.55                              | 17.67                               | 32.26                      | -                           | 0.00 |
| Finger blast incidence (%) | F₄          | -                    | -                 | 9.01                              | 18.27                               | 31.05                      | -                           | 0.00 |
|                 | F₅          | -                    | -                 | 8.89                              | 17.04                               | 32.64                      | -                           | 0.00 |
|                 | F₆          | -                    | -                 | 9.03                              | 17.10                               | 33.11                      | -                           | 0.00 |
Table 8 The best ten F6 finger millet RILs showing resistance to neck and finger blast disease incidence

| Identity of test RILs | Neck blast incidence (%) | Finger blast incidence (%) | Identity of test RILs | Finger blast incidence (%) | Neck blast incidence (%) |
|-----------------------|--------------------------|----------------------------|-----------------------|-----------------------------|--------------------------|
| 176                   | 7.15                     | 13.42                      | 349                   | 7.69                        | 10.72                    |
| 216                   | 7.19                     | 13.45                      | 347                   | 7.89                        | 10.22                    |
| 150                   | 7.29                     | 11.42                      | 47                    | 8.02                        | 9.62                     |
| 175                   | 7.35                     | 11.22                      | 65                    | 8.09                        | 10.69                    |
| 215                   | 7.39                     | 11.25                      | 49                    | 8.22                        | 9.02                     |
| 56                    | 7.42                     | 11.12                      | 317                   | 8.22                        | 12.22                    |
| 147                   | 7.49                     | 11.02                      | 345                   | 8.49                        | 13.32                    |
| 250                   | 7.72                     | 12.59                      | 45                    | 9.02                        | 10.62                    |
| 269                   | 7.75                     | 12.49                      | 304                   | 9.12                        | 10.72                    |
| 116                   | 7.82                     | 13.42                      | 67                    | 9.39                        | 8.69                     |
| PR 202 (Susceptible parent) | 35.88                   |                            | PR 202 (Susceptible parent) | 31.94                      |
| GPU 48 (Resistant parent) | 8.05                    |                            | GPU 48 (Resistant parent) | 8.81                       |
| Uduru mallige (Susceptible check) | 66.63                   |                            | Uduru mallige (Susceptible check) | 61.81                      |
| SEm±                  | 0.50                     |                            | SEm±                  | 0.52                        |
| CD @ P=0.05           | 1.22                     |                            | CD @ P=0.05           | 1.27                        |
Fig.1 Frequency distribution of response of RILs to neck blast incidence

Fig.2 Frequency distribution of response of RILs to finger blast incidence
**Fig. 3** Graph depicting parent-offspring correlation and regression of the F₄, F₅ and F₆ generations for neck and finger blast disease incidence.
Higher standardised range resulted in higher estimates of PCV and GCV. Neck blast and finger blast in F₄ (37.76% and 36.69%) and F₅ (39.58% and 38.89%) exhibited higher PCV. Narrow difference between GCV and PCV suggested limited influence of environment on neck and finger blast disease incidence expression.

Along with high GCV, RILs exhibited high broad-sense heritability and expected GAM for both neck and finger blast disease incidence over three generations (Table 4), suggesting the effectiveness of selection for blast resistance. Kiran Babu et al., (2013) also reported high PCV and GCV for neck and finger blast coupled with high heritability and expected GAM.

**Genetic interpretation of skewness and kurtosis**

Positive skewness was observed for both neck and finger blast disease incidence in all the three generations. Neck and finger blast disease incidence exhibited platykurtic distribution in all the three generations (Table 4). Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Pooni et al., 1977; Choo and Reinbergs, 1982; Kotch et al., 1992).

Negatively skewed platykurtic distribution is an evidence for involvement of large number of dominant genes with majority of them having increasing effects and duplicate type of epistasis in the inheritance of neck and finger blast disease incidence.

**Parent-offspring regression**

Parent-offspring regression for F₄:F₅ (0.58) and F₃:F₆ (0.46) for neck blast disease incidence and for F₄:F₅ (0.56) and F₅:F₆ (0.51) for finger blast disease incidence were found significant (Fig. 3). Narrow-sense heritability estimated based on parent-offspring (F₄:F₅ and F₅:F₆) regression was found low for both neck and finger blast disease incidence (Table 5). Low narrow-sense heritability suggested less additive genetic variance; therefore the selection should be stringent for identification of resistant RILs.

**Identification of blast disease resistant RILs**

Based on the evaluation of F₄, F₅ and F₆ RILs for response to blast disease incidence at Vizianagaram natural hotspot, few RILs were moderately resistant to neck and finger blast disease (Table 6). Moderately resistant RILs were more frequent in F₅ compared to those in F₄ and F₅ generations. Based on the mean blast disease index RILs were classified into different response groups.

One way ANOVA indicated significant difference between different disease response groups for mean disease index (Table 7) and thus indicated the efficiency of classification.

Among the 360 F₆ RILs evaluated at Vizianagaram, none of the RILs were found highly resistant or resistant to blast disease. However, a few RILs showed moderate resistance, among them top ten RILs were identified (Table 8). These RILs are suggested for use in breeding finger millet for enhanced resistant to neck and finger blast disease.

In a low value crop like finger millet, breeding for resistance is very useful. Identification of disease resistant RILs from the finger millet RIL population would permit use of many blast disease resistance RILs for future breeding efforts and to ensure a better chance of success in finger millet improvement in developing new cultivars.

These RILs which possess good level of partial resistance should be tested for agronomic value.

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