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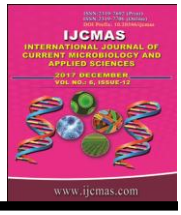

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Identification of Blast Disease Resistant Finger Millet [*Eleusine coracana* (L.) Gaertn] RILs Screened Under Natural Hot Spot

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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_6generations. Low and moderate GCV were observed for neck and finger blast respectively, in F_4 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.

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 *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*per cent* protein (Barbeau and Hilu, 1993) and is rich in calcium, iron, methionine, phosphorus, carbohydrate and other nutrients (Leung *et al.*, 1968).

Finger millet production is affected by a number of diseases like blast, foot rot, smut, streak and mottling virus (Govindu *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*

al., 2001) is the major production constraint. 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 F_4, F_5 and F_6 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 F_4, F_5 and F_6 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 F_4, F_5 and F_6 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.

$$\text{Neck blast incidence (NBI) (\%)} = \frac{\text{Number of ears showing infection on peduncle in each row}}{\text{Total number of ears in each row}} \times 100$$

$$\text{Finger blast incidence (FBI) (\%)} = \frac{\text{Number of infected fingers in each row}}{\text{Average number of fingers/ear} \times \text{Total number of ears in each row}} \times 100$$

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,

$\overline{X_j}$ = The mean NBI and FBI of check entries in j^{th} block

$\overline{X..}$ = 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,

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

PCV was estimated as phenotypic standardised deviation of NBI or FBI/mean 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 $h^2 = (Vg/Vp) \times 100$ 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_6generations (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			
	Temperature (˚C)		Relative humidity (%)	Average rainfall (mm)
	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

(AICRP, small millets)

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 (%)		
Parameters	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_{yx}) of the F_4:F_5 and F_5:F_6 generations, additive genetic variance (σ^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	$b_{(yx)}$	σ^2_A	h^2_{ns}	r	$b_{(yx)}$	σ^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 F_4, F_5 and F_6 RILs corresponding to different disease response groups in finger millet

Disease response groups	Number of RILs in F_4		Number of RILs in F_5		Number of RILs in F_6	
	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 F_4, F_5 and F_6 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 (%)	F_4	-	-	8.66	18.30	31.30	-	0.00
	F_5	-	-	8.31	17.59	32.08	-	0.00
	F_6	-	-	8.55	17.67	32.26	-	0.00
Finger blast incidence (%)	F_4	-	-	9.01	18.27	31.05	-	0.00
	F_5	-	-	8.89	17.04	32.64	-	0.00
	F_6	-	-	9.03	17.10	33.11	-	0.00

Table.8 The best ten F_6 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

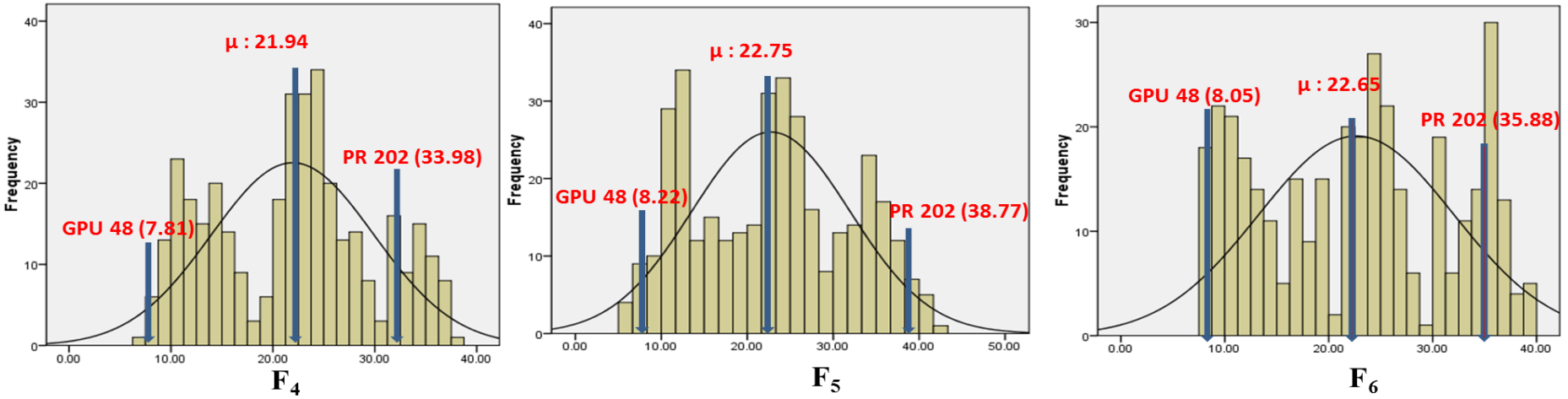


Fig.2 Frequency distribution of response of RILs to finger blast incidence

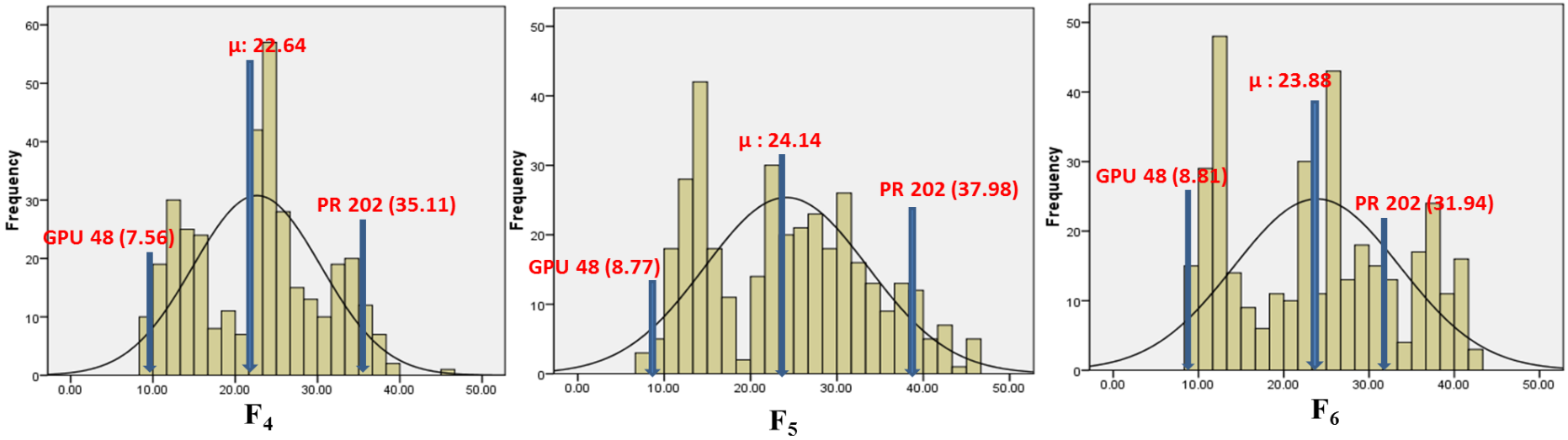
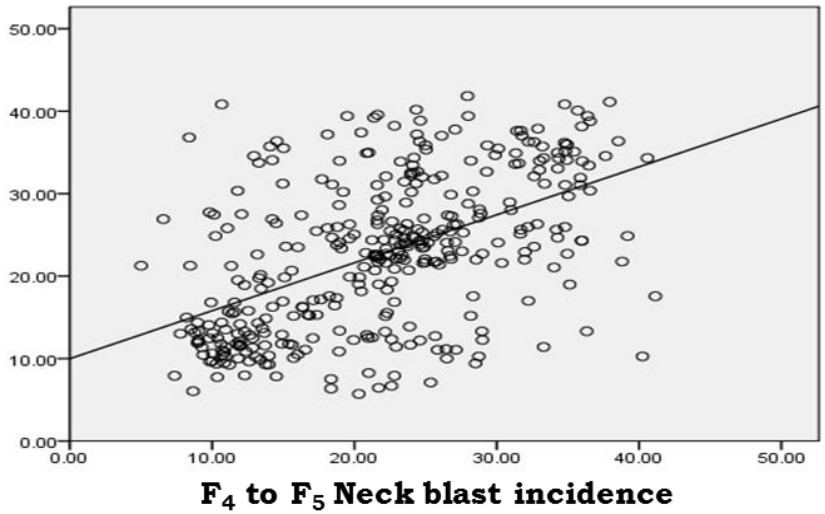
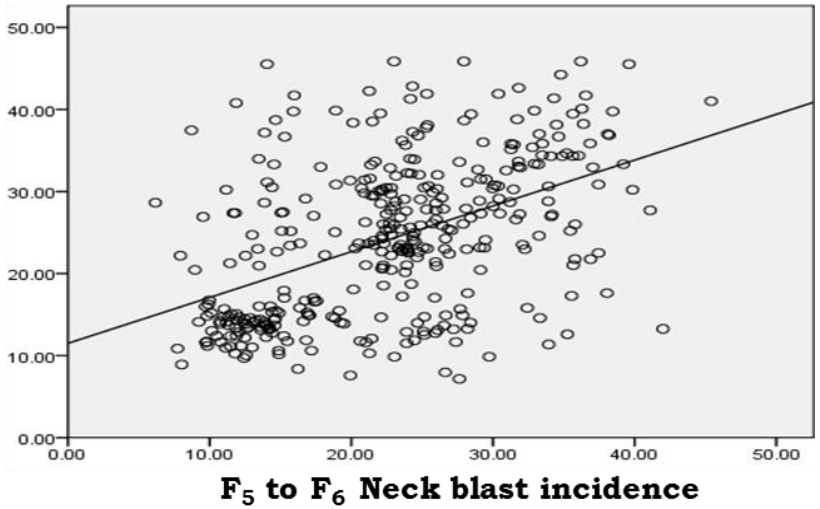


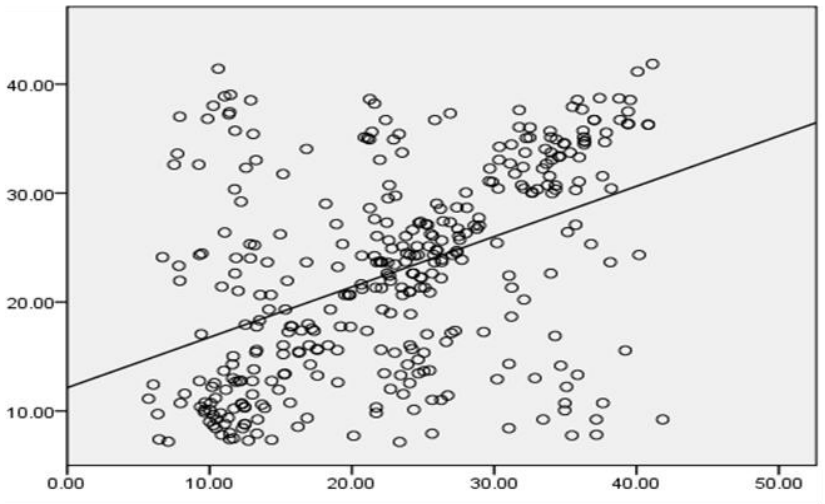
Fig.3 Graph depicting parent offspring correlation and regression of the F_4, F_5 and F_6 generations for neck and finger blast disease incidence



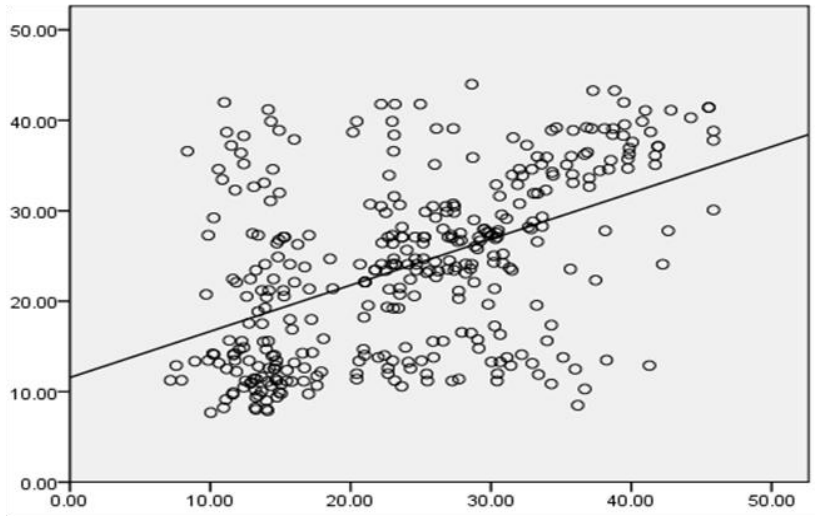
F_4 to F_5 Neck blast incidence



F_5 to F_6 Neck blast incidence



F_4 to F_5 Finger blast incidence



F_5 to F_6 Finger blast incidence

Higher standardised range resulted in higher estimates of PCV and GCV. Neck blast and finger blast in F_5 (37.76% and 36.69%) and F_6 (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 F_4:F_5 (0.58) and F_5:F_6 (0.46) for neck blast disease incidence and for F_4:F_5 (0.56) and F_5:F_6 (0.51) for finger blast disease incidence were found significant (Fig. 3).

Narrow-sense heritability estimated based on parent-offspring (F_4:F_5 and F_5:F_6) 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 F_4, F_5 and F_6 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 F_6 compared to those in F_4 and F_5 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 F_6 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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