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Selection of finger millet [*Eleusine coracana* (L.) Gaertn] blast resistant RILs

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Abstract
The 500 F_5, F_6 and F_7 RILs Derived from the cross between GPU48 x *Uduru mallige* were screened for neck and finger blast incidence, in F_5, one RIL were moderately resistant to neck blast, whereas in F_6, four RILs were moderately resistant to neck blast and two RILs were moderately resistant to finger blast whereas in F_7 six RILs were moderately resistant to neck blast and four RILs to finger millet blast. ANOVA showed the effects of checks, varieties and checks vs. varieties were all significant. Significant effects suggested that certain lines exhibited differential resistance and probably possess different resistance gene(s). Significant effects of the location suggests, weather conditions were more conducive for blast. There is lower differences in GCV and PCV, lower difference between GCV and PCV suggests narrow influence of environment on neck and finger blast expression. Kurtosis is positive, indicates the existence of gene interactions.

Keywords: finger millet, resistant, screening, *Pyricularia grisea*, neck blast, finger blast

Introduction

In world, finger millet stands fourth in position amongst millets after sorghum, pearl millet and foxtail millet. It is commonly grown in Africa and South Asia, and it is projected that some 10% of the world's 30 million tons of millet produced is finger millet, from the nutritional perspective, it is rich in minerals and its micronutrient density is higher than that of the world's major cereal grains; rice and wheat (Antony and Chandra, 1998) [4].

Finger millet is a rich source of calcium among cereals with up to 10- fold higher calcium content than brown rice, wheat or maize and three times than milk. Being rich in iron and fibre, makes this crop more nutritive as compared to other most commonly used cereals. Finger millet is enriched with the essential amino acids like lysine (Mc Donough *et al.*, 2000) [15] and methionine which are important in human health and growth but absent in most other plant foods. In addition, it contains ample of the two polyunsaturated fatty acids- linoleic acid and α-linolenic acid (Fernandez *et al*., 2003) [9], metabolized products of which facilitate normal development of the central nervous system (Jacobson *et al.*, 2008) [11]. It also contains both water soluble and lipo-soluble vitamins thiamine, riboflavin, niacin, and tocopherols (Belton and Taylor, 2002) [5].

Millets are important but underutilized crops in tropical and semiarid regions of the world due to their greater resistance to pests and diseases, good adaption to a wide range weather conditions and their good yields, can resist significant levels of salinity, short growing season, water logging, drought tolerant, requires little inputs during growth and with increasing world population and decreasing water supplies represents important crop for future human use. Finger millet is a hardy crop it is comparatively easy to grow under biotic and abiotic stress conditions, without compensating the net productivity. There is greater potential to process millet grains into value added foodstuffs and concoctions, and millets, do not contain gluten and hence it is desirable for stomach (abdominal) patients.

Finger millet blast is caused by *Magnaporthe grisea* (Hebert) Barr. The genus *Magnaporthe* collectively parasitizes more than 50 hosts. Major constraints in finger millet production include blast disease and abiotic stresses such as drought and low soil fertility. Blast is endemic wherever the crop is grown and is most destructive in almost all the finger millet growing regions of the world (Anilkumar *et al*., 2003) [2]. In India, blast was first reported from Tanjore delta of Tamil Nadu by Mc Rae (1920) [16]. The disease is seen on leaf, neck and on fingers, on fingers it occurs in more destructive form as compared to leaf and neck (Takan *et al*., 2012) [23]; The average loss due to blast has been reported to be around 28- 36 per cent (Nagaraja *et al*., 2007) [17], but could be as high as 80-90 per cent in endemic areas (Rao, 1990) [20].

Development of finger millet lines with enhanced genetic resistance to blast has enormous importance in disease prone areas. In order to develop the resistance varieties we crossed the one resistant parent GPU48 pure line into one agronomically superior cultivar *Uduru mallige*, RILs were screened in blast hot spot Vizianagaram (AP).

The objective of the present investigation was to select the neck and finger blast resistant recombinant inbred lines (RILs) because, in a crop like finger millet, breeding for resistance is very useful because use of resistant varieties is very cost effective and this crop is cultivated by small and marginal farmers and their total income is generally very low, hence use resistant varieties good for its cultivation.

Material and Methods

Material

The present study consisted of 500 F_5, F_6 and F_7 RILs derived from Uduru mallige × GPU 48. The checks are *Uduru mallige* (susceptible check) and GPU 48 as resistant. The seeds of 500 F_4, F_5 and F_6 RILs along with checks were sown in 25 compact blocks adopting augmented design (Federer, 1956) during 2015, 2016 and 2017 *kharif*, *rabi* and *kharif* seasons respectively. Every block contained of 20 RILs with checks. Two replications were maintained, with 3 meters row length at a spacing of 0.3 m in-between rows. After Ten days of sowing, population was maintained by thinning and 0.1 m length distance maintained between plants and also within row.

Infector-row method

For availability of adequate inoculum load to enable even disease spread, after every two rows of entries susceptible check was sown.

Blast disease scoring

Observations were recorded for neck blast incidence (NBI) and finger blast disease incidences (FBI) for every 500 F_5, F_6 and F_7 RILs. NBI and FBI were noted at dough stage. The per cent disease incidences of RILs and checks for neck blast (NB) and finger blast (FB) were scored and the data was expressed in *per cent* using the following formulae.

$$NBI = \frac{\text{Number of ears showing infection on peduncle in each row}}{\text{Total number ears in each row}} \times 100$$

$$FBI = \frac{\text{Number of infected ears in each row}}{\text{Average number of fingers X Total number ears in each row}} \times 100$$

The reactions of F_5, F_6 and F_7 RILs and checks to NB and FB disease infection under natural conditions were grouped using 1-6 rating scale (Table 1).

Table 1: Grouping of RILs based on NBI and FBI

Sl. No.	*Per cent* Disease Incidence (PDI)	Reaction group
1	0.00	HR
2	<5.00	R
3	5.01-10.00	MR
4	10.01-25.00	MS
5	25.01-50.00	S
6	>50.00	HS

(AICRP, small millets)

Legend: Where, HR= highly resistant, R= Resistant, MR= moderately resistant, MS= moderately susceptible, S= Susceptible and HS= highly susceptible

Statistical analysis

Analysis of variance (ANOVA)

ANOVA was done 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 of neck blast incidence and finger blast incidence of every 500 F_5, F_6 and F_7 RILs were adjusted for block effect. The effect of each block (B_j) was assessed as, $B_j = X_j - X$.

Where,

X_j = The mean neck blast incidence and finger blast incidence of check entries in j^{th} block

X. = The mean neck blast incidence and finger blast incidence of all the checks in all the blocks

The estimate of Bj was used to adjust the neck blast incidence and finger blast incidence of the RILs related to the block. Hence, the mean neck blast incidence and finger blast incidence of each RIL assessed in j^{th} block was adjusted by subtracting the block effect 'Bj' of the j^{th} block from actual neck blast incidence and finger blast incidence of the RILs.

Adjusted mean neck blast incidence and finger blast incidence values were used for estimating descriptive statistics such as mean neck blast incidence and finger blast incidence, phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) (Burton and De Vane, 1953) [6] was estimated.

PCV was calculated as phenotypic standardised deviation of neck blast incidence or finger blast incidence /mean neck blast incidence or finger blast incidence. GCV was estimated as genotypic standardised deviation of neck blast incidence or finger blast incidence /mean neck blast incidence or finger blast incidence.

Heritability in broad-sense (h2) was estimated as

$$h2 = (Vg/Vp) \times 100$$

Where, Vg = Genotypic variance, Vp = Phenotypic variance.

The mean scores of reactions of RIL to neck and finger blast disease infection were calculated based on 1-6 rating scale.

F_5, F_6 and F_7 RILs were classified into different response groups (Table 1).

The mean score of responses of F_5, F_6 and F_7 RILs classified into different response groups was computed

Coefficients of Skewness and kurtosis

Skewness the third degree statistics and kurtosis the fourth degree statistics were assessed (Snedecor and Cochran, 1994) to understand the nature of distribution of mean scores of the RILs. Genetic expectations of Skewness (-3/4 d2 h) reveal the nature of genetic controller of the traits (Fisher *et al.*, 1932). The parameters 'd' represents additive gene effects and 'h' represents dominance gene effects.

Kurtosis shows the relative number of genes governing the traits (Robson, 1956) [21]. The adjusted mean scores of response of each RIL to NB and FB disease were used to determine the coefficients of Skewness and kurtosis through 'SPSS' software.

Phenotyping data of finger millet population was analysed by using Window Stat software (Augmented, RBD).

Results and Discussion

In the present investigation, 500 lines were assessed at Vizianagaram has provided stable and differential reactions. ANOVA (Table 2) showed the effects of checks, varieties and checks vs. varieties were all significant (P<0.0001). Significant effects suggested that certain lines exhibited

Differential resistance and probably possess different resistance gene(s). Significant effects of the location suggested that the weather parameters were more conducive for blast.

In the present experiment, broad sense heritability for resistance to neck and finger blast ranged from 0.89 to 0.92 for F_5 generation, for F_6 generation broad sense heritability ranged from 0.89 to 0.92 and for F_7 generation it ranged from 0.61 to 0.85 indicating more importance of genetic than environmental variability in all the tests. The heritability for F_5 generation for, neck and finger blast was 0.89 and 0.92 respectively, for F_6 generation, it was 0.92 and 0.89 respectively, whereas for F_7 heritability for neck and finger blast was 0.61 and 0.85 respectively (Table 3). If heritability is more, selection will be more effective at a particular generation for that trait. The field experiment conducted at Vizianagaram showed high heritability, thus indicating that selection for finger millet blast can be more significant at this location. According to Johnson *et al*. (1955) [12] high heritability estimates are usually more helpful in predicting gain under selection.

These results shows there is significant differences among the RILs and they varied from the checks for NB and FB incidence. Lule *et al.*, (2012) [14]; Angadi *et al.*, (2017) [1] and Anjum (2015) also observed significant differences among finger millet lines for blast disease infection. The scores of reactions of F5, F6 and F7 RILs to NB and FB disease were normally distributed (Graph. 1 and 2).

Descriptive statistics of RILs to neck blast incidence and finger blast incidence

The estimations of means of F5, F6 and F7 recombinant inbred lines were comparable for NBI and FBI representing average response of population across all the generations. The estimates of standardised range were comparable in F5, F6 and F7 RILs for NB and FB incidence proposing occurrence of extreme RILs for blast (Table 3). Wide-ranging response of RILs from moderately resistant (MR) to susceptible reaction (S) for blast disease was observed. The variance of scores of response of RILs to neck and finger blast disease was lesser in F5 compared to those of F6 and F7 (Table 3) representing more likely fixation of alleles governing response of RILs to NB and FB disease. This was anticipated, as selfing generations advances, variance between RILs increases and variance within the RILs decreases.

GCV for NBI was low in F_7 RILs (31.42%) and high in F_6 (38.48%) and in F_5 RILs (37.98%). GCV for FBI was high in F5 (38.48 %%) and slightly lower in F6 (37.63) and F7 (37.45) Whereas, PCV was higher than the GCV *i.e* in F_5 (40.13%), F_6 (39.98%) and F_7 (40.11) (Table 3) for NBI and for FBI in F_5 (39.98%), F_6 (39.76%) and F_7 (40.56 %) respectively. These results indicates there is lower differences in GCV and PCV. Minor difference between GCV and PCV suggested narrow influence of environment on neck blast incidence and finger millet incidence.

Genetic elucidation of Skewness and Kurtosis

Positive Skewness was observed for NBI and FBI in three generations. Neck and finger blast disease incidence exhibited platykurtic distribution in all the three generations (Table 3). Kurtosis is positive this indicates the presence of gene interactions (Pooni *et al.*, 1977; Choo and Reinbergs, 1982; Kotch *et al.*, 1992 and Angadi *et al.*, (2017) [19, 7, 13, 1].

Parent-offspring regression

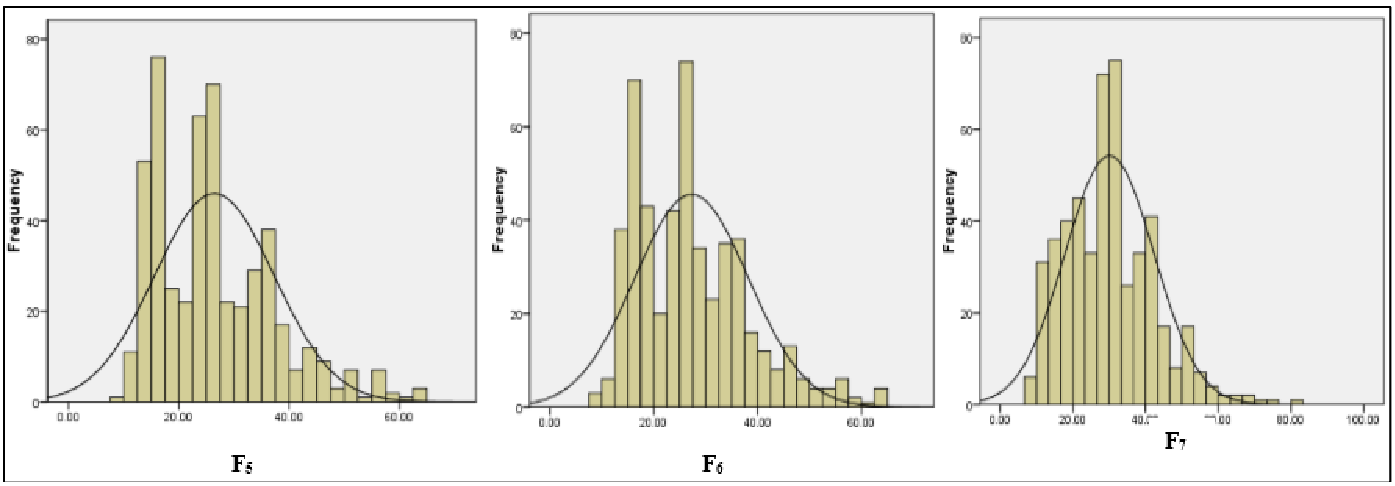
Parent-offspring regression for F_5: F_6 (0.96) and F_6:F_7 (0.75) for NBI and for F_5:F_6 (0.95) and F_6:F_7 (0.80) for FBI were found significant (Graph 3). Parent-offspring regression is a tool to determine the heritability of phenotypic traits; *i.e.*, the relative extent to which those traits are controlled by genetic factors. The results depicts parent offspring regression is significant and the parent offspring values ranged from 0.75 to 0.96 hence the genes which are governing the resistance to blast disease are influenced by the weather to some extent, but also by genetics, therefore parent offspring values are smaller than 1, but larger than 0.

Selection of NB and FB resistant RILs

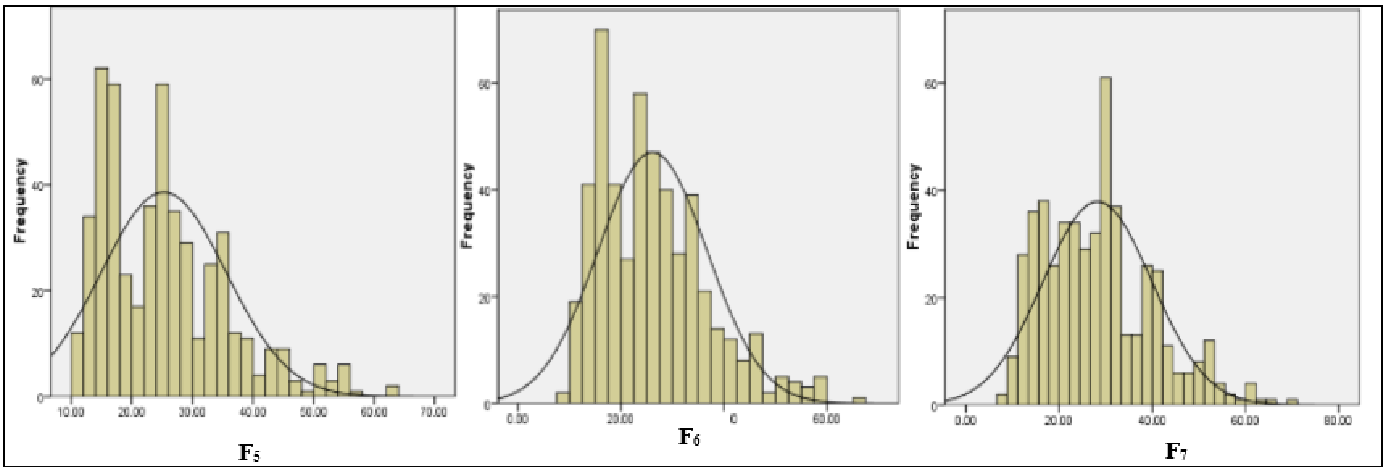
Based on the reactions of F5, F6 and F7 RILs to NBI and FBI at Vizianagaram none of RILs were highly resistant and resistant in three generations. Six RILs were MR to NB and four RILs were resistant to FB in F_7 generation (Table 4). Based on the mean blast disease index RILs were grouped into different groups.

Among evaluated RILs best ten RILs for NB and FB were identified (Table 6). These RILs can be used for further breeding programme for development of improved resistant to NB and FB disease.

The total number of moderately resistant, moderately susceptible, susceptible and highly susceptible RILs with their response were classified according to neck and finger blast and presented in table 4 and the assessments of mean NBI and FBI according to their response group are presented in table 5.



Graph 1: Frequency distribution of reactions of RILs to NBI



Graph 2: Frequency distribution of reactions of RILs to FBI

Table 2: Analysis of variance for response to blast disease incidence among F5, F6 and F7 RILs in finger millet

Source of variation	df	F5				F6				F7			
		Neck blast		Finger blast		Neck blast		Finger blast		Neck blast			
		Mean Squares	F Ratio	Mean Squares	F Ratio	Mean Square	F Ratio	Mean Square	F Ratio	Mean Square	F Ratio	Mean Square	F Ratio
Block (eliminating Check + Var.)	24	11.69	0.997	11.195	1.49	15.84	1.099	15.35	1.36	27.003	0.48	29.79	1.54
Entries (ignoring Blocks)	501	195.24	16.642	185.24	24.67	196.26	13.6	191.25	17.02	222.16	3.95	217.41	11.24
Checks	1	34065.71	2903.68	34539.8	4600.74	33556.02	2326.45	34726.67	3090.9	32837.17	584.08	36753.02	1901.66
Varieties	499	117.84	10.04	106.83	14.23	120.04	8.32	112.72	10.033	150.25	2.67	136.84	7.08
Checks vs. Varieties	1	4944.38	421.44	4954.34	659.92	4871.4	337.736	4843.35	431.09	3493.09	62.132	3885.92	201.06
ERROR	24	11.73		7.5		14.423		11.235		56.22		19.32	

Table 3: Descriptive statistics between F_5, F_6 and F_7 RILs reaction to NB and FB disease

Traits	Neck blast incidence (%)			Finger blast incidence (%)		
Parameters	F5	F6	F7	F5	F6	F7
Mean ± SE	26.44±0.48	27.27± 0.48	30.08±0.54	25.24±0.46	26.09±0.47	28.21±0.52
Skewness	0.93	0.89	0.69	0.99	0.88	0.68
Kurtosis	0.68	0.62	0.72	0.80	0.59	0.18
Minimum	9.35	9.50	7.20	10.25	9.55	8.20
Maximum	64.70	64.80	80.85	63.95	65.65	71.00
ECV (%)	12.95	10.85	24.92	10.85	12.84	15.581
GCV (%)	37.98	38.48	31.428	38.48	37.63	37.45
PCV (%)	40.13	39.98	40.113	39.98	39.76	40.56
h_2(bs)	0.89	0.92	0.61	0.92	0.89	0.85
Expected GAM (%)	74.05	70.76	50.72	76.30	73.37	71.24

In a crop like finger millet, breeding for blast resistance is very advantageous because use of resistant varieties is better because it is cost effective and this crop is cultivated by small and marginal farmers and their total income is very low. Selection of RILs which are resistant to blast from the population could permit use of many RILs for future breeding programmes and to make sure an improved chance of selection in finger millet in developing new varieties, resistant to blast disease.

The RILs which have good agronomic characters which are susceptible to blast should be selected for good agronomic traits for further improvement.

Table 4: Number of F_5, F_6 and F_7 RILs corresponding to different disease response groups in finger millet

Disease response groups	Number of RILs in F_5		Number of RILs in F_6		Number of RILs in F_7	
	NBI (%)	FBI (%)	NBI (%)	FBI (%)	NBI (%)	FBI (%)
HR (0)	0	0	0	0	0	0
R(<5.00)	0	0	0	0	0	0
MR (5.01-10.00)	1	0	4	2	6	4
MS (10.01-25.00)	252	279	218	258	165	213
S (25.01-50.00)	226	203	257	222	292	252
HS (> 50.00)	21	18	21	18	37	31

Legend: NBI= Neck blast incidence, FBI= Finger blast incidence, HR= highly resistant, R= Resistant MR= moderately resistant, MS= moderately susceptible, S= Susceptible and HS= highly susceptible.

Table 5: Estimates of mean blast disease response of F_5, F_6 and F_7 RILs classified into different disease response groups in finger millet

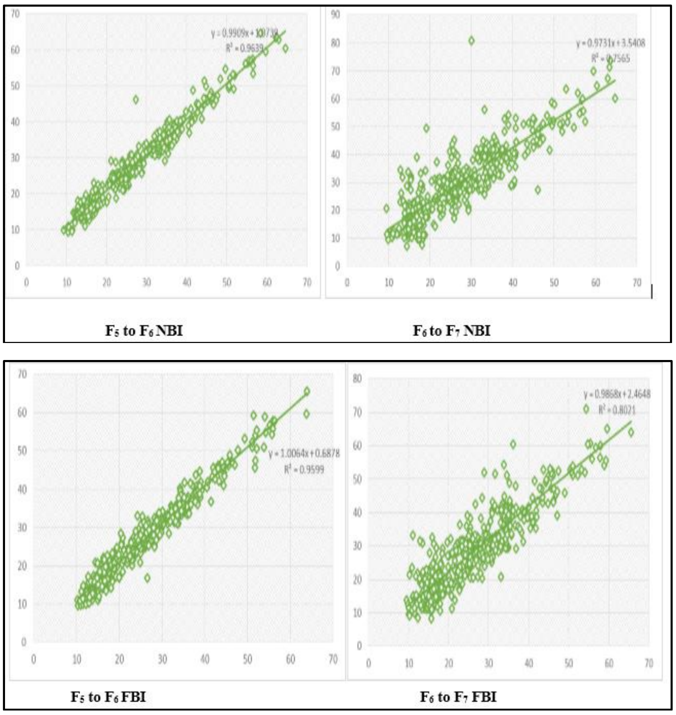
Response Groups	G	HR(0)	R (<5.00)	MR (5.01-10.00)	MS (10.01-25.00)	S (25.01-50.00)	HS (> 50.00)
Neck blast incidence (%)	F5	-	-	9.35	18.18	32.99	55.95
	F6	-	-	9.70	18.08	32.90	56.73
	F7	-	-	8.90	17.73	34.08	57.04
Finger blast incidence (%)	F5	-	-	-	17.98	32.63	54.95
	F6	-	-	9.70	18.20	33.12	55.51
	F7	-	-	8.83	17.95	33.87	55.24

Legend: G= generations HR= highly resistant, R= Resistant MR= moderately resistant, MS=moderately susceptible, S= Susceptible and HS= highly susceptible

Table 6: The best ten F_7 finger millet RILs showing resistance to neck and finger blast disease incidence

Identity of test RILs	Neck blast Incidence (%)	Identity of test RILs	Finger blast Incidence (%)
170	7.20	15	8.20
50	7.75	283	8.40
46	9.40	423	9.10
155	9.65	221	9.65
169	9.70	155	10.05
97	9.75	157	10.50
153	10.15	2	10.60
231	10.15	333	10.90
268	10.15	397	11.00
43	10.35	358	11.05
UM	72.3	UM	74.5
GPU48	10.3	GPU48	12.2

Legend: UM= Susceptible check GPU48= Resistant check



Graph 3: Graph showing parent offspring correlation and regression of the F_5, F_6 and F_7 for NBI and FBI

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