



RESEARCH PAPER

Identification of high yielding and blast disease resistant F₆ RILs in finger millet

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Abstract : Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the most important staple food crops in India. Blast disease caused by the fungus *Pyricularia grisea* (Cooke) is the most devastating biotic production constraint which affects different aerial parts of the plant at all plant growth stages. Development of pure-line varieties with high grain yield potential coupled with blast disease resistance is the major breeding objective of breeding finger millet. 360 F₆ Recombinant Inbred Lines (RILs) derived from the cross PR 202 × GPU 48 were evaluated at two locations during 2015 rainy season (Bengaluru and Mandya) for grain yield and response to blast disease reaction. Analysis of variance in F₆ RILs at both Bengaluru and Mandya locations revealed highly significant mean squares attributable to 'RILs' and 'check varieties' for all traits studied. High GCV and PCV were observed for grain yield plant⁻¹, neck blast incidence and finger blast incidence at Bengaluru and Mandya locations. All the traits studied exhibited higher broad sense heritability for both locations. The best ten high yielding RILs were identified.

Key Words : Recombinant inbred lines, Grain yield, Blast, Variability

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INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.], sub-species *coracana*, belongs to the family poaceae and the genus *Eleusine* under the tribe *Ergrostideae*. 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 millets 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 crop components for 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 is an important C₄ cereal crop for subsistence agriculture. Among the coarse cereals, finger millet accounts for 7

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per cent area and 11 per cent of production in India. It is grown in an area of 1.19 million hectares in India with a production of 1.60 million tons and productivity of 1.3 t ha⁻¹ (Anonymous, 2015).

Grain yield, for which the improvement is sought, is a complex character, which is not only influenced by its associated characters but also by the environment. This necessitates the separation of genetic variability from total variability to make selection. Even though finger millet is known to be one of the hardiest crops, it is affected by a number of diseases like blast, foot rot, smut, streak and mottling virus (Govindu *et al.*, 1970). Blast of finger millet is a major disease caused by the fungus *Pyricularia grisea* (Cooke) Sacc. (formerly *Pyricularia oryzae* Cavara.), an anamorph of *Magnaporthe grisea* (Hulbert *et al.*, 2001) Barr (Rossman *et al.*, 1990) that causes blast disease in rice. Blast in finger millet is affecting different aerial parts of the plant at all stages of its growth starting from seedling to grain formation with yield losses upto 28 per cent (Vishwanath *et al.*, 1986). Appearance of brown and also subsequently blackening of the area immediately below the ear is an indication of neck blast. An olive grey growth of the fungus may also appear in this (Patro and Madhuri, 2014). Finger blast usually begins from the apical portion and runs toward the base of the finger (Patro and Madhuri, 2014).

Crop improvement is a holistic activity in which biotic stress suppression is an integral component. In other words resistance breeding should not be an adjunct to the mainstream of breeding effort. Therefore, the emphasis should be not only to identify the stable resistant sources but also to understand genetic regulation of yield components and their association with disease characters in order to propose competitive methods of crop improvement. With this background, the present study was undertaken to identify the RILs which are high yielding and/or coupled with blast disease resistance.

Development of pure-line varieties with high grain yield potential coupled with blast disease resistance is the major breeding objective finger millet breeding. In this back ground, the present study was undertaken with an objective of identification of high yielding and blast disease resistant finger millet genotypes in F₆ generations derived from crosses involving blast susceptible and blast resistant parents.

MATERIAL AND METHODS

Experimental material :

The material for the present study consisted of 360 F₆ Recombinant Inbred Lines (RILs) derived from the cross PR 202 × GPU 48 following ear-to-row method. The female parent PR 202 is a blast disease susceptible released variety which is a pure-line selected from Peddapuram local while GPU 48 is a blast disease resistant from the cross GPU 26 × L 5. The seeds of the experimental material were procured from All India Co-ordinated Research Project (Small millets), Bengaluru, University of Agricultural Sciences (UAS), Bengaluru, India.

Layout of experiment :

The two separate experiments were conducted to (1) identify recombinant inbred lines (RILs) with high grain yielding ability, (2) screen RILs for response to blast disease incidence under natural conditions, at two locations. The experiments were laid out in augmented design (Federer, 1956). The 360 F₆ recombinant inbred lines (RILs), two parents (also used as checks) [PR 202, GPU 48] and check [GPU 28 (check for grain yield), KM 252 (susceptible check for blast disease)] were sown in 18 compact blocks. 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. The experiments were conducted under protective irrigation during 2015 rainy season at GKVK, Bengaluru and Zonal Agricultural Research Station (ZARS), Mandya, Karnataka.

Each entry was sown in a single row of 3 meters length and the spacing maintained was 30 cm between rows and 10 cm between plants within a row. Recommended crop production practices were followed during the crop growth period to raise a crop.

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.

Sampling of plants and data collection :

Data were recorded on five randomly chosen plants in each entry on days to 50 per cent flowering, (Days to 50 % flowering was taken from sowing date to the stage

when ears have emerged from 50 % of main tillers), tillers plant⁻¹ (Number of basal tillers bearing the mature ears were counted from each of the five plants and averaged plant⁻¹), plant height (cm) (The height of the main tiller was measured from the ground level to the tip of the panicle at dough stage in centimeters and expressed as plant height), finger length (cm) (Finger length was measured from base to tip of the longest finger (spike) on main tiller at dough stage and expressed in centimeters) and grain yield plant⁻¹(g) (Total grain yield of five plants were weighed and the mean value was computed and expressed as grain yield plant⁻¹ in gram).

Disease scoring :

Data were recorded for neck and finger blast disease incidences in each location. 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.

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

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

Statistical analysis :

Analysis of variance (ANOVA) was performed separately for both locations data to partition the total variance of entries into those attributable to RILs, checks and RILs vs checks as per augmented design. Quantitative trait means and neck/finger blast (NBI and FBI) incidence of each of the 360 RILs were adjusted for block effect. The effect of each block (B_j) was computed as:

$$B_j = \bar{X}_j - \bar{X}_{..}$$

where,

\bar{X}_j = Trait means of check entries in jth block

$\bar{X}_{..}$ = Trait mean of all the checks in all the blocks.

B_j was used to adjust the trait means of the RILs relevant to the block. Thus, trait means of each RIL evaluated in jth block was adjusted by subtracting the block effect B_j of the jth block from actual trait value of the RILs. Adjusted quantitative traits mean and neck/finger blast (NBI and FBI) incidence values were used for estimating descriptive statistics such as trait mean,

range, variance, skewness, kurtosis, phenotypic (PCV) and genotypic co-efficient of variation (GCV). Heritability in broad sense was estimated using the formula given.

PCV was estimated as phenotypic standardised deviation of trait/mean. GCV was estimated as genotypic standardised deviation of trait/mean. Heritability in broad-sense (h^2) was estimated as $h^2 = (Vg/Vp)$

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

Co-efficients 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 trait mean values of the RILs. Genetic expectations of skewness ($-3/4 d^2h$) 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 trait (Robson, 1956). The adjusted mean values of each RIL were used to estimate co-efficients of skewness and kurtosis using 'SPSS' software programme.

Based on neck/finger blast disease incidence, the response of RIL was assessed and RILs were classified into highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible. The significance of difference response of the RILs to neck/finger blast disease classified into different groups was tested using one way ANOVA.

Identification of high yielding and blast resistant genotypes :

Based on expression of grain yield plant⁻¹, the best ten high yielding RILs along with their respective blast disease reaction were identified in F₆ generations.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Analysis of variance :

Analysis of variance in F₆ RILs at both Bengaluru and Mandya locations revealed highly significant mean squares attributable to 'RILs' and 'check varieties' for all traits studied *viz.*, days to 50 per cent flowering, tillers plant⁻¹, plant height, finger length, grain yield plant⁻¹, neck

blast incidence and finger blast incidence (Table 1). Mean squares attributable to 'RILs vs check varieties' were significant for all traits. These results suggest significant differences among the F_6 RILs and they differed from the checks for all traits investigated at both locations. Frequency distributions of RILs were normal (Fig.1). This information indicates that sizable variability exists for all the characters studied and considerable improvement can be achieved in these characters by selection. However, the analysis of variance by itself is inconclusive in explaining all the inherent genetic variability in the RILs. This is evident by partitioning the total variability inherent in the RILs from the phenotypic variance. Ravikumar and Seetharam (1993); Satish (2003) and Angadi *et al.* (2016) also reported significant differences among the genotypes for the characters they studied in finger millet.

Descriptive statistics :

Detection of genetic variability assessing relative contribution of genetic and non-genetic sources is a prerequisite for formulating appropriate selection strategies to breed improved pure-line varieties. The estimates of traits range provide clues about the occurrence of genotypes with extreme expression. The traits ranges (Table 2) of the RILs were relatively higher for all the quantitative traits. The observed GCV and PCV were low for days to 50 per cent flowering and

plant height for Bengaluru and Mandya locations. Moderate GCV and PCV were observed for tillers plant⁻¹ and finger length for both Bengaluru and Mandya locations. High GCV and PCV were observed for grain yield plant⁻¹, neck blast incidence and finger blast incidence at Bengaluru and Mandya locations. Bedis *et al.* (2006); John (2006) and Angadi *et al.* (2016) also reported high GCV and PCV for grain yield and its component traits and for blast disease incidence. On the whole, co-efficient of variability values indicated existence of considerable amount of variability for most of the traits studied. Narrow difference between GCV and PCV for all the traits indicated less influence of environment on trait expression. Angadi *et al.* (2016) also observed narrow differences between PCV and GCV for days to 50 per cent flowering, plant height, grain yield plant⁻¹, neck blast incidence and finger blast incidence in finger millet.

Heritability is a quantitative measure which provides information about the correspondence between genotypic variance and phenotypic variance, *i.e.*, the ratio of variance due to heritable difference to the total phenotypic variance expressed as per cent. Knowledge on genetic advance that is expected by applying selection pressure to a population is useful in designing effective breeding programme. Heritability is a fraction of variance in phenotypic expression that arises from genetic effects. The nature of the selection units and sampling errors

Table 1: Analysis of variance for grain yield and its component traits among F_6 RILs of finger millet

Source of variation	Degrees of freedom	Mean sum of squares													
		Days to 50% flowering		Tillers plant ⁻¹		Plant height (cm)		Finger length (cm)		Grain yield plant ⁻¹ (g)		Neck blast incidence (%)		Finger blast incidence (%)	
		Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya
Blocks	17	1.14	0.61	0.01	0.02	1.43	1.32	0.13	0.02	1.41	0.23	1.32	2.45	7.71	0.49
Entries (RILs + checks)	362	6.73	9.16	0.40	0.19	103.37	71.59	0.59	0.31	20.13	14.32	114.89	205.62	107.43	193.82
Checks	02	340.13	245.40	2.44	1.78	1721.19	1780.93	3.62	0.73	336.74	56.56	7618.59	13434.50	6949.45	13555.82
RILs	359	4.76	7.66	0.38	0.18	87.22	59.09	0.56	0.32	14.90	13.94	30.87	48.51	21.22	31.10
Checks vs. RILs	01	47.39	74.46	2.69	1.34	2663.57	1143.07	4.01	0.92	1265.88	65.96	30152.86	30152.86	17370.13	31887.31
Error	34	0.91	0.55	0.01	0.02	2.62	2.87	0.109	0.02	1.71	0.14	1.32	2.45	7.71	0.49

* and ** indicate significance of value at P=0.05 and 0.01, respectively

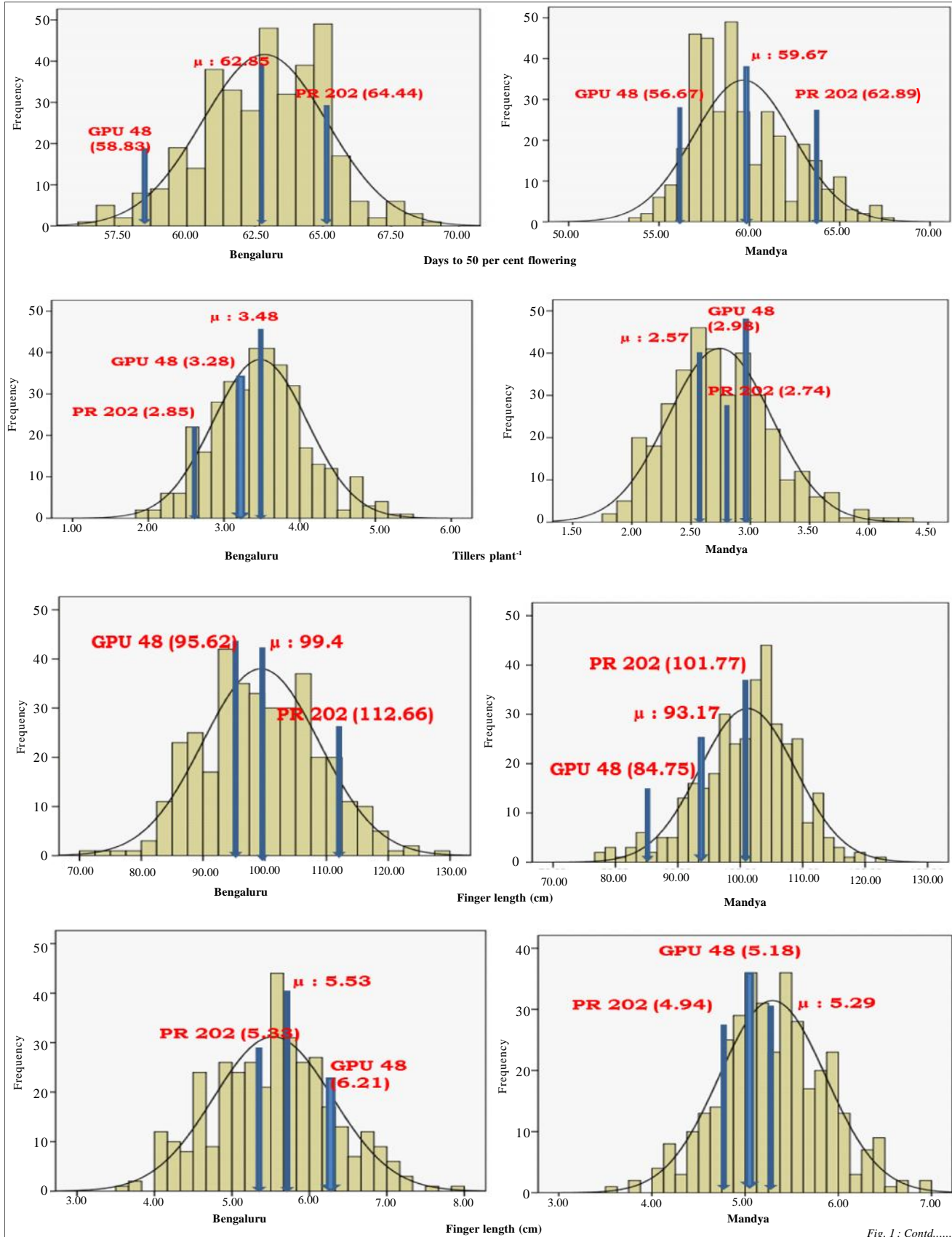


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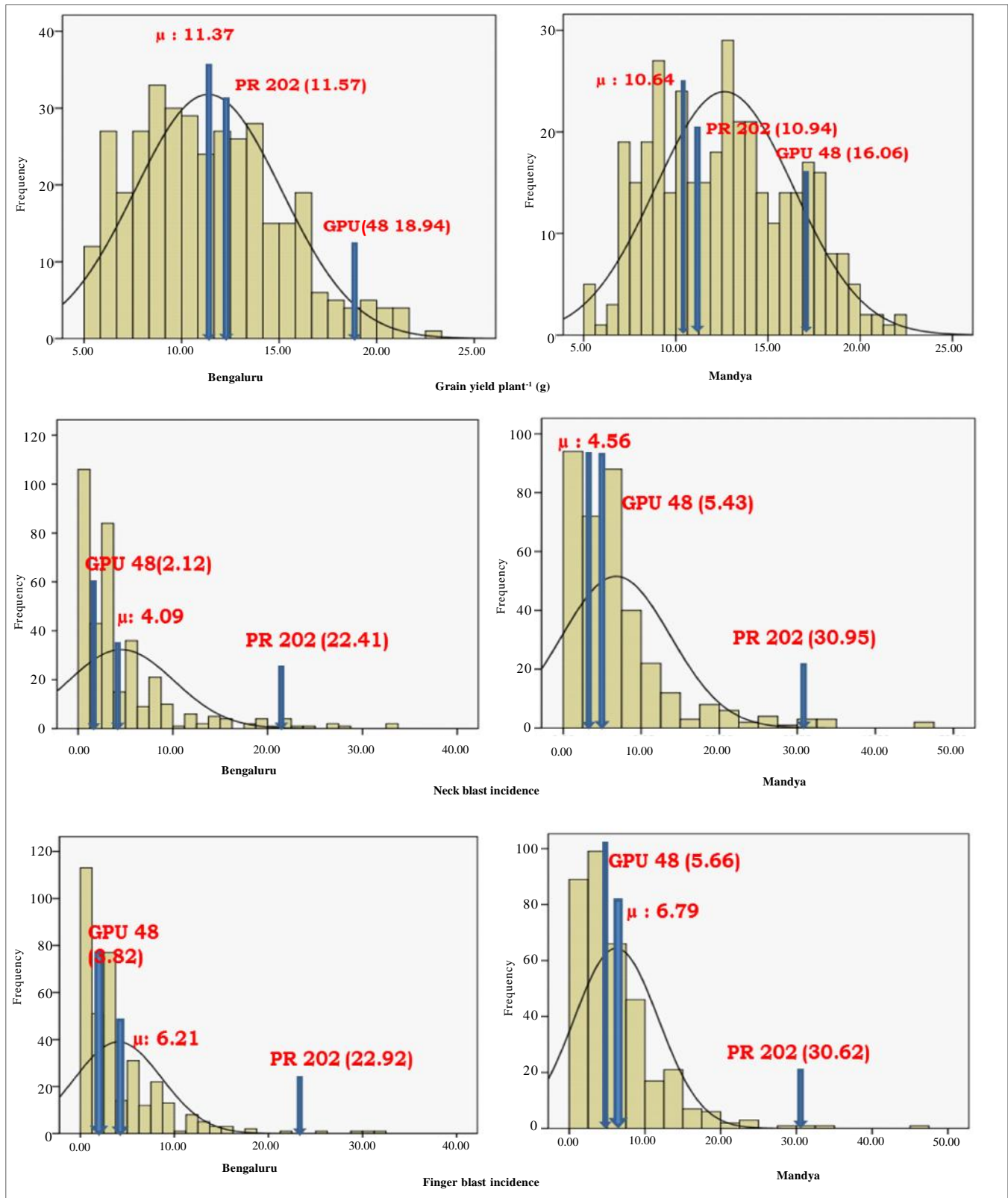


Fig. 1 : Frequency distribution of F₆ RILs for days to 50 per cent flowering, tillers plant⁻¹, plant height (cm), finger length (cm), grain yield plant⁻¹ and blast incidence (g) at Bengaluru and Mandya

also influences greatly the magnitude of heritability estimates. A most misleading estimate of phenotypic variance for disease and yield under natural conditions would have been estimated from a single test, resulting in the confounding of the interaction variances with progeny variance. The estimates of genetic advance may be biased upward, if phenotypic variance contains a fraction of genetic variance due to non-additive effects

(dominance or epistasis) if present (Hanson *et al.*, 1956). Therefore, it is necessary to minimize interaction by evaluating in different location. According to Johnson *et al.* (1955), heritability estimates along with genetic gain would be more useful than the former alone in predicting the effectiveness of selection. Therefore, it is essential to consider the predicted genetic advance along with heritability estimate as a tool in selection programme for

Table 2: Descriptive statistics for grain yield and its component traits among F₆ RILs of finger millet

Traits	Days to 50% flowering		Tillers plant ⁻¹		Plant height (cm)		Finger length (cm)		Grain yield plant ⁻¹ (g)		Neck blast incidence (%)		Finger blast incidence (%)	
	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya	Bengaluru	Mandya
Mean ± SE	62.85± 0.12	59.67± 0.15	3.48± 0.03	2.74± 0.02	99.38± 0.50	101.17± 0.40	5.53± .04	5.29± 0.03	11.37± 0.19	10.64± 0.20	4.56± 0.30	6.79± 0.37	4.05± 0.24	6.21± 0.30
Variance	5.29	7.59	0.39	0.19	89.27	58.9	0.59	0.33	14.17	14.04	30.87	48.51	21.22	31.10
Skewness	-0.23	0.64	0.24	0.53	0.08	-0.45	0.08	0.04	0.53	0.23	2.36	2.37	2.72	2.46
Kurtosis	-0.02	-0.19	0.04	0.30	-0.26	0.39	-0.25	-0.03	-0.25	-0.77	6.44	7.59	10.57	9.96
Minimum	56.18	53.93	1.94	1.78	71.31	76.87	3.59	3.62	5.03	5.02	0.00	0.00	0.00	0.00
Maximum	69.18	67.59	5.37	4.28	128.38	121.73	7.94	6.92	22.53	22.32	32.56	46.50	32.40	45.65
Standardised range	0.21	0.23	0.99	0.91	0.57	0.44	0.79	0.62	1.54	1.63	7.14	6.85	8.00	7.35
GCV (%)	2.97	4.25	16.76	14.34	8.81	7.06	11.62	9.76	30.42	27.99	114.56	96.29	69.32	83.92
PCV (%)	3.34	4.43	17.03	15.02	8.96	7.25	13.07	10.12	32.52	28.15	116.10	97.76	111.64	85.61
h ² _(bs)	0.79	0.92	0.96	0.91	0.96	0.94	0.79	0.92	0.87	0.91	0.97	0.97	0.39	0.96
Expected GAM (%)	5.45	8.42	33.99	28.21	17.85	14.14	21.29	19.38	58.63	57.35	232.86	195.35	88.67	169.48

Table 3: Mean of the best ten F₆ RILs for grain yield and its component traits at Bengaluru and their responses to blast disease

RILs identity	Grain yield plant ⁻¹ (g)	Days to 50% flowering	Tillers plant ⁻¹	Plant height (cm)	Finger length (cm)	Neck blast incidence (%)	Finger blast incidence (%)
281	22.53	64.85	3.47	98.87	5.57	3.25	6.50
143	21.36	62.18	2.68	96.35	6.09	0.56	0.58
324	21.33	66.18	2.64	115.18	6.95	0.52	0.50
81	20.99	64.52	3.91	87.71	6.17	0.00	0.20
104	20.93	61.18	3.97	113.31	6.56	1.20	0.56
94	20.79	59.52	3.91	112.38	5.67	8.40	9.58
158	20.36	61.18	3.33	88.35	6.69	0.00	0.52
229	20.29	66.18	3.33	102.21	6.70	8.25	3.50
355	20.24	64.18	3.64	80.84	6.53	0.00	0.25
102	19.93	65.18	3.64	106.31	4.86	0.56	1.20
PR 202 (Susceptible parent)	11.57	66.44	2.85	112.66	5.33	22.41	22.92
GPU 48 (Resistant parent)	18.94	58.83	3.28	95.62	6.21	2.12	3.82
Ckeck (GPU 28/KM 252)	19.17	66.28	3.58	112.46	5.94	43.26	43.11
S.E.±	0.20	0.12	0.03	0.50	0.04	0.29	0.24
C.D. (P=0.05)	0.52	0.29	0.10	1.47	0.11	0.72	0.58

better efficiency.

All the traits studied exhibited higher broad sense heritability for both locations (Table 2). The traits such as days to 50 per cent flowering exhibited low GAM at both locations. Moderate GAM was observed for plant height and finger length at Mandya. The remaining traits like number of tillers, grain yield plant⁻¹, neck blast incidence and finger blast incidence at both locations and finger length at Bengaluru location exhibited higher

GAM.

The grain yield and its components traits like, finger length and tillers plant⁻¹ and blast disease incidences showed higher GAM coupled with high heritability indicated that, the variations are highly heritable and selection would be effective for these traits. The higher estimates of heritability coupled with high GAM was reported by earlier researchers for finger length and grain weight (Ganapathy *et al.*, 2011; Nandini *et al.*, 2010 and

Table 4: Mean of the best ten F₆ RILs for grain yield and its component traits at Mandya and their responses to blast disease

RILs identity	Grain yield plant ⁻¹ (g)	Days to 50% flowering	Tillers plant ⁻¹	Plant height (cm)	Finger length (cm)	Neck blast incidence (%)	Finger blast incidence (%)
103	22.32	60.26	3.51	110.63	5.88	4.26	3.45
182	22.32	62.93	3.18	104.63	5.68	5.36	3.38
231	21.45	62.93	2.68	104.93	4.88	5.35	3.57
114	21.02	64.26	2.51	105.53	5.08	0.00	0.57
110	20.82	58.26	2.11	98.23	5.18	8.68	8.49
101	20.22	64.26	2.91	104.03	4.88	13.20	5.91
184	20.02	61.93	2.98	102.53	4.38	3.65	3.11
126	19.88	56.59	2.21	94.33	5.22	7.21	4.54
106	19.82	58.26	2.11	102.23	5.48	4.26	3.56
102	19.62	60.26	2.71	107.63	6.48	2.63	1.54
PR 202(Susceptible parent)	11.6	62.89	2.57	101.77	4.94	28.45	30.28
GPU 48(Resistant parent)	14.06	56.67	2.98	84.75	5.18	5.43	5.66
Ccheck(GPU 28/KM 252)	15.47	63.22	3.18	102.18	5.34	63.09	62.44
S.E.±	0.20	0.15	0.02	0.40	0.03	0.37	0.29
C.D. (P=0.05)	0.53	0.38	0.05	0.98	0.10	0.91	0.71

Table 5: Number of F₆ RILs corresponding to different disease response groups in finger millet

Disease response groups	Neck blast incidence (%)		Finger blast incidence (%)	
	Bengaluru	Mandya	Bengaluru	Mandya
Highly resistant (0)	53	46	12	1
Resistant (<5.00)	195	120	243	187
Moderately resistant (5.01-10.00)	76	128	78	112
Moderately susceptible (10.01-25.00)	31	53	23	56
Susceptible (25.01-50.00)	5	13	4	4
Highly susceptible (> 50.00)	0	0	0	0

Table 6 : Estimates of mean blast disease response of RILs classified into different disease incidence groups in F₆ generation of finger millet

Disease response groups		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 (%)	Bengaluru	0.00	2.32	6.96	16.61	29.28	-	0.00
	Mandya	0.00	2.97	6.93	14.79	32.18	-	0.00
Finger blast incidence (%)	Bengaluru	0.00	1.91	7.11	13.92	29.57	-	0.00
	Mandya	0.00	2.63	6.99	14.68	35.03	-	0.00

Sonnad *et al.*, 2007). Nandini *et al.* (2010) reported in moderate to high broad sense heritability for days to 50 per cent flowering, finger length and high broad sense heritability for plant height in F₂ generation of four crosses of finger millet.

The traits like days to 50 per cent flowering, finger length and grain yield plant⁻¹ exhibited negative kurtosis value which means these traits displays platykurtic distribution and are governed by many numbers of genes. However, tillers plant⁻¹, displays the positive kurtosis value indicating the leptokurtic distribution and are governed by few numbers of genes. Positive skewness values were observed for number of tillers, grain yield plant⁻¹, neck blast incidence and finger blast incidence at both locations (Table 2). Dhanalaxmi (2009) reported negative skewness and positive kurtosis value for days to 50 per cent flowering and plant height. While, negative skewness and kurtosis value were observed for grain yield plant⁻¹. Based on the F₆ RILs performance at two locations ten best RILs were identified for grain yield at Bengaluru (Table 3) and Mandya (Table 4) locations.

Based on blast disease indices F₆ RILs were grouped into different response groups. Most of the RILs fell into resistant and moderately resistant groups at Bengaluru. A very few (12 and 1) RILs fell into highly resistant group and most (243 and 187) of RILs fell into resistant groups for neck and finger blast, respectively (Table 5) at Mandya. The differences at the two locations could be due to differences in environmental conditions, since Kiran *et al.* (2013) indicated that blast pathogen depends on weather variables such as temperature and relative humidity for infection and spread of disease. One way ANOVA indicated significance of differences in mean disease indices of RILs into different response groups (Table 6) and further suggested efficiency of classification of response groups.

Conclusion :

In a low value crop like finger millet, breeding for resistance is very useful. Identification of high yielding and disease resistant RILs from the finger millet RIL population would permit a better chance of success in finger millet improvement in developing new cultivars. They could be used in breeding finger millet for higher grain yield potential with blast disease resistance.

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