

## Development of a Field Screening Technique and Identification of Blast Resistance in Finger Millet Core Collection

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### Abstract

Effective management of blast disease in finger millet can best be achieved through host-plant resistance. In this study, field screening technique was developed and core collection evaluated to identify sources of resistance to blast. The field screening technique involved: use of systematic susceptible checks after every four test rows, artificial spray inoculation at pre-flowering stage with an aqueous conidial suspension ( $1 \times 10^5$  spores  $ml^{-1}$ ) of *Magnaporthe grisea* fm strain multiplied on oatmeal agar medium at  $27 \pm 1^\circ C$  for 10 days, and maintaining high humidity and leaf wetness through sprinkler irrigation twice a day for 4 weeks following inoculation. Neck blast was recorded on a 1–5 scale and finger blast as severity percentage on all the tillers of selected 10 plants in a row at physiological maturity. The finger millet core collection consisting of 622 accessions was evaluated for neck and finger blast resistance. Among the core collection, 402 accessions were found resistant to neck blast, 436 to finger blast and 372 had combined resistance to both the diseases. Blast resistant accessions belonged to one wild and four cultivated races of finger millet that originated from 19 countries indicating the wide geographical diversity among resistant accessions. Most of the accessions from Asian origin were susceptible to neck and finger blasts while, those from African origin were resistant. A significant strong positive correlation ( $r = 0.85$ ,  $P < 0.0001$ ) was found between neck blast and finger blast ratings. Core collection accessions with stable resistance to blast would be useful for finger millet breeding programs.

**Keywords:** Core collection, finger millet, blast resistance, field screening technique

### Introduction

Finger millet [*Eleusine coracana* (L) Gaertn.] is the main staple food of millions of poor people in the arid and semi-arid tropical regions of Africa and Asia besides having high value for the straw. It is widely cultivated in India, Srilanka, Malaysia, China, Myanmar, Nepal and Japan in Asia, and Kenya, Uganda, Tanzania, Ethiopia, Eritrea, Rwanda, Democratic Republic of Congo, Zaire, Eritrea, and Somalia in Africa. As production statistics for the nine cultivated millets are often combined, reliable estimates of the areas sown to individual species are difficult to find. It was estimated that finger millet accounts for 10% of 38 million ha sown to millets globally (Mgonja *et al.*, 2007). In India, the important finger millet growing states are Karnataka, Odisha, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttarakhand, Uttar Pradesh and Bihar. The total area under

finger millet (ragi) in India is about 2.8 million ha with an annual production of about 2.78 million tons (Nagaraja *et al.*, 2007) and nearly half of the area is in Karnataka (Nagaraja *et al.*, 2008). Nutritionally, finger millet is equal or superior to other staple cereals, especially in minerals. This cereal is important for pregnant women, nursing mothers and children (National Research Council 1996) and could also help and sustain the malnourished people as it is recognized as an important dietary supplement for HIV positive people. Upadhyaya *et al.*, (2011) identified accessions with high protein, calcium, iron and zinc from the core collection of finger millet for use in crop improvement programs.

Of the several diseases that afflict finger millet, blast disease caused by *Magnaporthe grisea* (anamorph-*Pyricularia grisea* (Cooke) Sacc.) is a major problem and most

destructive disease in India and Africa causing substantial yield losses to the crop. Blast affects finger millet at all stages of growth and most of the landraces and a number of other varieties are highly susceptible to it. Average loss has been reported to be around 28-36% (Vishwanath *et al.*, 1986 and Nagaraja *et al.*, 2007), and yield losses could be as high as 80-90% in endemic areas (Bisht, 1987 and Rao, 1990). Use of host plant resistance is the most feasible and economical means of managing this disease as the crop is mainly cultivated by resource-poor farmers. Development of efficient and effective screening techniques based on the basic knowledge of pathogen biology and epidemiology for evaluation of germplasm is critical to a successful breeding program for blast resistance. The success of such a program depends on the identification of stable resistance sources and its subsequent utilization in breeding. Availability of adequate genetic variation is a prerequisite for genetic improvement of any crop species. Germplasm accessions collected and maintained in gene banks represent vast genetic variation that can be utilized in crop improvement. To overcome the need for large-scale evaluation of germ plasm collections against various biotic and abiotic stresses, Frankel and Brown (1984) proposed the concept of a core collection (10% of the entire collection) to minimize repetitiveness within the collection and to represent the genetic diversity of a crop species. Following the methods described by Frankel and Brown (1984) and Brown (1989), Upadhyaya *et al.*, (2006) established a core collection in finger millet, which consists of 622 accessions representing geographical regions and biological races from the entire collection of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Therefore, in this study we developed field screening technique and evaluated finger millet core collection to identify sources of resistance to blast.

## Materials and methods

The culture of *M. grisea* was obtained from the diseased sample collected from the finger millet fields at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The pathogen was purified through single-spore isolation and maintained on Oat-meal agar medium for further use. Mass multiplication of fungal spores for inoculation was achieved by growing the fungus (9 discs/plate) on OMA medium at  $27\pm 1^\circ\text{C}$  for 10 days. The conidial concentration in the suspension was adjusted to  $1\times 10^5$  spores  $\text{ml}^{-1}$  and Tween 20 (0.02%) (Jia *et al.*, 2003) was added to the suspension just before the inoculation.

The experiment consisting of 622 accessions with standard checks (VL 149, VR 708, RAU 8 and PR 202) was conducted in field by artificial inoculation with the blast

pathogen at pre-flowering stage of the crop during the rainy season 2009 at ICRISAT, Patancheru, and blast severity was recorded on 619 accessions (3 accessions could not get established).

**Field screening.** Each accession was grown in one row of 2 m length with row-to-row spacing of 60 cm and plant-to-plant spacing within the row of 10 cm with two replications in completely randomized block design. Plants were thinned to 20 plants/row at 15 days after planting and other agronomic practices were followed as per local practices. Systematic susceptible checks (VR 708 and VL 149) were planted on every 5<sup>th</sup> row alternately. Plants were spray-inoculated at pre-flowering stage with an aqueous conidial suspension (about  $1\times 10^5$  spores  $\text{ml}^{-1}$ ) of *M. grisea*. High humidity was provided by perfo-irrigation twice a day on rain-free days, 30 min each between 10:00 and 12:00 noon, and 4:00 and 6:00 P.M. to promote disease development. The neck and finger blast severity was recorded (10 individual plants in each replication) at dough stage using a 1–5 progressive scale for neck blast (1 = no lesions to pin head size of lesions on the neck region, 2 = 0.1–2.0 cm; 3 = 2.1–4.0 cm; 4 = 4.1–6.0 cm and 5 = >6.0 cm of lesions on the neck region) and per cent finger blast severity across all tillers in selected individual plants in a row.

## Results and discussion

Under favourable conditions, foliar blast occurred in a number of accessions at the seedling stage, which did not correlate well with crop growth stages and maturity of the plants, probably because of buildup of adult plant resistance. Hence, neck and finger blast that are more destructive were considered as measures of blast resistance. Neck blast scores ranged from 1.0 to 5.0 on a 1–5 scale in core collection compared to the 4.7 and 4.9 on the susceptible checks, VR 708 and VL 149, and 1.7 and 1.9 on resistant checks PR 202 and RAU 8 respectively. Finger blast severity ranged from 0 to 64% compared to 30.7 and 30.1% on the susceptible checks, VL 149 and VR 708, and 8.5 and 10.5% on resistant checks PR 202 and RAU 8 respectively. In general, neck and finger blast severity in susceptible checks was quite high indicating adequate disease pressure for an effective field screening.

**Resistance to neck blast.** Of 619 accessions, 11 were found highly resistant (score 1.0 on a 1–5 scale), 391 resistant (score 1.1–2.0), 171 moderately resistant (score 2.1–3.0), 35 susceptible (score 3.1–4.0) and remaining 11 were highly susceptible (score 4.1–5.0) to neck blast (Table 1).

**Resistance to finger blast.** Of 619 accessions, 57 were highly resistant (0–1%), 379 resistant (2.0–10%), 133

**Table 1. Race/subrace of finger millet core collection and their reaction to blast under field conditions during the rainy season 2009 at ICRISAT, Patancheru**

Race/subrace	No. of accessions <sup>a</sup>	Neck blast reaction <sup>b</sup>					Finger blast reaction <sup>c</sup>				
		HR	R	MR	S	HS	HR	R	MR	S	HS
<i>Compacta</i>	75	1	54	17	2	1	9	54	11	-	1
<i>Elongata</i>	50	5	29	13	2	0	3	26	14	3	3
<i>Laxa</i>	16	2	11	3	-	-	1	11	4	-	-
<i>Reclusa</i>	21	3	9	7	1	-	2	12	1	2	3
<i>Sparsa</i>	13	-	9	3	1	-	-	3	9	1	-
<i>Plana</i>	102	1	79	18	1	1	18	70	11	-	1
<i>Confundere</i>	81	1	64	15	-	-	15	58	7	-	-
<i>Grandigluma</i>	5	-	3	1	-	-	1	2	1	-	-
<i>Seriata</i>	16	-	12	2	1	1	2	10	3	-	1
<i>Vulgaris</i>	379	4	224	115	28	8	27	221	92	26	13
<i>Digitata</i>	122	2	83	27	10	-	7	82	22	10	1
<i>Incurvata</i>	163	2	95	60	4	2	16	96	43	6	2
<i>Liliacea</i>	34	-	16	11	7	-	2	13	11	6	2
<i>Stellata</i>	60	-	30	17	7	6	2	30	16	4	8
<i>Africana</i>	16	-	5	8	2	1	1	7	5	1	2
Total	622	11	391	171	35	11	57	379	133	30	20

<sup>a</sup> Three entries data not available

<sup>b</sup> Neck blast reaction based on 1-5 scale: 0-1.0: Highly resistant (HR); 1.1-2.0: Resistant (R); 2.1-3.0: Moderately Resistant (MR); 3.1-4.0: Susceptible (S); 4.1-5.0: Highly Susceptible (HS)

<sup>c</sup> Finger blast severity (%): 0-1.0: Highly resistant (HR); 2.0-10: Resistant (R); 11-20: Moderately Resistant (MR); 21-30: Susceptible (S); >30: Highly susceptible (HS)

moderately resistant (11–20%), 30 susceptible (21–30%) and 20 highly susceptible (>30%) to finger blast (Table 1).

**Resistance to both neck and finger blast.** A total of 372 accessions had combined resistant to both neck and finger blast. The resistant accessions belongs to five basic races of finger millet, *compacta* 53 out of 75, *plana* 76 of 102, *vulgaris* 212 out of 379, *elongata* 26 out of 50, *africana* 5 out of 16 (Table 1). Among the 76 resistant accessions in race *plana* belongs to three subraces, *confundere* (62), *grandigluma* (2), *seriata* (12). Of the 212 accessions in race *vulgaris* represented four subraces, *digitata* (80), *incurvata* (91), *liliacea* (14), *stellata* (27). Of the 26 resistant accessions in race *elongata* belongs to 3 sub-races, *laxa* (12), *reclusa* (11), *sparsa* (3).

Blast resistant accessions in the core collection originated from 19 countries indicating the wide geographical diversity among the resistant accessions (Table 2). Among the 402

neck-blast resistant accessions, 290 of the 365 accessions (79.5%) from Africa, 85 of the 223 accessions (38.1%) from Asia, 4 of the 5 (80%) from America, 6 of the 7 (85.7%) were of the European origin and the remaining 17 were of unknown origin. Of the 436 finger blast resistant accessions, 314 of the 365 accessions (86%) from Africa, 92 of the 223 (41.2%) from Asia, 4 of the 6 (66.6%) from America, 6 of the 7 (85.7%) were from Europe and the remaining 20 of unknown origin. Most of the accessions from Asian origin were found susceptible to neck and finger blast. A total of 372 accessions (60%) had combined resistance to neck and finger blast originating from Burundi, Ethiopia, Germany, India, Italy, Kenya, Malawi, Mozambique, Nepal, Nigeria, Senegal, Srilanka, Tanzania, United Kingdom, Uganda, United States of America, Zaire, Zambia and Zimbabwe (Table 2).

A significant strong positive correlation ( $r = 0.85$ ,  $P < 0.0001$ ) was observed between neck blast and finger blast ratings

**Table 2. Origin of finger millet core collection and their reaction to blast disease under field conditions during the rainy season 2009 at ICRISAT, Patancheru**

Country of origin	No. of accessions	Neck blast severity (1-5 scale)		Finger blast severity (%)	
		Range <sup>a</sup>	No. <sup>b</sup>	Range <sup>a</sup>	No. <sup>b</sup>
<b>Africa</b>	<b>365</b>	-	<b>290</b>	-	<b>314</b>
Burundi	3	1.2- 4.7	2	1.1-42.7	1
Cameroon	1	3.0	-	40	-
Ethiopia	3	1.0-3.0	1	1.5-22.5	1
Kenya	107	1.0-3.4	78	0-20.5	94
Malawi	25	1.0-2.5	21	1.5-12.5	21
Mozambique	1	1.7	1	7	1
Nigeria	5	1.0-1.8	5	0-12	4
Senegal	1	1.4	1	4	1
South Africa	1	2.4	-	18.5	-
Tanzania	3	1.2-2	3	3-12.5	2
Uganda	81	1.0-3.0	68	0-19.5	75
Zaire	1	2.0	1	4.5	1
Zambia	21	1.0-2.6	17	0-18.5	19
Zimbabwe	112	1.1-2.6	92	0-18.5	94
<b>Asia</b>	<b>223</b>	-	<b>85</b>	-	<b>92</b>
India	149	1.2-4.9	50	0.5-64	58
Maldives	1	2.8	-	31	-
Nepal	70	1.1-4.9	34	2.0-60	32
Pakistan	1	2.5	-	11	-
Sri Lanka	2	1.5-2.1	1	3.2-10.5	2
<b>Americas (USA)</b>	<b>5</b>	<b>1.3-2.2</b>	<b>4</b>	<b>3.5-11</b>	<b>4</b>
<b>Europe</b>	<b>7</b>	-	<b>6</b>	-	<b>6</b>
Germany	1	2.0	1	1.5	1
Italy	3	1.5-1.9	3	1-7.5	3
United Kingdom	3	1.8-2.2	2	4.5-11	2
<b>Unknown</b>	<b>22</b>	<b>1.3-2.3</b>	<b>17</b>	<b>1-13</b>	<b>20</b>
Total	622	-	402	-	436
SE m $\pm$ <sup>c</sup>	-	0.31	-	4.18 <sup>c</sup>	-

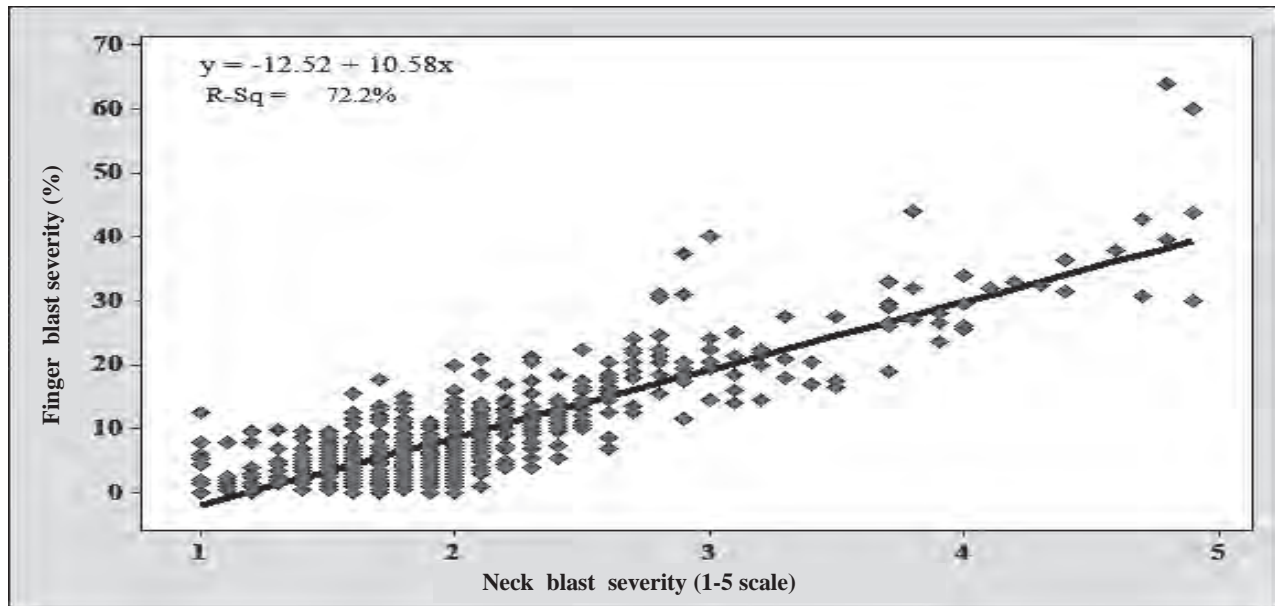
<sup>a</sup> Based on the mean of two replications

<sup>b</sup> No. = Number of resistant accessions

<sup>c</sup> Standard error (SE) of individual accession mean over replications

(Figure 1). Recording the blast severity using these two scales provided realistic data under field conditions at the right stage of the crop (physiological maturity) and also possible ability of the same gene(s) to induce resistance to

both neck and finger blast. It could be suggested that there is no isolate or strain specificity for causing neck or finger blast. This is an important finding on the significant role of neck infection to the finger blast development. Thus for rapid



**Figure 1. Relationship of neck and finger blast severity of finger millet core collection under field conditions during the rainy season 2009 at ICRISAT, Patancheru**

evaluation of finger millet lines either of the two recordings should suffice for resistance evaluation. Such information on correlation between neck and finger infection of this disease is very limited in the literature.

In this study, we developed the field screening technique for neck and finger blast whereby finger millet germplasm can be effectively screened in the field conditions. Field screening has been reported (Govindu *et al.*, 1971) to identify blast resistance in finger millet under natural infection (Mantur and Madhukeshwara, 2001; Takan *et al.*, 2004; Kumar *et al.*, 2006; Nagaraja and Mantur, 2007; Kumar and Kumar, 2009; Nagaraja *et al.*, 2010) and no artificial inoculation was made. Screening under natural infection condition may provide escapes and the true resistance may not be identified. In this study, we developed field screening technique involved artificial inoculation of plants at appropriate stages and favourable conditions (temperature and relative humidity) were provided for disease development that greatly minimize the chances of escape from infection.

Proper and precise disease assessment and evaluation procedures are critical for identification of resistant genotypes. Artificial inoculation usually generates high disease pressure that allows easy distinction of genotypes into different groups and it is important to develop a rating method that fully describes the range of infection responses. The neck and finger blast were routinely assessed at dough stage of the crop based on percentage of ears showing

infection on the neck and fingers over total number of neck and fingers in a row (Kumar and Kumar, 2009; Nagaraja *et al.*, 2010).

We developed a more precise 1–5 rating scale for neck blast and estimation of finger blast severity (%) based on severity under field conditions to categorize accessions into highly resistant, resistant, moderately resistant, susceptible and highly susceptible. These scales were very effective, easy, and convenient and provided good correlation between neck and finger blast severity. This is a significant step towards simplifying the screening process in terms of improving precision of disease scoring and economizing on time and resources. This procedure would also increase the pace of screening germplasm accessions and improve efficacy of blast resistance breeding in finger millet.

Sources of blast resistance have been reported in finger millet, and efforts have been made to incorporate resistance into improved cultivars and elite breeding lines (Seetharam and Halaswamy, 2003; Nagaraja *et al.*, 2008; Nagaraja and Mantur, 2007). Although, good number of high yielding blast varieties like GPU 28, GPU 45 and GPU 48 are released for cultivation and it is likely that resistance may break down owing to development of new pathotypes. Levy *et al.*, (1993), in case of rice-blast pathosystem indicated that to understand the mechanisms of frequent breakdown of resistance in blast resistant cultivars, studies on the extent of genetic diversity present in the population of *M. grisea* in a specific geographical region is important. Development



of durable blast resistance for environments highly conducive for the disease should be possible, if breeding programs are based on a complete understanding of pathogen diversity in the target area. Substantial work has been done with the rice-blast pathosystem on pathogenic and genetic diversity, epidemiology and disease management through host-plant resistance. However, such studies are very limited with the finger millet-blast pathosystem.

There are several reports, where core or mini-core collections (10% core or 1% entire collection; Upadhyaya and Ortiz, 2001) have successfully been used to identify resistance to diseases (Holbrook and Anderson, 1995; Franke *et al.*, 1999; Neill and Bauchan, 2000; Grunwald *et al.*, 2003; Pande *et al.*, 2006; Silvar *et al.*, 2009; Damicone *et al.*, 2010; Sharma *et al.*, 2010). Xia *et al.* (2010) identified 188 rice blast resistant accessions from primary core collection of Chinese rice germplasm. Utilizing a core collection enables a subset of accessions to be screened more efficiently for disease resistance (Franke *et al.*, 1999). The core collection can be used as a starting point to screen accessions for resistance to a particular disease. It would be desirable to screen the core collection at different locations in India and elsewhere and confirm the resistance under greenhouse conditions. A subsample of the core collection possessing stable resistance to blast can be useful for finger millet breeding programs.

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