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Resistance to blast (*Magnaporthe grisea*) in a mini-core collection of finger millet germplasm

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Abstract Blast caused by *Pyricularia grisea* [teleomorph: *Magnaporthe grisea*] is an economically important and widespread disease of finger millet in the world. Host resistance is the most economical and effective means of combating this disease as finger millet is predominantly grown by resource-poor and marginal farmers. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), we evaluated a finger millet mini-core collection of 80 germplasm accessions (about 1 % of the total germplasm collection representing major trait variability) for blast resistance both in the field and greenhouse. Field evaluation was done using a refined screening technique that included new improved rating scales for leaf, neck and finger infection. Sixty six of the 80 accessions showed combined resistance to leaf, neck and finger blast in two seasons (2009 and 2010) of field screening. A highly significant and positive correlation was found between neck and finger blast ratings (r=0.92), whereas small but significant correlations were found between leaf blast and neck blast (r=0.25) and between leaf blast and finger blast (r=0.30). These accessions were also screened for leaf blast resistance in the greenhouse by artificial inoculation of seedlings to confirm field observations. Fifty-eight of the 80 accessions were resistant to leaf blast in the greenhouse screen as well. These resistant accessions represented one wild (*africana*) and four cultivated races (*vulgaris*, *plana*, *elongate* and *compacta*) of finger millet that originated from 13 countries in Asia and Africa and exhibited considerable diversity for agronomic traits, such as maturity period, plant height and panicle type. These blast resistant accessions from the mini-core collection would be useful in finger millet disease resistance breeding programs.

Keywords Blast disease · Finger millet · Host plant resistance · Mini-core collection and rating scales

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Introduction

Finger millet [*Eleusine coracana* (L.) Gaertn.], a widely grown traditional and highly nutritious grain cereal cultivated in the semi-arid areas of Eastern and Southern Africa and South Asia, is a staple food for millions of poor people. The precise data on world area under finger millet are not available, because it is frequently reported with other millets including pearl millet (in the FAO database). However, as per the estimate by the Consultative Group on International Agricultural Research (CGIAR), finger millet contributes to about 10 % of

the total area (34.6 mha) planted to millets (FAO 2004). In India, finger millet ranks next to pearl millet and is cultivated on 2.6 mha area with a production of about 3.0 mt (www.indiastat.com). Finger millet is being increasingly recognized as a highly nutritious food for the weak and immuno-compromised people (Takan et al. 2012). The grains are rich source of protein (Malleshi and Klopfenstein 1998), fibre, minerals (calcium, iron, zinc, and manganese) and amino acids (tryptophan, cystine, and methionine), which are crucial to human health and growth, and these are deficient in most cereals. The nutritional quality of finger millet grain makes it an ideal food for expectant women, lactating mothers, children, the sick, and diabetics (National Research Council 1996). Thus, finger millet plays the key role in the livelihood of small-holder farmers in the semi-arid areas of Africa and Asia. However, diseases and insects, in addition to abiotic stresses, are major impediments toward realizing the high yield potential of finger millet cultivars.

Among the diseases, blast caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Hebert) Barr.] is very prominent and affects the productivity, utilization and trade of finger millet within Eastern and Southern Africa (Mgonja et al. 2007) and South Asia. The average loss due to blast has been reported to be around 28–36 % (Nagaraja et al. 2007), and in certain areas yield losses could be as high as 80–90 % (Vishwanath et al. 1986; Rao 1990). The disease affects the crop at all growth stages, and neck and panicle blast are the most destructive form of the disease (Pande et al. 1995; Takan et al. 2004, 2012). The most susceptible stage for leaf blast is seedling stage, whereas for neck and finger blast it is pre-flowering stage. Growing cultivars with durable resistance is the best means of combating the blast disease of finger millet. Resistance is often assessed at the seedling stage, which did not correlate well with neck and finger infection. Hence, neck and finger blast which are more destructive than leaf blast were considered important parameters for blast resistance (Nagaraja et al. 2007). Resistance in finger millet to *M. grisea* is often evaluated in the field under natural infection (Somashekhara et al. 1991; Takan et al. 2004; Mgonja et al. 2007; Nagaraja et al. 2007, 2010) and no systematic artificial inoculation was made. Screening under natural infection condition may provide escapes and the true resistance may not be identified (Thakur et al. 2009). The neck and finger blast are routinely assessed at the dough stage of the crop as per cent disease incidence *i.e.*, number of plant infected (incidence does not differentiate levels of susceptibility—number and size of lesions across test lines).

The finger millet core collection (about 10 % of the total germplasm collection) consists of 622 accessions representing geographical regions and biological races from the entire germplasm collection available at ICRISAT (Upadhyaya et al. 2006). Further, a mini-core (10 % of core and 1 % entire collection) was developed (Upadhyaya and Ortiz 2001) to facilitate easy evaluation of germplasm accessions for various traits, including disease resistance. This mini-core evaluation approach has successfully been used to identify resistance sources for various diseases (Pande et al. 2006; Sharma et al. 2010, 2012a, b), drought (Kashiwagi et al. 2005; Krishnamurthy et al. 2010) and salinity (Vadez et al. 2007). Similarly, a finger millet mini-core consisting of 80 accessions (representing genetic diversity of the core collection and entire collection) developed at ICRISAT (Upadhyaya et al. 2010) was used in this study. The prime objective of this study were to develop a comprehensive disease severity assessment (rating scales) for leaf, neck and finger blast based on the qualitative and quantitative differences of lesions observed on finger millet plants infected with *M. grisea* and to evaluate the mini-core collection of finger millet in field and greenhouse by artificial inoculation in order to identify accessions having resistance to blast disease that could be utilized in resistance breeding programs.

Materials and methods

Seed source

Seed of the 80 germplasm accessions of the finger millet mini-core comprising of four cultivated races—*vulgaris* (53), *plana* (13), *elongata* (6) *compacta* (7) and one wild race *africana* (1), and sub races—*liliacea* (5), *stellata* (7), *incurvata* (23) and *digitata* (18) in race *vulgaris*; *seriata* (2), *confundere* (9) and *grandigluma* (2) in race *plana*; *laxa* (1), *reclusa* (4), and *sparsa* (1) in race *elongata* was obtained from Genebank, ICRISAT, Patancheru, India. The race *compacta* has no subraces (Prasada Rao and deWet 1997).

The finger millet mini-core was developed from a core collection of 622 accessions (www.icrisat.org). The core collection was evaluated for five qualitative and 15 quantitative traits at five agroecologically diverse

locations in India. The hierarchical cluster analysis of data using phenotypic distances resulted in 40 clusters. A mini-core collection of 80 accessions (sampling one to four accessions from 40 clusters) was formed using the sampling strategy of 10 % or a minimum of one accession from each cluster (Upadhyaya et al. 2010).

Pathogen and inoculum preparation

Inoculum was prepared from a single-spore representative culture of *M. grisea* isolated from blast infected samples collected from the finger millet fields during the rainy season 2008 at ICRISAT, Patancheru, India. Mass multiplication of fungal spores for inoculation was achieved by growing the fungus (nine discs per plate) on oat meal agar (OMA) medium at 26±1 °C for 10 days. Spores were harvested by flooding the plates with sterilized distilled water and scrapping the growth by a spatula. The spore suspension was adjusted to desired concentration (1×10^5 spores/ml) with the help of haemocytometer and a drop of a surfactant (Tween 20) was added to ensure the uniform dispersal of spores. The suspension was used for field and greenhouse inoculations.

Field screening

The 80 finger millet mini-core accessions along with checks (VR 708, VL 149, RAU 8 and PR 202) were evaluated in the finger millet blast nursery during the 2009 and 2010 rainy seasons in field by artificial inoculation at ICRISAT, Patancheru, India. Each accession was grown in two rows 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 a randomized complete block design (RCBD). In order to increase the disease pressure, the susceptible check variety (VR 708) was planted on every 5th row alternatively. Plants were thinned to 20 plants/row at 15 days after emergence. Thirty day-old-seedlings were inoculated by spraying the inoculum (1×10^5 spores/ml) on the foliage using a Knapsack power sprayer during the evening hours. High humidity (>90 % relative humidity) and leaf wetness was provided by sprinkler irrigation twice a day for 30 min each between 10:00 a.m. and 12:00 noon, and 4:00 p.m. and 6:00 p.m. to promote disease development. For neck and finger blast, plants were spray-inoculated at pre-flowering stage with an aqueous conidial suspension and procedure for providing favourable conditions as explained above up to physiological maturity.

Disease severity assessment

Leaf blast severity Infected leaves exhibiting uniform lesion types were photographed to make colour plates to aid in the classification of disease development. The leaf blast severity was recorded at 10 days after inoculation (DAI) using a progressive 1–9 scale, where 1 = no lesions to small brown specks of pinhead size (0.1–1.0 mm), less than 1 % leaf area affected; 2 = typical blast lesions covering 1–5 % leaf area covered with lesions; 3=6–10 %, 4=11–20 %, 5=21–30 %, 6=31–40 %, 7=41–50 %, 8=51–75 % and many leaves dead; and 9 = typical blast lesions covering >75 % leaf area or all the leaves dead (Fig. 1).

Neck blast severity Based on the relative lesion size on the neck a 1–5 progressive rating scale was

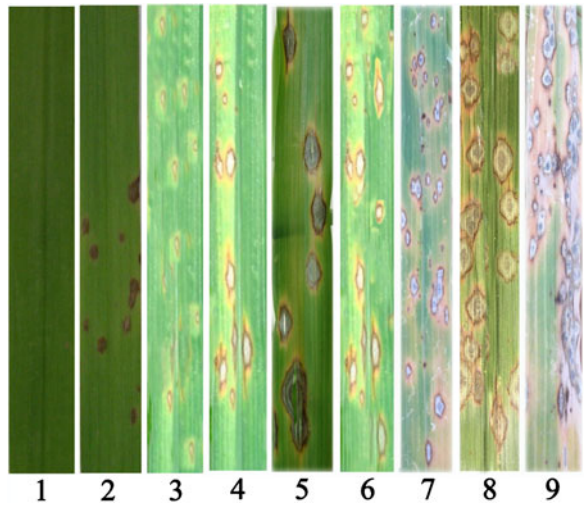

Fig. 1 Disease rating scale for leaf blast of finger millet seedlings infected with *M. grisea*. Disease rating scales are based on the per cent leaf area covered with blast lesions observed on 10 days after inoculation under field and greenhouse conditions. The 9-class disease rating scale is classified into four general categories of resistant (1.0–3.0), moderately resistant (3.1–5.0), susceptible (5.1–7.0) and highly susceptible (7.1–9.0) responses. Class 1 is the immune response in which no lesions were observed, class 2 consists of typical lesions covering 1–5 % of leaf area, class 3 consists 6–10 %, class 4 consists of 11–20 %, class 5 consists of 21–30 % class 6 consists of 31–40 %, class 7 consists of 41–50 %, class 8 consists of 51–75 % and class 9 consists of typical blast lesions covering >75 % of leaf area or all the leaves dead. The leaves depicted in the plates are 1.0× their original sizes

developed where, 1 = no lesions to pin head size of lesions on the neck region, 2=0.1–2.0 cm size of typical blast lesion on the neck region, 3=2.1–4.0 cm, 4=4.1–6.0 cm, and 5 = >6.0 cm size of typical blast lesion on the neck region (Fig. 2). Data were recorded in field at the physiological maturity on 10 randomly selected individual plants of each accession.

Finger blast severity The finger blast severity estimate was recorded as visual percentage of blasted florets across all tillers of a plant (Fig. 3) on the same 10 randomly selected plants that were earlier rated for the neck blast severity in each row.

Agronomic traits Data were also recorded for agronomic traits, such as days to flowering (DF) (time of full panicle emergence in 50 % of the plants in a row), plant height (measured from the base of the plant to the tip of the panicle at maturity), and spike type (compactness of the panicle at maturity *i.e.* top curved, incurved and long open) during 2010 by following the finger millet descriptor (IBPGR 1985).

Greenhouse screening

Resistance to leaf blast in mini-core germplasm accessions along with checks was confirmed under greenhouse screening. The seedlings were raised in 15-cm diameter plastic pots (10 seedlings/pot) filled with sterilized soil-sand-farm yard manure mix (2:1:1 by volume) in a greenhouse bay maintained at 28±2 °C. The 12-day-old seedlings were inoculated by using an atomizer to spray the conidial suspension on the leaves of finger millet plants. The conidial inoculum was applied just until the beginning of runoff from the foliage. Inoculated plants were placed in a moist chamber at 23± 1 °C. After inoculation for 48 h in the moist chamber, the plastic pots containing 10 inoculated finger millet plants, were transferred to a greenhouse bay and exposed to high humidity (>90 % RH) under misting for 10 days. Ten seedlings of each accession were tested in three replications (10 seedlings/pot) in a completely randomized design (CRD) and repeated twice to confirm the results. Leaf blast severity was recorded for 10 days after inoculation.

Data analysis

Data on leaf, neck and finger blast severity were recorded from each plot in the field experiments during 2009 and 2010. Statistical analysis was performed following the Residual Maximum Likelihood (REML) on GENSTAT statistical package (edition 14.0; Rothamsted Experiment Station, Harpenden, Herts AL52JQ, UK) for both years separately and on the combined data. For combining data across 2 years, Bartlett's test for homogeneity of error variance was done, which indicated that the error variances were homogeneous. Here, the year (environment) were considered as a fixed and genotype as random effect. Variance components due to genotype (σ_g^2), genotype ×

Fig. 2 A 1–5 rating scale based on lesion size on neck region for recording neck blast severity in finger millet infected with *M. grisea*. Neck blast rating scale based on relative size of lesions on the neck region under field conditions at the physiological maturity on 10 randomly selected individual plants of each accession. The 1-to-5 rating scale was classified into four infection types (*i.e.* 1.0–2.0 = resistant; 2.1–3.0 = moderately resistant; 3.1–4.0 = susceptible and 4.1–5.0 = highly susceptible). Class 1 = no lesions to pin head size of blast lesions on the neck region, class 2=0.1–2.0 cm size of typical blast lesion on the neck region, class 3=2.1–4.0 cm, class 4=4.1–6.0 cm and class 5 = >6.0 cm size of typical blast lesion on the neck region

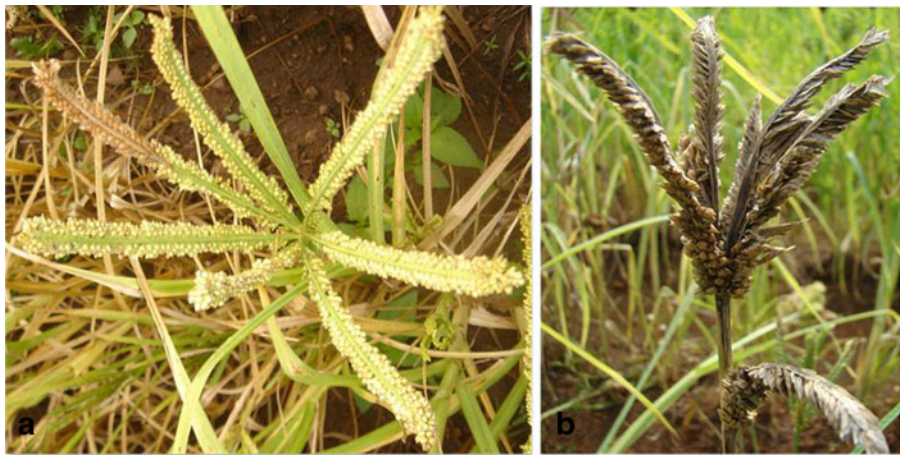

Fig. 3 The per cent finger blast severity for finger millet plants infected with *M. grisea*. **a** Out of 7 fingers, only half of the portion of finger is infected so, the per cent finger blast severity is 7 %. **b** Photograph showing more than 90 % finger blast severity

environment (years) interaction (σ_{ge}^2) and their standard errors were determined. The significance of the years (environment) was assessed using the Wald statistic (Wald 1943) that asymptotically follows a χ^2 distribution. Environment-wise best linear unbiased predictors (BLUPs) for the leaf, neck and finger blast severity of mini-core accessions were calculated. The associations between pairs of variables such as – leaf, neck and finger blast, plant height, days to flowering and spike type were determined in terms of Pearson's correlation coefficients using the PROC CORR procedure in SAS (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC).

Results

Development of new rating scales

For assessing leaf blast severity, a 1–9 rating scale was developed based on per cent leaf area covered with lesions (Fig. 1). The 1-to-9 rating scale was classified into four general categories of resistant (R) (1.0–3.0 score, Fig. 1-1–1-3), moderately resistant (MR) (3.1–5.0 score, Fig. 1-4 and -5), susceptible (S) (5.1–7.0 score, Fig. 1-6 and -7) and highly susceptible (HS) (>7.0 score, Fig. 1-8 and -9). This rating scale was used both in field and greenhouse screens. For assessing neck blast severity, a 1–5 rating scale was developed based on the lesion size on the neck region just below the fingers (Fig. 2). Although the size of lesions on neck region varied almost continuously, four general classes (1.0–2.0 = resistant; 2.1–3.0 = moderately resistant; 3.1–4.0 = susceptible and 4.1–5.0 = highly susceptible) were distinguished based on the relative size of lesions (Figs. 2.1–2.5). The finger blast severity percentage was classified into resistant (1.0–10 %), moderately resistant (10.1–20 %), susceptible (20.1–30 %) and highly susceptible (>30 %) (Fig. 3).

Field resistance to blast

Of the 80 finger millet mini-core germplasm collection evaluated for blast resistance (leaf, neck and finger) in field during the rainy seasons 2009 and 2010, blast severity was recorded on 78 accessions in 2009 (two accessions did not established) and on all accessions in 2010. The REML analysis indicated significant ($P<$ 0.001) variation among the accessions for leaf, neck and finger blast reactions in both the environments (2009 and 2010 seasons) separately as well as in the pooled data (Table 1), indicating high variation among the genotypes for blast resistance. However, in the pooled analysis, variance component due to genotype (σ_g^2) was non-significant for leaf blast, but highly significant for neck and finger blast. Although there was significant interaction between accession and years, the variance components due to genotypes (σ_g^2) was very high compared to G × E interaction (σ_{ge}^2) for neck and finger blast, indicating that differences in blast severity were mainly contributed by the accessions (Table 1).

Based on mean leaf blast severity for the two experiments (2009 and 2010), 78 accessions were found resistant (score 1.0–3.0 on 1–9 scale) and

Table 1 Estimates of variance due to genotype (σ^2g), genotype × environment ($\sigma^2g \times e$), and error (σ^2e) and their standard errors for leaf, neck and finger blast in the finger millet mini-core collection during 2009 and 2010 rainy seasons, ICRISAT, Patancheru, India

Disease	Season	σ^2g	s.e.	$\sigma^2g \times e$	s.e.	σ^2e	s.e.	Wald statistics (season)	F prob
Leaf blast	2009	0.094	0.031			0.161	0.026		
	2010	0.895	0.161			0.249	0.040		
	Pooled	0.034	0.067	0.526	0.101	0.203	0.023	11.07	0.002
Neck blast	2009	0.798	0.013			0.098	0.016		
	2010	0.987	0.161			0.101	0.016		
	Pooled	0.850	0.101	0.035	0.015	0.100	0.011	61.00	<0.001
Finger blast	2009	180.354	28.827			7.591	1.240		
	2010	141.282	22.516			7.157	1.139		
	Pooled	155.371	24.790	4.593	1.372	7.339	0.834	14.99	<0.001

two moderately resistant (score 3.1–5.0). Based on mean neck blast severity, 68 accessions were resistant (score 1.0–2.0 on a 1–5 scale), six moderately resistant (score 2.1–3.0), two susceptible (score 3.1–4.0) and four highly susceptible (score >4.0) compared to 4.6 and 4.4 score in susceptible checks VR 708 and VL 149, and 1.5 and 2.0 in resistant checks PR 202 and RAU 8, respectively. Sixty-nine accessions were resistant (finger blast severity 1–10 %), four moderately resistant (10.1–20 %), two susceptible (20.1–30 %) and five highly susceptible (>30 %) to finger blast compared to 76.8 and 40.7 % severity in susceptible checks VR 708 and VL 149, and 7.0 and 9.6 % in resistant checks PR 202 and RAU 8, respectively (Table 2).

Out of eight mini-core accessions, sixty-six were found to have combined resistance to all three phases of blast (leaf, neck and finger) in both the years and data of those accessions along with checks are presented in Table 2. These resistant accessions belong to four cultivated races of finger millet, *vulgaris* (43 out of 53), *plana* (10 out of 13), *compacta* (7), *elongata* (5 out of 6) and wild race *africana* (1) (Table 2). The finger millet mini-core accessions originated from 13 countries, which is an ideal pool of geographical diversity of resistance sources. Of the 66 resistant mini-core accessions, 21 accessions originated from Zimbabwe, 11 from India, 10 from Uganda, five each from Kenya and Nepal, four and three from Malawi and Zambia, respectively, and one each from Burundi, Germany, Nigeria, Senegal and United States of America (USA) and two were of unknown origin (Table 2).

Correlation between disease severity and agronomic traits

A significant moderate correlation was observed between leaf blast with neck (r=0.25, P<0.001) and finger blast (r=0.30, P<0.001) whereas, neck and finger blast ratings had a high correlation (r=0.92, P<0.001) (Table 2; Fig. 4). The blast resistant accessions exhibited wide diversity for agronomical traits such as days to 50 % flowering (DF), plant height and spike type. The significant differences were observed for DF and plant height between the mini-core accessions. The DF ranged between 45 and 92 days with the mean of 70.2 days and plant height ranged between 70 and 137 cm (mean 105.8 cm). Diversity for spike type such as top curved (TC), incurved (I) and long open (LO) was also observed in mini-core accessions (Table 2). Leaf, neck and/or finger blast severity was negatively correlated with plant height (r=−0.21, −0.26 and/or −0.27) and DF (r=−0.19, −0.55 and/or −0.57) whereas, it was positively correlated with spike type (r=0.17, 0.06 and/or 0.07).

Confirmation of leaf blast resistance under greenhouse conditions

Leaf blast severity scores of 80 accessions in greenhouse screen showed 58 accessions as resistant, 20 moderately resistant and two susceptible compared with 8.0 and 7.1 scores on susceptible checks VR 708 and VL 149, and 3.0 and 2.4 on resistant checks PR 202 and RAU 8, respectively (Table 2). Significant and moderate correlation (r=0.44, P<0.0001) was found between greenhouse and field screening for leaf blast.

Table 2 Leaf, neck and finger blast severity, origin, race type and variable agro-morphological characters of blast resistant finger millet accessions selected from mini-core collection

IE No.	Origin	Race	Leaf blast severity (1–9 scale)[ab]				Neck blast severity (1–5 scale)[c]			Finger blast severity (%)[d]			Agronomic traits[e]		
			Greenhouse	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	DF	Height (cm)	Spike type
501	India	*Vulgaris*	3.2	1.5	2.0	1.8	4.8	5	4.9	64.0	47.0	55.5	48	81	TC
518	India	*Vulgaris*	3.6	2.0	2.5	2.3	2.8	3.2	3.0	18.5	23.5	21.0	60	110.8	TC
1055	Unknown	*Vulgaris*	2.1	1.5	1.6	1.5	2.1	1.3	1.7	6.6	3.4	4.9	62	121.2	TC
2034	India	*Vulgaris*	4.1	1.5	2.4	2.0	1.5	1.0	1.3	7.6	1.2	4.3	89	85	TC
2042	India	*Vulgaris*	3.5	1.8	2.0	2.0	2.1	1.9	2.0	9.1	9.0	9.0	60	105	TC
2217	India	*Vulgaris*	2.8	1.5	2.0	1.7	2.1	1.7	1.9	4.2	12.4	8.3	64	90	TC
2296	India	*Vulgaris*	2.9	1.5	1.6	1.5	1.9	1.1	1.5	1.7	6.8	4.2	72	93	TC
2312	India	*Elongata*	3.9	1.5	2.0	1.7	1.9	1.2	1.6	5.1	5.6	5.3	82	110	LO
2430	Kenya	*Vulgaris*	3.2	1.2	2.0	1.5	1.6	1.1	1.3	2.2	2.4	2.2	74	130	TC
2437	Kenya	*Plana*	2.0	1.0	2.0	1.5	2.6	2.2	2.4	7.0	5.5	6.3	75	123	TC
2457	Kenya	*Compacta*	1.1	1.5	2.0	1.7	2.0	1.1	1.5	2.7	0.2	1.3	78	115	TC
2572[f]	Kenya	*Plana*	2.0	–	3.0	3.0	–	1.0	1.0	–	1.0	1.0	92	83	LO
2589	USA	*Plana*	2.0	1.2	2.0	1.5	1.4	1.0	1.2	4.2	0.4	2.2	71	137	TC
2606	Malawi	*Vulgaris*	3.9	1.5	2.0	1.7	1.6	1.0	1.3	3.7	4.6	4.1	80	95	TC
2619	Malawi	*Vulgaris*	2.7	1.2	2.0	1.5	1.7	1.3	1.5	2.2	3.4	2.7	82	105	TC
2710	Malawi	*Plana*	1.6	1.2	2.0	1.5	1.2	1.0	1.1	2.7	0.4	1.5	78	110	I
2790	Malawi	*Elongata*	2.0	1.2	1.6	1.3	1.8	1.0	1.4	5.1	3.8	4.4	79	70	LO
2821	Nepal	*Compacta*	2.1	1.2	2.4	1.7	1.1	1.0	1.0	2.2	3.6	2.8	62	113	I
2871	Zambia	*Compacta*	1.1	1.5	1.6	1.5	1.4	1.0	1.2	0.7	0.2	0.4	83	106	I
2872	Zambia	*Vulgaris*	1.0	1.2	1.6	1.3	1.5	1.0	1.3	3.7	1.4	2.5	60	103	TC
2911	Zambia	*Vulgaris*	1.0	1.5	1.1	1.3	1.1	1.0	1.0	1.2	0.2	0.6	83	129	TC
2957	Germany	*Vulgaris*	1.9	1.5	2.0	1.7	2.0	1.0	1.5	1.8	0.9	1.2	70	91	TC
3045	India	*Vulgaris*	2.1	1.5	2.0	1.7	1.8	1.6	1.7	7.2	7.5	7.3	62	114	LO
3077	India	*Vulgaris*	3.2	1.2	2.0	1.5	1.3	1.2	1.2	5.2	5.3	5.2	62	104.2	TC
3104	India	*Vulgaris*	2.3	1.5	2.0	1.8	3.8	5.0	4.4	48.0	45.5	46.8	55	86	I
3317	Zimbabwe	*Vulgaris*	2.6	1.5	1.1	1.3	1.6	1.3	1.4	3.7	1.4	2.5	76	110	TC
3391	Zimbabwe	*Vulgaris*	3.2	1.5	1.6	1.5	1.2	1.0	1.1	2.7	0.4	1.5	79	122	TC
3392	Zimbabwe	*Compacta*	2.0	1.2	2.0	1.5	1.8	1.0	1.4	3.7	1.4	2.5	66	95	TC
3470	India	*Vulgaris*	3.7	1.5	1.6	1.5	1.4	1.3	1.3	6.2	6.0	6.0	62	109	TC
3475	India	*Vulgaris*	4.7	1.2	2.4	1.7	1.6	1.4	1.5	3.7	5.1	4.3	74	101	I
3614	Unknown	*Plana*	2.1	1.5	2.0	1.7	1.4	1.0	1.2	3.7	0.2	1.8	75	123	TC
3721	Uganda	*Compacta*	2.2	1.2	2.0	1.5	1.9	1.0	1.5	0.3	0.9	0.5	83	134	TC

Table 2 (continued)

IE No.	Origin	Race	Leaf blast severity (1–9 scale)[ab]				Neck blast severity (1–5 scale)[c]			Finger blast severity (%)[d]			Agronomic traits[e]		
			Greenhouse	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	DF	Height (cm)	Spike type
3945	Uganda	*Plana*	2.0	1.2	2.0	1.5	1.8	1.0	1.4	1.8	2.1	1.8	79	123.2	TC
3952	Uganda	*Plana*	1.1	1.5	1.6	1.5	1.6	1.2	1.4	0.3	0.9	0.5	79	77	TC
3973	Uganda	*Vulgaris*	1.7	1.5	1.6	1.5	2.0	1.3	1.6	2.3	1.2	1.6	76	85	TC
4028	Uganda	*Vulgaris*	2.9	1.2	1.6	1.3	1.4	1.1	1.3	4.7	3.8	4.2	74	130	TC
4057	Uganda	*Plana*	3.2	1.5	2.4	2.0	1.0	1.1	1.0	1.3	1.4	1.2	70	105	TC
4073	Uganda	*Elongata*	1.5	1.7	1.6	1.8	1.1	1.0	1.1	0.6	0.2	0.3	79	96	TC
4121	Uganda	*Plana*	1.9	1.2	1.6	1.3	1.7	1.5	1.6	7.2	5.6	6.3	62	114	TC
4329	Zimbabwe	*Vulgaris*	3.2	1.5	1.6	1.5	1.2	1.1	1.1	2.7	0.7	1.6	74	114	TC
4491	Zimbabwe	*Elongata*	2.3	1.8	1.6	1.7	2.0	1.4	1.7	10.1	6.3	8.1	62	125	TC
4497	Zimbabwe	*Vulgaris*	2.5	1.2	1.6	1.3	1.8	1.4	1.6	5.7	5.8	5.7	70	110	TC
4545	Zimbabwe	*Compacta*	3.2	1.2	1.1	1.1	1.9	1.3	1.6	10.9	7.7	9.4	74	110	TC
4565	Zimbabwe	*Elongata*	2.2	1.7	2.0	2.0	1.3	1.0	1.2	4.5	0.7	2.6	73	125	TC
4570	Zimbabwe	*Plana*	1.5	1.2	1.1	1.1	1.2	1.2	1.1	2.1	2.1	2.1	62	118	TC
4622	Zimbabwe	*Compacta*	2.3	2.3	1.6	2.2	1.3	1.3	1.3	3.1	1.7	2.3	75	102	I
4646	Zimbabwe	*Plana*	3.6	1.4	1.1	1.3	1.7	1.2	1.4	4.0	5.1	4.5	79	93	TC
4671	India	*Vulgaris*	1.4	2.0	1.1	1.7	2.3	1.2	1.7	12.8	3.6	8.2	67	95	TC
4709	Burundi	*Africana*	1.2	1.9	1.9	2.2	1.3	1.6	1.4	0.2	0.4	0.1	45	85	LO
4734	India	*Vulgaris*	2.5	1.5	1.5	1.5	4.9	4.8	4.9	48.0	48.0	48.0	51	98	I
4757	India	*Vulgaris*	2.3	1.5	2.0	1.8	3.2	3.3	3.2	20.0	12.8	16.4	62	110.2	TC
4795	Zimbabwe	*Vulgaris*	2.2	1.7	1.6	1.7	2.1	1.1	1.6	9.9	8.0	9.0	73	105.2	TC
4797	Maldives	*Vulgaris*	6.6	1.5	6.5	4.0	2.8	1.9	2.3	31.0	21.8	26.4	74	90	TC
4816[f]	India	*Elongata*	1.5	–	1.5	1.5	–	1.0	1.0	–	2.0	2.0	85	90	LO
5066	Senegal	*Vulgaris*	1.6	1.4	1.6	1.5	1.4	1.1	1.2	4.0	1.2	2.6	62	107	TC
5091	Zimbabwe	*Vulgaris*	2.0	1.2	2.0	1.5	1.9	1.4	1.7	8.9	2.9	5.9	62	110.2	TC
5106	Zimbabwe	*Vulgaris*	1.9	1.2	1.6	1.3	2.1	1.1	1.6	5.0	1.2	3.1	70	105	TC
5201	India	*Vulgaris*	1.4	1.4	2.0	1.7	1.7	1.0	1.4	1.6	7.7	4.7	80	109.8	LO
5306	Zimbabwe	*Vulgaris*	2.5	1.4	1.1	1.3	1.7	1.5	1.6	3.5	2.6	3.1	75	110	TC
5367	Kenya	*Vulgaris*	2.0	1.5	2.0	1.8	2.9	1.2	2.1	20.5	10.3	15.4	74	115	TC
5537	Nepal	*Vulgaris*	1.6	1.4	2.0	1.7	1.6	1.6	1.6	6.5	8.2	7.4	60	105	TC
5817	Nepal	*Vulgaris*	1.35	1.5	1.5	1.5	2.5	1.9	2.2	12.0	9.8	10.9	67	100	I
5870	Nepal	*Vulgaris*	3.1	1.5	4.0	2.8	3.8	3.4	3.6	32.0	28.5	30.3	55	100	I
6059	Nepal	*Vulgaris*	1.15	1.5	3.5	2.5	2.3	2.2	2.2	12.0	11.8	11.9	62	130	TC

Table 2 (continued)

IE No.	Origin	Race	Leaf blast severity (1–9 scale)[ab]				Neck blast severity (1–5 scale)[c]			Finger blast severity (%)[d]			Agronomic traits[e]		
			Greenhouse	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	DF	Height (cm)	Spike type
6082	Nepal	*Plana*	5.2	1.5	6.5	4.0	4.9	5.0	5.0	60.0	43.0	51.5	62	105	I
6154	Nepal	*Vulgaris*	1.0	1.5	1.6	1.5	1.4	1.5	1.5	4.0	8.0	6.0	77	95.4	TC
6165	Nepal	*Vulgaris*	1.6	1.2	1.1	1.1	1.4	1.4	1.4	4.0	5.3	4.7	79	90	I
6221	Nepal	*Vulgaris*	1.0	1.2	1.6	1.3	1.6	1.3	1.5	4.5	4.1	4.3	74	100	I
6240	Zimbabwe	*Vulgaris*	1.3	1.2	2.0	1.5	1.3	1.2	1.3	2.6	3.6	3.1	71	115.2	TC
6294	Zimbabwe	*Vulgaris*	3.2	1.8	1.6	1.7	1.7	1.2	1.4	5.0	2.6	3.8	74	110	TC
6326	Zimbabwe	*Vulgaris*	3.0	1.2	1.1	1.1	1.6	1.2	1.4	5.5	0.2	2.8	75	95	TC
6337	Zimbabwe	*Vulgaris*	1.7	1.8	1.6	1.7	1.7	1.0	1.4	1.1	0.2	0.6	70	92	TC
6350	Zimbabwe	*Vulgaris*	1.9	1.8	1.6	1.7	1.4	1.0	1.2	4.5	1.2	2.8	79	95	TC
6421	Uganda	*Vulgaris*	1.0	1.2	2.0	1.5	2.0	1.1	1.5	9.4	4.6	7.0	70	110	TC
6473	Uganda	*Plana*	2.0	1.2	2.0	1.5	1.2	1.0	1.1	1.6	0.7	1.1	76	123	TC
6514	Zimbabwe	*Vulgaris*	2.1	1.5	2.0	1.7	1.8	1.0	1.4	10.9	4.6	7.8	76	115	TC
6537	Nigeria	*Vulgaris*	3.5	1.3	2.0	1.6	1.8	1.0	1.4	−0.2	0.2	0.2	78	95	TC
7018	Kenya	*Vulgaris*	1.4	1.8	2.0	2.0	1.8	1.0	1.4	6.5	0.2	3.3	70	121	TC
7079	Kenya	*Vulgaris*	3.5	1.8	2.0	2.0	1.4	1.2	1.3	5.5	3.1	4.3	62	115	TC
7320	Kenya	*Vulgaris*	4.5	1.2	1.6	1.3	1.2	1.1	1.1	4.0	3.1	3.6	62	128.2	TC
VR 708-Check	India	*Vulgaris*	8.0	1.2	4.7	2.8	4.7	4.4	4.6	70.1	83.6	76.8	55	90	TC
PR 202-Check	India	*Vulgaris*	3.0	1.8	2.0	2.0	1.7	1.3	1.5	8.4	5.6	7.0	62	110	TC
RAU 8-Check	India	*Vulgaris*	2.4	1.2	2.0	1.5	1.9	2.2	2.0	8.4	10.7	9.6	62	95	TC
VL 149-Check	India	*Compacta*	7.1	1.2	4.7	2.8	4.5	4.2	4.4	42.2	38.5	40.7	60	87.2	TC
LSD ($P<0.05$)[g]	–	–	0.86	0.68	1.1	0.72	0.83	0.74	0.56	7.7	6.3	4.9	4.15	0.7	–

[a] Mean of two replications, 10 plants/replication at 40 DAS for leaf blast and at physiological maturity for neck and finger blast

[b] Leaf blast severity on 1–9 scale where 1 = no infection and 9 = >75 % leaf area covered with lesions

[c] Neck blast severity on 1–5 scale where 1 = no infection and 5 = >6 cm lesions on the neck region

[d] Finger blast severity (%) across all panicles/all tillers in a row

[e] DF = days to flowering and Height = Plant height (cm), Spike type: TC = Top curved, I = Incurved, LO = Long open

[f] Two accessions could not get established in 2009

[g] Trial least significant difference

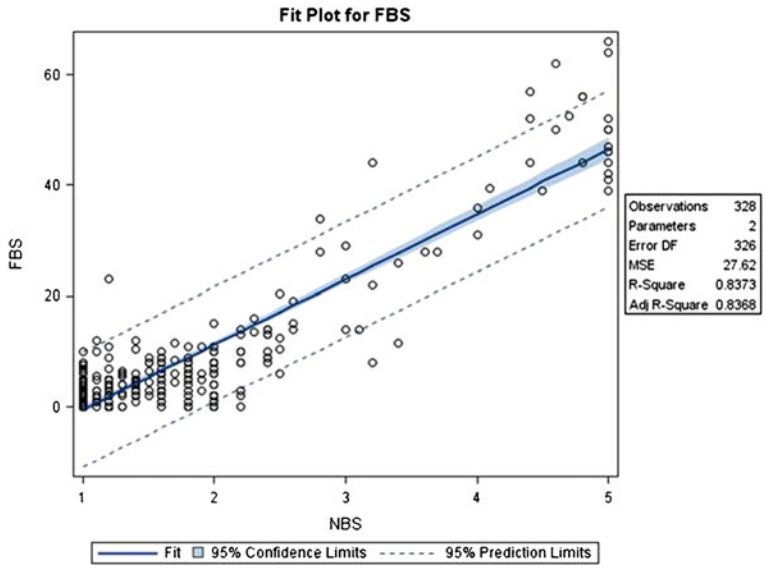

Fig. 4 Relationship between neck (NBS on 1–5 scale) and finger blast severity (FBS - %) of finger millet mini-core evaluated for blast resistance under field conditions during the rainy season 2009 and 2010 at ICRISAT, Patancheru, India

Discussion

In this study, the rating scales were developed or refined for accurately assessing the leaf, neck and finger blast under field and greenhouse conditions. Leaf blast severity in finger millet has been routinely assessed using the rice blast rating scale (Takan et al. 2004, 2012; Mgonja et al. 2007; Nagaraja et al. 2007, 2010). A literature search revealed that there is no specific rating system to finger millet for recording the leaf blast severity. Thus, we developed a new 1–9-class leaf blast rating scale and classified these based on per cent leaf area diseased, which is illustrated by colour plates (Fig. 1). The neck and finger blast are routinely assessed at the dough stage of the crop as disease incidence *i.e.*, percentage of ears showing infection on the neck and fingers over total number of neck and fingers in a row (Somashekhara et al. 1991; Mgonja et al. 2007; Nagaraja et al. 2007, 2010). This type of scoring does not provide the extent of infection and the damage caused by the pathogen in the lines being evaluated. To overcome this limitation, we developed a new rating scale (1–5 scale for neck blast and per cent finger blast severity) for recording the neck and finger blast severity (Figs. 2 and 3). The use of this scale will facilitate estimation of exact damage caused by neck and finger blast phases of the disease. The given lesion dimensions in neck blast rating scale were not intended to be a rigid or fixed criterion upon which the rating scale are classified. Rather, they were given as supplemental information for describing and separating infection types and for general comparison by other researchers. We never intended, nor do we ever expect, any user of this scale to actually measure lesions for assessment of neck blast infection responses. It simply would not be practical to measure individual lesions for the routine evaluation of finger millet accessions for resistance to neck blast. Instead, one can use the colour plates as a guide to quickly and easily estimate the relative size of lesions present on finger millet lines as needed for the classification of the neck blast. The newly developed rating scale for recording neck and finger blast adequately provide the actual disease severity on an individual plant and not merely the per cent disease incidence. We consider this as a significant step towards simplifying and improving the precision of disease scoring for identification and utilization of resistance sources.

The significant effect of year, as detected by Wald statistics that occurred in leaf, neck and finger blast infection levels between 2 years of experiment could be due to variable weather conditions. Such differences in weather conditions between 2 years could influence disease level is a known fact (Koutroubas et al. 2009). Environmental conditions, especially relative humidity and temperature could strongly affect the sporulation, release and germination of blast conidia (Ou 1985). In this study, a highly significant and strong positive correlation (r=0.93) for neck and finger blast severity found between 2009 and 2010 suggest that the significant year effect didn't cause much

impact on disease severity and reaction. Under favourable conditions, foliar blast occurred in a majority of accessions at the seedling stage, which did not correlate well with crop growth stages and maturity of the plants, probably because of the build-up of adult plant resistance. Hence, neck and finger blast reactions that are more destructive than leaf blast were considered important parameters for blast resistance (Takan et al. 2004; Nagaraja et al. 2007, 2010).

The negative correlations between blast severity and plant height indicates that tall and late maturing accessions might escape blast infection due to less favourable microclimatic conditions (Thakur et al. 2009). The role of plant height in rice blast resistance is well documented (Torres and Teng 1993; Koutroubas et al. 2009). A significant negative correlation was found between blast severity and days to flowering (DF) suggesting that early flowering accessions are more susceptible than the late ones (Pande et al. 1995; Mgonja et al. 2007). For instance, the accessions (IE 501, -3104, -4734, -5870 and -6082) that were earliest to flower (mean 54.2 days and range 48 to 62 days) recorded highest neck blast severity (score 3.5–4.9 on a 1–5 scale and mean 4.5) and 30–55 % finger blast severity with a mean of 46 % compared to highly resistant accessions with DF ranging from 60 to 92 (mean 72.5 days) (Table 2). In this study, positive association between spike type (top curved, incurved and long open) and blast severity was found. However, accessions with dark coloured seeds and compact heads have been reported to be more resistant compared to white seeded and open headed varieties (Takan et al. 2004).

Significant moderate correlations between leaf blast with neck and, finger blast suggests that a high level of leaf blast severity achieved by early inoculation may not result in severe neck or finger blast during the later stages of plant development. Poor correlation has been observed for leaf blast with neck blast (r=0.04) and finger blast (r=0.27) infection in finger millet (Somashekhara et al. 1991). It has been reported that seedlings of finger millet are more susceptible to leaf blast than mature plant (Rachie and Peters 1977). However, no relationship is known between the intensity of seedling infection and that of later neck and finger infection. Rather, prevailing weather conditions at a particular stage of crop development determine the intensity of blast infection (Esele 2002). Contrasting responses between the vegetative stage and reproductive stage often occur, indicating differential gene expression for resistance to leaf, neck and/or finger blast infection. The gene(s) responsible for leaf blast resistance have not been found effective at the reproductive stage in rice (Wu et al. 2004), indicating that researchers should not rely solely on the seedling reaction for assessing potential adult plant resistance. In contrast, a significant strong positive correlation found between neck and finger blast severity suggested that recording the blast severity using these two severity scales provided realistic data under field conditions at the right stage of the crop *i.e.* physiological maturity and also possible ability of the same gene(s) to induce resistance to both neck and finger blast. Significant positive correlation between neck and finger blast incidence has earlier been reported in finger millet (Nagaraja et al. 2010).

Developing improved blast resistant varieties is an important breeding goal in finger millet improvement (Mgonja et al. 2007). In this study, we refined the field screening technique for finger millet blast whereby germplasm can be effectively screened in the field and resistance confirmed through greenhouse screening. Large scale screening at the seedling stage for leaf blast resistance could be more economical and rapid in the greenhouse than in the field. The inoculated plants in the seedlings stage were observed up to the dough stage for neck and finger blast under greenhouse conditions. However, no neck and finger infection was observed in this experiment suggesting that prevailing weather conditions and availability of pathogen inoculum at a particular stage of crop development determine the intensity of blast infection (Esele 2002).

Among the resistant accessions, nine (IE 1055, -28 21, -2872, -4121, -4491, -4570, -5066, -5091, and -5537) had desirable agronomic traits, such as early flowering (<65 days), medium plant height (105–125 cm), semi-compact to compact inflorescence. These would be desirable sources of resistance for a finger millet breeding program. Of the resistant accessions, IE 3392 is a rich source of iron (Fe), IE 2957 of calcium (Ca) and IE 6537 of Ca and protein (Upadhyaya et al. 2011). Analysis and exploitation of such accessions would be an important and logical step towards breeding varieties with combined traits of high grain nutrient density, blast resistance and desirable agronomic traits. The information on clusters to which particular accessions with traits of interest belong will assist in looking extensively for more accessions with similar traits in the larger subsets, core collection and, eventually entire collection. For example, the mini-core accession IE 4709, only *africana* type finger

millet found highly resistant to blast, agronomically desirable characters (Upadhyaya et al. 2007) and also been reported for rich source of grains nutrients such as Fe, Ca, Zinc (Zn) and protein contents (Upadhyaya et al. 2011). This type of accession could be involved in hybridization with agronomically superior accessions/breeding lines to combine blast resistance, grain nutrients, and farmer and consumer preferred traits. Similarly, IE 1012 was referred by Gowda et al. (1986) as an African cultivar exploited in India as a source of blast resistance. Introduction of micronutrient and protein-dense blast resistant finger millet cultivars would help to reduce the losses caused by blast disease and more importantly malnutrition due to micronutrients and protein deficiency of resource-poor people, who consume finger millet-based diet in large quantities on a daily basis. It would be useful to evaluate the remaining *africana* type accessions from the core and entire collection that were not included in the mini-core collection to identify additional sources of resistance to blast and grain nutrients, and test their resistance stability through multilocation testing in India and elsewhere. The disease scales illustrated in Figs. 1, 2 and 3 may be useful criterion to evaluate a large number of finger millet germplasm for leaf, neck and finger blast. Identification of disease resistant accessions from the finger millet mini-core would permit use of diverse resistance sources for future breeding efforts and to ensure a better chance of success in finger millet improvement in developing cultivars with a broad genetic base.

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