



Screening of Finger Millet (*Eleusine coracana*) Germplasm for the Blast Disease-Resistance in Sri Lanka

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ABSTRACT

Finger millet is the third important cereal crop in Sri Lanka, mostly cultivated in rainfed uplands in dry and intermediate zones. Blast disease (leaf, finger, and neck blast) caused by *Magnaporthe grisea* is economically devastating to rice and finger millet worldwide. Host resistance is the most viable option for managing the disease as it is mainly grown in low-input systems. One hundred thirty-nine finger millet accessions collected from the Plant Genetic Resources Center in Sri Lanka were field screened for leaf blast disease in three seasons under artificial inoculation at the Field Crops Research and Development Institute at Mahailuppallama, Sri Lanka. The results revealed that almost all the tested accessions were susceptible to leaf blast at the early seedling stage. However, 5–6 weeks after sowing, the severity of leaf blast disease decreased drastically, and all the germplasm were resistant or moderately resistant. The finger blast and neck blast severity, scored under natural infection, was low under both high and low-density planting. Seven and 87 finger millet accessions consistently showed immune reactions for finger blast and neck blast, respectively. A negative correlation was observed between the days to flowering and blast disease. The early flowering accessions, namely, Ac12968 and Ac2384 from Jaffna, TVFM, 13-1, TVFM-04, and TVFM-02 from Killinochchi, showed a higher severity score for leaf, finger, and neck blast compared to other accessions. A significant positive correlation ($P < 0.05$) was found between finger blast and neck blast, however, these two diseases did not show a significant correlation ($P > 0.05$) with leaf blast.

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INTRODUCTION

Finger millet, [*Eleusine coracana* L. (Gaertn)] is a traditional cereal of many south Asian and African countries. It is renowned as a nutritious cereal, rich in calcium (1.8–4.8 g/kg), iron (21.7–65.23 mg/kg), zinc (16.5–25 mg/kg) and protein (6–11%) (Upadhyaya *et al.*, 2011). Finger millet thrives in altitudes from 0 to 2,400 m above mean sea level in a diverse range of soils from poor to fertile. The grains are attributed to health properties such as anti-diabetic, anti-tumorogenesis, anti-atherosclerogenic, anti-oxidant, and anti-microbial reactions in the human body (Serna and Rooney, 1995; Devi *et al.*, 2014). Hence, the demand for finger millet exceeds its' annual production, about 8,060 mt in 2018, and 3,084 mt were imported in the same year (AgStat, 2019).

Even though finger millet can withstand many biotic and abiotic stresses, blast disease caused by *Magnaporthe grisea* has become devastating biotic stress during cultivation. Blast has caused yield losses ranging from 28–36 % in Asia (Nagaraja *et al.*, 2007), 10–90 % in Uganda (Bisht, 1987), 64 % in Kiboko in Kenya (Pande *et al.*, 1995), and 10.1–41.4% in Ethiopia (Getachew *et al.*, 2014). Damage to finger millet due to blast is characterized by the appearance of lesions on the leaves, nodes, and heads. Lesions on the leaves are typically spindle-shaped, wide in the center, and pointed towards either end. Large lesions usually develop a grayish center, with a brown margin on older lesions.

Magnaporthe grisea is a filamentous, heterothallic ascomycete fungus, which infects a wide variety of grasses. The fungus produces greyish mycelium, and the disease is polycyclic. It produces millions of conidia within a short period. High relative humidity (>89%) and temperature ranging from 25 to 38 °C favor vigorous infection of the disease (Ruiz, 2003). Hence, the crop is highly vulnerable to blast disease during the rainy and winter seasons. The disease occurs at all growth stages of the plant starting from the seedling stage (leaf blast) to flowering and then to the grain filling stage (finger blast) and panicle stalk (neck blast). The fungus

appears in live plant parts and dead plant debris in the soil (Uddin and Soika, 2000).

Finger millet is normally grown in low input cropping systems in Sri Lanka at a subsistence level. Therefore, attention on disease management is poor or negligible. Further, finger millet is mainly cultivated in the *Maha* season, which favors disease infection and spread due to wet weather. Such circumstances necessitate host resistance as the most desirable and durable way of disease management. Therefore, the development of blast-resistant varieties is of utmost importance to finger millet farmers to sustain their livelihood. It urges accurate information on blast-resistant finger millet germplasm available in the country. Therefore, this study attempted to identify blast-resistant finger millet germplasm from the local and exotic finger millet germplasm collection available at the Plant Genetic Resources Center (PGRC) at Gannoruwa, Sri Lanka.

METHODOLOGY

Hundred and thirty-nine finger millet accessions were evaluated against blast disease at the Field Crops Research and Development Institute (FCRDI) at Mahailuppallama in Sri Lanka (altitude of 117 m amsl; longitude - 80°28"E; latitude - 8°07"N). One hundred local accessions, 17 exotic and 9 of unknown origin, were collected from the Plant Genetic Resources Center (PGRC) and farmer cultivars from Mahiyanganaya (9) and Killinochchi (4) (Table 3). Experiments were conducted in three *Maha* seasons (October to February) in 2016/17, 2017/18, and 2019/20, the most appropriate season for disease screening of upland cereals.

Field establishment of finger millet accessions for disease screening

The high-density nursery bed (1 m long double rows, 2–3 cm between plants, and 10 cm between rows), which are generally used to raise seedlings until transplanting, were prepared during *Maha* seasons 2016/17 and 2019/20. Only the leaf blast screening was

done in *Maha* 2016/17. In *Maha* 2019/20, similar high-density nurseries were established to represent the seed broadcasting method commonly practiced by farmers in the dry zone of Sri Lanka. Comparatively lower seeding density and large plot size (3 m long; single row; 60 cm between rows; 30–40 plants per row) were used in *Maha* 2017/18 to provide adequate spacing for plant growth and panicle formation to screen for finger and neck blast. The susceptible check variety *Rawana* (Ac10326) was planted in every 10th row and borders on both sides of a row in all three experiments. Basal application comprising 65 kg of urea/ha, 55 kg of tripe super phosphate/ha, and 85 kg of muriate of potash/ha, and a top dressing of fertilizer at 40 days after seeding (DAS) consisting of 150 kg of urea/ha were applied. Experiments were arranged in a Randomized Complete Block Design (RCBD) with two replicates. During 2017/18, sprinklers were operated for 2 hours during the morning and evening (starting at 7.30 am and 3.00 pm, respectively) to maintain high humidity in the field to trigger the disease infection. However, during *Maha* 2016/17 and 2019/20 nursery beds were covered with polythene (30 cm height) during nighttime to create high relative humidity (RH).

Artificial inoculation

Finger millet leaves infected with *M. grisea* were collected from the diseased plot maintained at the FCRDI. One kg of infected leaves with spores was chopped and extracted in 1 L tap water. Then 1 L of concentrated spore extraction was mixed with 10 L of water and sprayed (20 L / 500 m²) using a Knapsack sprayer at 22 DAS in 2016/17, 20, 32, and 63 DAS in 2017/18, and 13 and 32 DAS in 2019/20. The spore concentration of the sprayed extract was 1 x 10⁵ cfu/ml.

Screening of finger millet accessions for leaf blast, finger and neck blast

Finger millet accessions were screened for leaf blast during 2016/17, 2017/18, and 2019/20 under artificial inoculation. Whereas, accessions were screened for finger

and neck blast during 2017/18 and 2019/20 at flowering and panicle stages under natural disease infection. During 2019/20, one planting row was removed in each accession after scoring for leaf blast severity to provide minimum space (20 cm between rows) for plant growth and panicle formation. Figure 1 showed the blast screening fields at FCRDI during three seasons.

Data recording and analysis

Leaf blast was scored in randomly selected 5 plants at 29 and 34 DAS in 2016/17 and 2017/18, respectively. In 2019/20, the blast disease incidence was recorded at 22, 28, and 39 DAS on a Standard Evaluation System (SES) at 0–5 scale (Anonymous, 1995; Patro et al., 2018).

The disease severity index (DSI) was calculated using Equation 1 (Waller et al., 2002). The accessions were categorized into five groups based on DSI (Table 2).

$$DSI (\%) = \frac{\sum WN_s}{TN_m} * 100 \dots\dots\dots \text{Equation 1}$$

Where, DSI = Disease severity index, W = Number of affected plants, N_s = Severity scale, T = Total number of observations, N_m = Maximum scale number (5).

Finger blast and neck blast counts were converted to percentages from total fingers/plot or ears/plot, accordingly (Equation 2). The disease severity was rated based on the percentage of infected plants, and accessions were categorized into five groups as shown in Table 4 (Ravikumar, 1988).

$$\text{Finger blast or neck blast (\%)} = \frac{\text{Total No. of infected fingers or ears}}{\text{Total No. of fingers or ears per plot}} * 100 \dots\dots\dots \text{Equation 2}$$

The agronomic traits such as days to 50% flowering were recorded. Grain yield was measured only in the 2017/18 season. The plot yield was measured and estimated as mt/ha. Analysis of variance was done for DSI and agronomic traits. Spearman's rank correlation coefficients were obtained for the

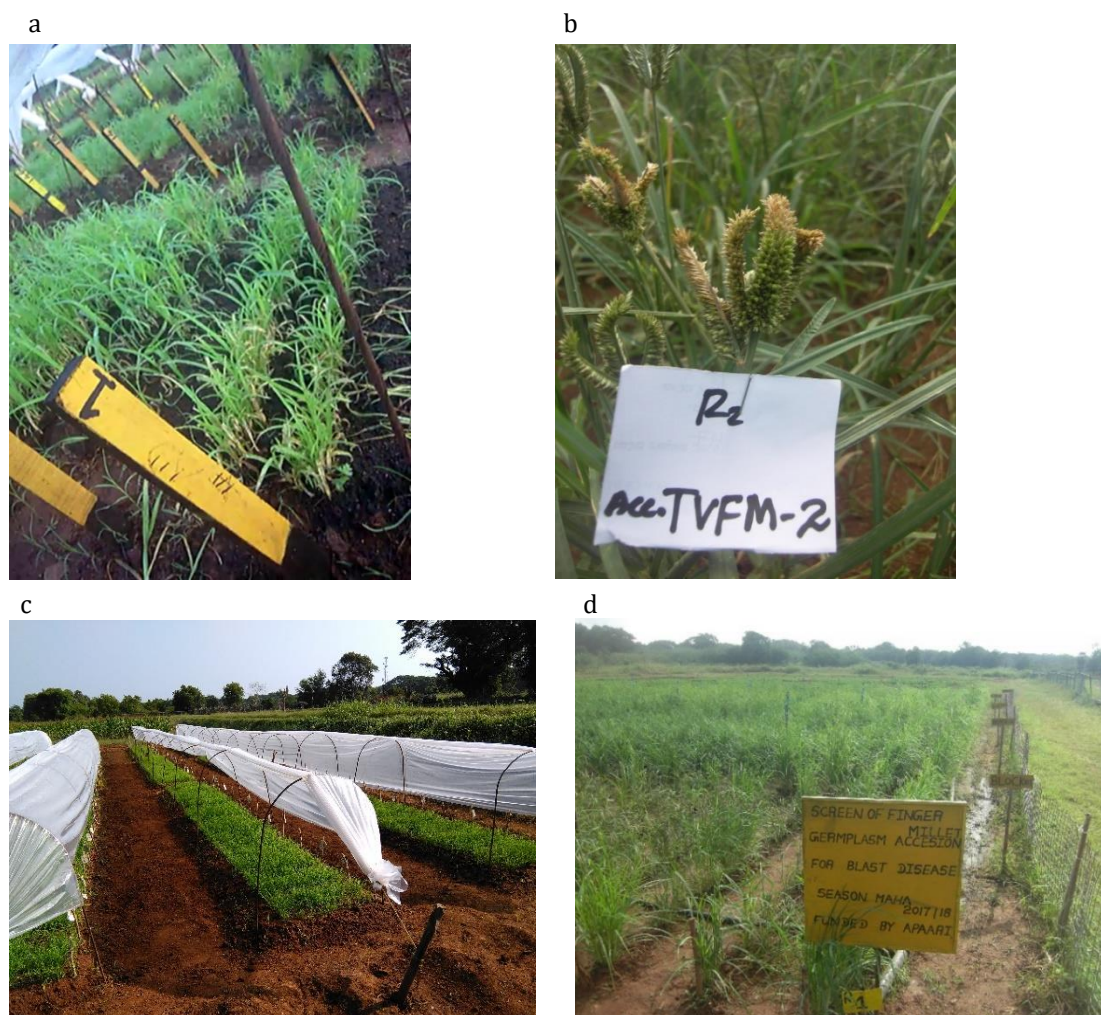


Figure 1: Blast disease screening fields at Field Crops Research and Development Institute, Mahailuppallama. a) Leaf blast screening in high-density nurseries in *Maha* 2019/20, b) Finger blast infected ear in *Maha* 2017/18, c) Leaf blast screening in high-density nurseries in *Maha* 2016/17, d) Finger and neck blast screening field in *Maha* 2017/18

average DSI and agronomic traits of 139 accessions. Monthly total rainfall, number of rainy days, and relative humidity (RH) were collected from the weather station located at Mahailuppallama experimental site as the important parameters to understand the prevailing weather conditions during the study (Table 1).

RESULTS AND DISCUSSION

Screening of finger millet accessions for leaf blast

Leaf blast screening results are summarized in Table 2. The disease severity was inconsistent depending on the environmental

factors that prevailed during the different seasons. Disease severity was high in 2019/20 where none of the accessions were identified as resistant (R) or moderately resistant (MR) at 28 DAS. Whereas, 67 accessions in 2016/17 (29 DAS) and 138 in 2017/18 (34 DAS) were collectively grouped into R and MR categories.

In 2019/20, 125 accessions were in the leaf blast-susceptible category (either MS, S, or HS) rated at 22 DAS. However, at 39 DAS, all 139 accessions were rated either as R or MR. Generally, resistance/tolerance of accessions to leaf blast increased gradually with the aging of seedlings. One of the reasons for higher leaf blast severity at the early seedling

Table 1: Monthly total rainfall, number of rainy days, monthly average relative humidity (RH%), minimum and maximum temperature during the three evaluation periods

Season	Date of sowing	Month	Monthly total rain fall (mm)	No. of rainy days/month	RH%±SD	Temperature (°C)±SD	
						Maximum	Minimum
Maha 2016/17	24.12.2016	November	336.3	17	89±4.6	31±1.5	22±1.2
		December	43.4	8	90±6.2	30±1.4	21±2.2
		January	154.0	10	90±4.6	30±1.7	20±2.6
		February	55.0	4	90±4.4	31±0.9	20±2.3
Maha 2017/18	02.11.2017	November	234.3	19	89±5.0	30±1.6	22.6±0.9
		December	153.0	13	90 ±3.9	30±1.4	21.6±1.4
		January	17.0	4	90±3.7	30±0.8	20±2.1
		February	20.0	5	90±3.7	30±0.83	20±2.1
Maha 2019/20	14.11.2019	November	248.5	20	88±5.3	31±1.4	23±0.51
		December	369.4	17	92±4.7	29±1.4	23±0.9
		January	19.8	6	87±4.0	31±0.7	21±2.0
		February	+0	1	84±5.6	32±0.6	21±1.6

SD – Standard Deviation

Table 2: Number of finger millet germplasm accessions under different leaf blast resistant categories during three seasons, 2016/17, 2017/18 and 2019/20

Disease reaction for leaf blast	Reaction disease rating (%)	No. Accessions				
		2016/17	2017/18	2019/20		
		29 DAS	34 DAS	22DAS	28 DAS	39 DAS
Resistant (R)	0 – 5% infection	2	0	0	0	15
Moderately resistant (MR)	6- 25% infection	65	138	14	0	124
Moderately Susceptible (MS)	26–50% infection	69	1	75	12	0
susceptible (S)	51-75% infection	3	0	47	75	0
Highly susceptible (HS)	>75% infection	0	0	3	52	0
Total		139	139	139	139	139

DAS - Days after sowing

stage would be the highly conducive environment conditions (17 days of wet days during December 2019 together with 90-95% RH) (Table 1). Further, the high plant density in the nursery also would have favored the spread of the disease faster. Even though the same weather conditions continued until 39 DAS, the leaf blast disease severity reduced drastically. It may be due to the rapid vegetative growth and also tillering of plants that started from the 4th week onwards. Accordingly, with time, plants become physiologically stronger and build up adult-plant resistance (Takan 2004; Nagaraja et al., 2007; Nagaraja et al., 2010). Further, Manyasa et al. (2019) reported that

the general decline in leaf blast disease development after 51 DAS could be due to the physiological changes of the plant, thus, showing the expression level of mature resistance, which was also reported for rice (Li et al., 2007).

In 2017/18, the leaf blast score of finger millet accessions was comparatively low compared to the other two seasons and DSI ranged from 8-25% (moderate resistance; Table 3). The reason for this lower disease severity index may be the changes in the microclimate around plants due to the low planting density (60 cm x 10 cm) adopted in the year 2017/18 compared to 2016/17 and 2019/20 seasons. Further, tillering of plants

was also increased due to this reason. Even though favorable conditions were provided for pathogen development such as high humidity levels (by operating sprinklers) and artificial inoculation, the disease development was less severe during 2017/18 compared to high-density nursery beds in 2016/17 and 2019/20.

According to ANOVA, the finger millet accessions showed a significant difference in leaf blast severity (Table 3). The accessions with a consistently higher leaf blast severity score in all three seasons were TVFM013-1, TVFM-04, and TVFM-02 from Killinochchi, Ac12968, and Ac2384 from Jaffna, and Ac1233 from Matale. The germplasm from the northern region (Jaffna and Killinochchi) were short-age varieties. Further, the yield of those varieties was comparatively lower than the rest. Even though TVFM-01 from Killinochchi showed a higher leaf blast score in 2019/20 at 28 DAS, its yield was at a moderate level (2.3 mt/ha in 2016/17). The variety *Rawana* (Ac10326) was used as a susceptible check, but it gave a comparatively higher yield (3.4 mt/ha). The moderately-resistant variety *Oshadha* (Ac108) was also susceptible to leaf blast. Fernando *et al.* (2016) reported that the variety *Oshadha* is a moderately-susceptible variety to blast disease. A total of 48 germplasm accessions showed an appreciable higher yield range (2.5–3.6 mt/ha) during 2016/17 in the disease screening plots (Table 3).

Screening of finger millet accessions for Neck blast and finger blast

Screening of neck and finger blast was done under natural infection. However, high RH was maintained in the field by operating sprinklers. The finger blast and neck blast infections were low during both seasons, and most of the studied germplasm showed immune or resistant reactions (Table 4).

Seven accessions, namely, Ac12038, Ac5047, Ac11818, Ac12282, Ac11334, Ac12181, and Ac10098, showed consistent immune reactions for finger blast during both seasons (Table 3).

More than 80% of the accession flowered at >72 DAS. The weather conditions at flowering (i.e. mid to end of January in the *Maha* seasons 2017/18 and 2019/20) were comparatively dry (Tables 1 and 3). Thus, the natural weather conditions that prevailed have not favored the disease development and spread under open-field conditions. Hence, the finger and neck blast incidences may not be that severe under farmer's cultivation conditions during the *Maha* season, especially for medium and long-age varieties.

Correlation of finger millet leaf, finger and neck blast DSI with other agronomic traits

A significant positive correlation was observed between neck blast and finger blast (Table 5). However, finger blast or neck blast did not correlate significantly ($P > 0.05$) with the leaf blast. These results agreed with the findings of the previous studies conducted by Babu *et al.* (2013). However, Manyasa *et al.* (2019) found positive correlations among the leaf, finger, and neck blast. However, in another study on rice blast, no linkage was reported between leaf blast and neck blast (Bonman *et al.*, 1989). Bonman *et al.* (1989) further reported that some rice germplasm accessions that were susceptible to leaf blast, exhibited resistance to neck blast and finger blast. The seedlings of finger millet were reported to be more susceptible to blast than mature plants and the severity of finger and neck blast was primarily determined by prevailing weather conditions at a particular crop growth stage (Esele, 2002). High RH (85–99%) and minimum and maximum temperature (21–29 °C) coincide with the flowering and maturity stages of finger millet crops and favor disease development (Patel and Tripathi, 1998). A study on rice revealed contrasting responses between the vegetative and reproductive stages, which can often occur due to differential gene expression for resistance to leaf, neck, and/or finger blast infection (Wu *et al.*, 2004). Hence, researchers should not rely solely on the seedling reaction in assessing the potential adult-plant resistance.

7610 (KN)	2.4	79	11	2	0	64	1	0	2383 (JF)	1.1	59	15	1	4	79	6	0
927 (IN)	2.4	63	15	7	3	50	5	1	12927(MN)	1.0	60	11	7	0	66	3	1
6587(HM)	2.3	77	16	0	0	54	3	0	7111(ZM)	1.0	66	11	5	0	53	9	0
11347 (KR)	2.3	74	23	2	0	77	2	1	1329(RT)	0.9	72	17	15	6	48	3	1
12449 (KR)	2.3	77	15	2	0	60	2	0	910(IN)	0.8	55	14	8	6	52	3	1
192 (HM)	2.3	81	11	3	0	69	3	0	2384(JF)	0.7	51	15	4	8	55	1	0
2953 (MN)	2.3	76	21	4	1	49	1	0	TVFM-02	0.6	53	14	13	3	77	4	1
TVFM -01	2.3	66	17	9	7	81	1	0	12968 (JF)	0.6	56	21	28	35	55	4	2
11332 (MN)	2.3	84	22	2	0	69	0	0	TVFM-04	0.4	53	17	12	7	77	4	2
8470 (MN)	2.3	80	17	1	0	66	1	0	TVFM013-1	0.1	52	9	16	2	86	5	2
11369(MN)	2.3	75	20	0	0	58	1	1									
Mean	2.2	75	16	3.4	1	58	2	0.2	MSS	0.8**	116**	33*	33*	25**	453*	7.7**	0.37*

Accession - PGRC accession number and district/country where germplasm collected, * significantly different at $P < 0.05$, ** significantly different at $p < 0.001$, DF- days to 50% flowering, LB - Leaf blast, FB - Finger blast, NB - Neck blast, MSS - Mean Sums of Square of accessions

AN - Anuradhapura, AP - Amparai, BT - Batticaloa, IN - Exotic India, NP - Exotic Nepal, ZM - Exotic Zimbabwe, UN - unknown origin, HM - Hambantota, KR - Kurunegala, JF - Jaffna, KAN - Kandy, MN - Monaragala, MT - Matale, NE - Nuwaraeliya, PL - Polonnaruwa, RT- Ratnapura. TVFM - farmer cultivar from Killinochchi and Jaffna, M1-M9 farmer cultivars from Mahiyanganaya,

Table 4: Number of finger millet germplasm accessions identified under different finger blast and neck blast resistance category during 2017/18 and 2019/20 Maha seasons

Disease reaction category	Finger blast			Neck blast		
	Disease Reaction rating (%)	No. of accessions		Disease Reaction rating (%)	No. of accessions	
		2017/18	2019/20		2017/18	2019/20
Immune (I)	0	20	23	0	96	114
Resistant (R)	0.1 -10	108	116	0.1- 5	40	25
Moderately susceptible (MS)	10.1 - 20	10	0	5.1-10	1	0
Susceptible (S)	20.1-30	1	0	10.1- 25	1	0
Highly susceptible (HS)	>30	0	0	>25	1	0
Total No. germplasm		139	139		139	139

Table 5: Spearman's rank correlation coefficient among blast disease, days to 50% flowering and grain yield

	Spearman rank correlation coefficient									
	2017/18				2019/20					
	DF	LB	FB	NB	DF	LB1	LB2	LB3	FB	NB
LB1 - 3 WAS					0.311*					
LB2 - 4 WAS	-0.107				0.26*	0.60*				
LB3 - 4 WAS					-0.043	0.144	0.219*			
FB	-0.46*	0.02			-0.55*	-0.24*	-0.27*	-0.1		
NB	-0.40*	-0.09	0.456*		-				0.344	
YLD	0.37*	-0.042	-0.175	0.26	0.365*	0.082	0.208*	0.269*	0.054	0.27*

*Significant at $P < 0.01$

DF - days to 50% flowering, WAS - weeks after sowing; YLD - grain yield, LB - Leaf blast, FB - finger blast, NB - neck blast, LB1 - Leaf blast 3 WAS, LB2 - Leaf blast 4 WAS, LB3 - Leaf blast 5 WAS

A significant negative correlation ($P < 0.05$) was observed in days to 50% flowering with finger blast and neck blast in both *Maha* seasons 2017/18 and 2019/20. The early flowering accessions (50 - 60 days to 50% flowering) were more vulnerable to finger and neck blast infection compared to late flowering due to the continuation of favorable microclimate (rainy weather with high RH) at the flowering stage. According to Table 3, from mid-December to early January, the rainy weather coincided with flowering in early-flowering accessions. Thakur *et al.* (2009) also reported that late-maturing accessions would escape from favorable weather conditions that help blast infection.

According to Table 3, the grain yields were lower in short-duration finger millet varieties. Kumari *et al.* (2018) also reported a significant negative correlation between days to flowering and grain yield of the same finger millet accessions. The leaf blast severity was also found to be negatively correlated with days to flowering. Hence, the negative correlation between yield and leaf blast severity was indirect in the present study.

CONCLUSIONS

Most of the studied finger millet accessions were susceptible to leaf blast at the seedling stage. The severity of leaf blast decreased gradually in the post-vegetative stages. The higher severity of neck blast and finger blast was not noticeable in the two recommended finger millet varieties (*Oshadha* and *Rawana*), farmer varieties (M1–M9), and other PGRC accessions, which flower at >72 DAS. Leaf blast, finger blast, and neck blast incidences were comparatively high in short-age (50-60 days to flowering) finger millet cultivars grown in the northern region (Killinochchi and Jaffna) than in the rest. Seven accessions, namely, Ac12038, Ac5047, Ac11818, Ac12282, Ac11334, Ac12181, and Ac10098 consistently showed immune reactions for finger blast during 2017/18 and 2019/20. About one-third (48) of accessions showed an appreciable grain yield of 2.5–3.6 mt/ha in the disease-screening plots.

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