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Screening of Maize Genotypes for their Resistance to Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) under Artificial Infestation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was undertaken to identify resistant maize genotypes against fall armyworm, which were screened in a net house under artificial infestation In a randomized complete block design at Winter Nursery Centre, ICAR-Indian Institute of Maize Research, Rajendranagar, Hyderabad during *rabi* 2023–24. The experimental material consisted of 19 diverse maize genotypes (Table 1). Each genotype was sown in a 2 m row and replicated thrice under

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randomized complete block design (RBD) in an insect screening net house facility. The tested genotypes were cultivated according to the recommended agronomic practices, with 60 and 20 cm spacing between rows and plants, respectively. Each genotype was released with 20 fall armyworm neonate larvae using a camel hair brush at the V5 phenological stage. The observations on leaf damage rating were taken on the 7th,14th, and 21st days after larval release based on leaf damage rating on a 1 to 9 scale. Among the tested genotypes, the leaf damage score ranged from 3.53 to 7.50 at 14 days after larval infestation. The genotypes namely CML 71 (3.53) and DMRE 63 (3.76) were found to be resistant while the genotypes namely HUZM 189 (4.13), CML 144 (4.36), CML 67 (4.50) CML 73 (4.56), CML-563 -B (4.60), CML 426 (4.66), CML-547 (W) (4.66), MIL 7-38-3 (4.66), CML 327 (5.03), VSL 16 (5.06), QIL 7-273 (5.26), MIL 7-102-2 (5.33), CML 33 (5.40), and MIL 7-16-163 (5.63) were found moderately resistant. At the same time, the remaining genotypes V 372 (6.06), CM 202 (7.33), and BML 6 (7.50) were found susceptible. The identified resistant genotypes (DMRE 63 and CML 71) can be used in resistant breeding.

Keywords: Artificial infestation; fall armyworm; leaf damage rating; maize; resistance.

1. INTRODUCTION

“Globally, maize is known as the "queen of cereals" because of its high genetic yield potential among cereals” [1]. “In India maize is grown in an area of 9.3 mha with a production of 35.67 million tonnes. It is used as food, feed, fodder, industrial purposes, and recently for ethanol production. Several biotic and abiotic stresses hinder the production of maize. Among biotic stresses, insect pests are the major ones affecting maize production. A total of 139 insects cause damage to maize. Out of them, fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] predominantly causing significant yield losses up to 60% if not well managed” [2]. Fall armyworm (FAW) is native to tropical and subtropical regions of the Americas and has been known as a sporadic pest since 1797, which was first recorded in Georgia in 1797. Outside America, FAW first invaded Africa in 2016 Georgen et al., [3] and by January 2018, the pest was reported in over 40 African countries [4]. In India, it was first reported in May 2018 from the Shivamogga district of Karnataka Sharanabasappa et al*.,* [5] and later it spread to major maize-growing states including Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, and Odisha. “Between 2018 and 2021, the pest rapidly spread across Asia–Pacific region and has been reported from Yemen, Bangladesh, Myanmar, China, Thailand, Sri Lanka, Nepal, Philippines, Vietnam, Indonesia, Australia, South Korea, Cambodia, Papua New Guinea, New Caledonia, Jordan and Syria” [6].

FAW consists of two genetically and behaviorally distinct strains that coexist across North and South America [7]. The "corn" strain (C) is known to preferentially damage maize, sorghum, and cotton, while the "rice" strain (R) primarily infests rice, alfalfa, pasture, and forage grasses [8]. FAW is a polyphagous insect pest and it prefers maize compared to other cereals and millets. The larvae cause extensive damage to the leaf, stem, and cob of the maize plant [9]. Indiscriminate use of insecticides to control fall armyworm may result in the development of resistance, resurgence, toxicity to natural enemies, and environmental pollution. Therefore, among several pest management strategies, host plant resistance-based approaches are the most promising as these are sustainable and environmental friendly. Host plant resistance (HPR) involves the development and utilization of crop varieties that possess natural or induced mechanisms to deter or tolerate insect pests. The development of resistant genotypes reduces insecticide applications which results in a decrease in the cost of production for insect management. It also helps to maintain a sufficient natural enemy population under field conditions [10]. Screening diverse maize germplasm, including landraces and improved varieties, is crucial for identifying genotypes with potential FAW resistance. Evaluating a broad genetic pool early on increases the likelihood of finding promising genotypes and achieving effective selection [4,11]. Studies on screening maize germplasm for insect pests identify sources of resistance and susceptibility, paving the way for developing pest-resistant varieties. Comprehensive screening for FAW-resistant maize germplasm has been conducted by Wiseman et al. [12], Widstrom et al. [13] and Smith [14]. Screening will facilitate gene introgression and the creation of high-yielding, resistant varieties within an IPM strategy. Screening methods vary widely and one of them uses the leaf injury rating caused by plant pests. The present study aimed to identify resistant maize genotypes against fall armyworm under artificial infestation.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present study was conducted at Winter Nursery Centre, ICAR-Indian Institute of Maize Research, Rajendranagar during the *rabi* 2023-24. The experimental area comes under Telangana's Southern Zone (Zone-3). It is located between 17.184°N latitude and 78.240°E longitude, at an elevation of 494 meters above Mean Sea Level (MSL).

2.2 Rearing of FAW Culture

Initially, fall armyworm neonate larvae were collected from infested plants at the Winter Nursery Centre (WNC) in Hyderabad, Telangana, India. Larvae were then reared in plastic jars containing a 2 to 3 mm layer of chickpea flour-casein-based artificial diet at the jar's bottom for five days in groups of 50-100 neonates at 28±1°C, 65±10% RH, under a 16-hour light/8-hour dark photoperiod [15]. To prevent cannibalism, larvae were subsequently transferred individually to 12-well plates (HiMedia), each cell measuring 2.5 cm in diameter and 2.3 cm in depth, where they remained until pupation. Pupae were sterilized using a 2% sodium hypochlorite solution and grouped in plastic jars with soil, numbering 25-50 per group. Upon adult emergence, 10 pairs of fall armyworm moths were introduced into a 30 cm long and 23 cm diameter oviposition cage. Adult moths were given a 10% honey solution on a cotton swab while blotting paper strips served as the oviposition substrate within the cage. These strips were replaced daily, and the eggs collected were sterilized using a 10% formalin solution. Subsequently, hatched eggs were transferred with a hairbrush to plastic jars containing an artificial diet. These lab-raised neonate larvae were utilized for artificial infestation to standardize the screening technique.

Table 1. Details of maize genotypes selected for screening against fall armyworm

Sl. No	IIMR Code	Name of the Genotype	Pedigree	Colour of the Seed
1	IMR-1	CML 71	ANTGP2-5-#-1-2-1-1-5-5-7-B	Yellow
2	IMR-2	CML 73	ANTGP2-5-#-1-2-3-B-1-1-1-B	Yellow
3	IMR-3	CML 144	P62-C5-FS182-2-1-2-B-B-3-1-B	White
4	IMR-4	CML 563-B	WLCY2-7-1-2-1-5-B*5	Yellow
5	IMR-5	CML 67	(ANTGP2-5-#-1/ANT38586-1)-6-B-4-2-2-5-B	Yellow
6	IMR-6	BML 6	SRRL 65-B96-1-1-2- # - 2-2-1-Ä-1-1-Äb-Äb	Yellow
7	IMR-156	CML 327	P45-C6-F83-3-1-B	White
8	IMR-363	CML 33	P28TSR-B-B-21-3-1-3-1-B	Yellow
9	IMR-367	CML 426	P31-C4-B*6-38-#-#-2-B	Yellow
10	IMR-392	MIL7-16-163	Brazil 117 X ESM 113-1-1-3-4	Yellow
11	IMR-405	CML547(W)	DRB-F2-60-1-1-1-B	White
12	IMR-416	V 372	V 372	Yellow
13	IMR-461	MIL7-38-3	DKC 8144-3-1-1-2	Yellow
14	IMR-469	MIL7-106-2	LM-13 X DML 170-1-3	Yellow
15	IMR-541	VSL 16	VSL 16	White
16	IMR-576	HUZM 189	HUZM 189	Yellow
17	IMR-630	QIL7-273	BML 6 X DQL 1017) X UMI 1220)-1	White
18	DMRE 63 (Resistant check)	DMRE 63	CM 500 SEL	Yellow
19	CM 202 (Susceptible check)	CM 202	ZSR923-B*4-5-1-B	Yellow

Table 2. Leaf Damage Rating (LDR) scale to categorize maize germplasm for resistance to FAW (Soujanya et al., 2022)

Ratings	Description/Symptoms	Response
1	Healthy plant/Nodamage/VisibleSymptoms	Resistant
2	Few short/pin sizes holes/scraping on a few leaves (1-2)	Resistant
3	Short/pin size holes/scraping on several leaves (3-4)	Resistant
4	Short/pin size holes/scraping on several leaves (5-6) and a few long-elongated lesions (1–3 Nos) up to 2.0 cm length present on whorl and or adjacent fully opened leaves	Resistant
5	Several holes with elongated lesions (4-5Nos) upto 4.0 cm in length and uniform/irregularly shaped holes present on whorl and or adjacent fully opened leaves	Moderately Resistant
6	Several leaves with elongated lesions (6-7Nos) up to 6.0 cm i n length and uniform/irregularly shaped holes present on whorl and adjacent fully opened leaves	Moderately Resistant
7	Several long lesions (>7 Nos) up to 10 cm in length and uniform/irregularly shaped holes common on one-half of the leaves present on whorl and adjacent fully opened leaves	Susceptible
8	Several long lesions >10 cm in length and uniform/irregularly shaped holes common on one-half to two-thirds of leaves present on whorl and adjacent fully opened leaves	Susceptible
9	Complete defoliation of whorl of the plant	Susceptible

2.3 Sowing and Artificial Infestation

This experiment includes 19 maize genotypes (Table 1) and each genotype was sown in a 2 m row and replicated thrice under RBD in an insect screening net house facility. Except for crop protection practices, all the tested maize genotypes were cultivated with recommended agronomic practices, with a 60 cm × 20 cm spacing between rows and plants, respectively. Each plant genotype was released with 20 fall armyworm neonate larvae using a camel hair brush at V5 phenological stage. The observations on leaf damage rating were recorded at the 7th,14th, and 21st DAI based on an LDR scale of 1 to 9[16] (Table 2).

2.4 Statistical Analysis

The LDR data pertaining to the screening of maize germplasm was subjected to RBD analysis using OPSTAT software. The mean values were separated by using DMRT.

3. RESULTS AND DISCUSSION

The mean leaf damage score of the maize genotypes is based on the 1-9 leaf damage rating scale detailed by Soujanya et al. [16]. The LDR Scale of tested genotypes ranged from 2.13 to 4.76 at seven days after larval release (Table 4). The mean leaf damage score per plant was lowest in CML 73 (2.13) followed by DMRE 63 (2.33), CML 71 (2.36), CML 67 (2.73), HUZM 189 (2.73), CML 144 (3.26), MIL 7-38-3 (3.76). The LDR score between 4.1 to 6.0 was observed in genotypes viz., MIL 7-102-2 (4.10), CML 327 (4.20), CML 563 -B (4.20), VSL 16 (4.30), QIL 7-273 (4.33), CML 547 (W) (4.43), BML 6 (4.46), CML 33 (4.56), V 372 (4.56), CM 202 (4.76), MIL 7-16-163 (4.90), and CML 426 (4.93).

At 14 days after larval infestation, the leaf damage rating score ranged from 3.53 to 7.50 and differed significantly among the tested genotypes. The genotypes namely CML 71 (3.53) and DMRE 63 (3.76) recorded LDR <4.0 and were found to be resistant while the genotypes namely HUZM 189 (4.13), CML 144 (4.36), CML 67 (4.50) CML 73 (4.56), CML 563 -B (4.60), CML 426 (4.66), CML 547 (W) (4.66), MIL 7-38-3 (4.66), CML 327 (5.03), VSL 16 (5.06), QIL 7-273 (5.26), MIL 7-102-2 (5.33), CML 33 (5.40), MIL 7-16-163 (5.63), recorded LDR Between 4.1 TO 6.0 and were found moderately resistant. At the same time, the remaining genotypes V 372 (6.06), CM 202 (7.33), and BML 6 (7.50) exhibited LDR between 6.1 to 9.0 and were found susceptible.

At 21 days after larval infestation, the leaf damage score ranged from 3.23 to 7.40 among the tested genotypes (Table 4). The mean leaf damage score per plant was lowest in DMRE 63 (3.23), followed by CML 71 (3.46), CML 327 (3.60), and CML 33 (3.90). The LDR score between 4.1 to 6.0 was observed in genotypes *viz.,* CML 563 -B (4.06), CML 547 (W) (4.10), CML 426 (4.13), MIL 7-16-163 (4.16), CML 67 (4.20), VSL 16 (4.33), CML 144 (4.46), CML 73 (4.50), V 372 (4.50), QIL 7-273 (4.73), MIL 7-38-3 (5.06), CM 202 (5.06), MIL 7-102-2 (5.63), and HUZM 189 (5.63). The remaining genotype, BML 6 (7.40) exhibited an LDR of > 6.

The pooled mean data of leaf damage score per plant varied from 3.11 in DMRE 63 and 5.72 in CM - 202, respectively, and differed markedly from each other (Table 4). The mean leaf damage score per plant was lowest DMRE 63 (3.11) followed by CML 71 (3.13), CML 73 (3.73), CML 67 (3.76). The LDR score between 4.1 to 6.0 was observed in genotypes *viz.,* CML 144 (4.06), CML 327 (4.10), CML 563-B (4.30), HUZM 189 (4.16), CML 547(W) (4.40), MIL 7-38-3 (4.49), VSL 16 (4.56), CML 426 (4.57), CML 33 (4.62), QIL 7-273 (4.77), MIL 7-16-163 (4.90), MIL 7-102-2 (5.02), V 372 (5.04), CM 202 (5.72). The remaining genotype, BML 6 (6.46) exhibited an LDR of > 6.0.

The current results align with Wiseman et al. [17], who reported differential damage by fall armyworm between moderately resistant and susceptible genotypes which might be due to antixenosis and or antibiosis mechanisms, Ni et al*.* [18] observed two genotypes, Mp 708 and FAW 706, as resistant to fall armyworm based on foliar damage rating. Conversely, Ab24E and EPM 6 were found to be the most susceptible genotypes. Varma et al. [19] similarly identified resistant and susceptible genotypes against fall armyworm, with GAYMH 3 (2.39), GAYMH 1 (2.60), GAWMH 2 (3.07), NARMADA MOTI (3.66) and GM 6 (3.74) as resistant genotypes whereas GSCH 0918 (5.80) as susceptible genotype. Furthermore, the current study's findings are consistent with those of Soujanya et al. [16], who noted that genotypes such as DMRE-63, DML163-1, CML 71, CML 141, CML 337, CML 346, and the wild ancestor *Z. mays* spp*. parviglumis* exhibited reduced leaf damage due to fall armyworm. These observations suggest their potential utility in maize breeding programs to enhance resistance. Moreover, the present study observations align with earlier findings of Gowda et al. [20], Where, CML 71, CML 67, DMRE 63, CML 561, AEBY-1, CML 335, CML 345, and CML 337 showed moderate resistance with mean LDR of 3.93, 4.00, 4.17, 4.36, 4.42, 4.57, 4.72, and 4.80, respectively. Matova et al*. [21]* found peak fall armyworm foliar damage at the V6 whorl leaf stage in maize genotypes. The decrease in LDR score was observed in genotype CML543/CML334 from the 4th week (6.43) to the 8th week (5.70) after germination. This is in accordance with the current study in the majority of genotypes which was noticed from 14th to 21st DAI. Tiwari et al. [22] observed that maize genotype ADV-768 (2.72) recorded minimum leaf damage, whereas SWEET GLORY (8.52) had maximum leaf damage. Darshan et al. [23] reported that Kaveri minchu recorded the highest mean leaf damage score (4.62) whereas, the hybrid PMH 224 recorded the lowest mean leaf damage score (0.73).

In the present study, LDR was recorded at the 7th,14th and 21st DAI, but for categorizing the genotypes into resistant, moderately resistant, and susceptible LDR of 14 DAI was considered. Assessing damage at 7 days after infestation (DAI) is premature because larvae in the mid-growth stages (first to third instar) have not reached their peak damage potential. These early instar larvae of the fall Armyworm are small and consume only 2 % of foliage during their life cycle. In contrast, fourth, fifth, and sixth instar larvae consume 4.7 %, 16.3 %, and 77.2 % respectively, heavily defoliating crops [24]. By the time larvae pupate around 21 DAI, plants begin to recover from initial damage, making the rating at this stage not fully indicative of the larvae's impact. Therefore, assessing damage at 14 DAI provides a more accurate reflection of both the complete leaf damage caused by FAW and the host plant's response to the infestation. Furthermore, the lower feeding rate of early instars (first and second) Ren et al., [25] is likely to get a low damage rating at 7 DAI. By 14 DAI, the larvae will attain the fourth and fifth instars and are feeding heavily, leading to increased damage levels. According to Zalucki et al. [26], mortality in the early larval stages is generally high in Lepidoptera. As per Davis et al*.,* [27], the 7-day scale reduces leaf damage from mid-instar FAW larvae migration, while the 14-day scale shows overall feeding damage and helps detect any delayed plant resistance to insects that may not be evident at 7 days.

Table 3. Response of various maize genotypes to fall armyworm infestation

Sl.no	Genotype	7 DAI	14 DAI	21 DAI	Mean	Response
1	CML 71	2.36^{gh}±0.067	3.53^{i}±0.120	3.46^{ef}±0.088	3.13^{f}± 0.367	R
2	CML 73	2.13^{h}±0.088	4.56^{defgh} ±0.088	4.50^{bcde}±0.058	3.73^{ef}± 0.817	MR
3	CML 144	3.26^{ef}±0.033	4.36^{fghi}±0.033	4.46^{bcdef}± 0.088	4.06^{cdef}± 0.384	MR
4	CML 563-B	4.20^{cd}±0.120	4.60^{defgh}±0.306	4.06^{cdef}± 0.549	4.30^{cde}± 0.153	MR
5	CML 67	2.73^{fg}±0.351	4.50^{efgh} ±0.058	4.20^{cdef} ± 0.100	3.76^{def}±0.289	MR
6	BML 6	4.46^{abc}±0.208	7.50^{a}±0.058	7.40^{a}± 0.115	6.46^{a}± 0.984	S
7	CML 327	4.20^{cd}±0.346	5.03^{cdef}±0.176	3.60^{def}± 0.306	4.10^{cdef}±0.406	MR
8	CML 33	4.56^{abc}±0.441	5.40^{bcd}±0.153	3.96^{cdef}± 0.441	4.62^{bcde}±0.433	MR
9	CML 426	4.93^{a}±0.416	4.66^{defg}±0.584	4.13^{cdef}± 0.546	4.57^{cde}±0.265	MR
10	MIL7-16-163	4.90^{a}±0.067	5.63^{bc}±0.433	4.16^{cdef}± 0.426	4.90^{bcd}±0.404	MR
11	CML-547(W)	4.43^{abc}±0.088	4.66^{defg}±0.433	4.10^{cdef}± 0.361	4.40^{cde}±0.176	MR
12	V 372	4.56^{abc}±0.186	6.06^{b}±0.219	4.50^{bcde}± 0.265	5.04^{bc} ±0.484	S
13	MIL 7-38-3	3.76^{de}±0.058	4.66^{defg}±0.219	5.06^{bc}± 0.601	4.49^{cde}±0.361	MR
14	MIL 7-102-2	4.10^{cd}±0.145	5.33^{bcde}±0.088	5.63^{b}± 1.241	5.02^{bc}±0.513	MR
15	VSL 16	4.30^{bcd}±0.176	5.06^{cdef}±0.406	4.33^{cdef}± 0.176	4.56^{cde}±0.233	MR
16	HUZM 189	2.73^{fg}±0.260	4.13^{ghi}±0.722	5.63^{b}± 0.633	4.16^{cdef}±0.837	MR
17	QIL 7-273	4.33^{bc}±0.033	5.26^{bcde}±0.491	4.73^{bcd}± 0.120	4.77^{bcde}±0.260	MR
18	DMRE 63	2.33^{gh}±0.120	3.76^{hi}±0.367	3.23^{f}± 0.133	3.11^{ef}±0.406	R
19	CM 202	4.76^{ab}±0.167	7.33^{a}±0.033	5.06^{bc}± 0.296	5.72^{ab} ±0.821	S
SE(m)		**0.209**	**0.295**	**0.445**	**0.400**	
***F* value**		**21.542**	**12.332**	**4.632**	**4.012**	
***P* value**		**<0.001**	**<0.001**	**0.00005**	**0.00019**	
CD		**0.601**	**0.849**	**1.279**	**1.151**	
CV		**9.360**	**10.091**	**16.980**	**15.444**	

Means with the same letter are not significantly different. Each value represents the mean of three replications
R, Resistant; MR, Moderately Resistant; S, Susceptible. DAI-Days After Infestation

Table 4. Classification of maize genotypes against fall armyworm based on leaf damage rating (1-9 scale)

Sl.No.	Genotypes	LDR	Categorization	Number of Genotypes
1	CML -73, DMRE 63	1-4	Resistant	2
2	CML 71, CML 67, CML 144, HUZM 189, CML-563 -B, CML 426, CML-547 (W), MIL 7-38-3, CML 327, VSL 16, QIL 7-273, MIL 7-102-2, CML 33, MIL 7-16-163	4.1-6	Moderately resistant	14
3	V 372, BML 6, CM 202	6.1-9	Susceptible	3

4. CONCLUSION

The study revealed that among the 19 genotypes screened, the genotypes namely CML 71 and DMRE 63 were found to be resistant, while, the genotypes namely HUZM 189, CML 144, CML 67, CML 73, CML 563 -B, CML 426, CML 547 (W), MIL 7-38-3, CML 327, VSL16, QIL 7-273, MIL 7-102-2, CML 33, MIL 7-16-163, were observed as moderately resistant. At the same time, the remaining genotypes V 372, CM 202, and BML 6 were found susceptible. The resistant genotypes identified in this study can be used in breeding programs aimed at developing hybrids resistant to Fall Armyworm (FAW).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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