Evaluation of Inbreds for Resistance to Post-Flowering Stalk Rot Disease in Maize (*Zea mays*.)

ABSTRACT

Maize (*Zea mays*) is the most influential crop in the world, which can grow in temperate as well as tropical conditions. It is a major source for food, livestock, the beverage industry and cosmetics. In recent days maize production decreased drastically due to many diseases. Fusarium stalk rot is one among them. To evaluate the genotypes resistance against PFSR, 40 maize genotypes were subjected against five virulent isolates of *Fusarium* using the same artificial inoculation method. Varying disease reactions among the inbreds, with some exhibiting premature drying and lodging. After 40 days post-inoculation, internode infection was observed, and disease severity was assessed based on lesion spread within or across internodes. The inbreds were categorized according to their resistance levels, ranging from highly resistant to highly susceptible based on internode infection. Only one inbred showed high resistance to both FUR11 and Raichur isolates. None of the inbreds exhibited high resistance or resistance against the F1 isolate. Eight inbreds showed resistance against multiple isolates. One inbred, DML-1802, displayed high resistance to FUR11 and resistance to Raichur isolate, while another, A8-6, was highly susceptible to all tested isolates, showing infection spreading up to two internodes and observed xylem vessel rotting and hollow stems. Eleven resistant inbreds against Fusarium stalk rot were selected for a breeding program aimed at developing Fusarium stalk rot-resistant hybrids. The identification of variability among Fusarium species causing stalk rot in maize provided valuable insights for future research due to the limited understanding of Fusarium heterogeneity in maize stalk rot.

Keywords: Inbreds; Fusarium; PFSR; Resistance.

1. INTRODUCTION

Maize (*Zea mays* L.) is popularly known as the 'Queen of cereals' because of its large area coverage, good production, productivity, feed, forage, industrial usage, cosmetics and biofuel manufacturing. Maize is cultivated all over the world. It is the third most significant crop after rice and wheat cultivated in India. In India, Karnataka stands first in the area with 1.3 M ha having production of 3.75 MT, while Madhya Pradesh stands first in Production and having 1.26 M ha with a production of 4.13 MT (According to Directorate of Economics and Statistics, 2019-20). The maize crop is susceptible to many diseases like southern corn blight (Bipolaris maydis), smut (*Ustilago maydis*), Northern corn blight (*Exserohilum turcicum*) and Post flowering stalk rot (*Fusarium* spp, *Macrophomina* spp. and *Cephalosporium* spp.). Among these diseases, Fusarium stalk rot (*Fusarium* spp.) disease is more prevalent (10-42%) in all most maize growing regions of India i.e. Jammu and Kashmir, Punjab, Haryana, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka [1]. The major pathogens associated with the disease are *Fusarium subglutinans*, *Fusarium verticilliodes*, *Fusarium proliferatum* [2] *Fusarium moniliformae* [3]. The disease is more severe in water stress conditions. Symptoms are majorly drooping leaves, lodging, poor grain formation and stalks appeared brownish to blackish discolouration when stalks split open, spreads to next internodes, dropping of ears, stalk breakage, premature drying of stalks and causes economic yield loss. Pathogens also produce toxins Fumonisins B1, Fumonisins B2, Deoxynivalenol and zearalenone [4]. Breeding for the resistance genotypes which provides the valuable insights to manage disease.

2. MATERIALS AND METHODS

Screening of maize genotypes against most virulent isolates of Fusarium spp. was conducted during summer season 2021 in the RLBCAU, Jhansi farm. The five most virulent isolates of Fusarium (FUR11, Raichur, Chokhla, F1, F59) were found in pathogenicity tests conducted during Kharif 2020 and Rabi 2020 season were inoculated to 40 different inbred lines of maize

obtained from the ICAR-Indian Institute of Maize Research, Ludhiana. Artificial inoculation was done by using toothpick inoculation method as described in materials and method section. Stems split open longitudinally at the harvest and were observed for dark brown discoloration and lesions after 30 days inoculation. Resistance and susceptible reactions and disease severity will be calculated based on lesion length by using the disease scale given by Payak & Sharma [5]. Susceptible and resistant inbred lines are identified. The genotypes having resistance reactions are selected for future breeding programs for resistance against the Fusarium stalk rot of Maize.

2.1 Toothpick Inoculation Method

The isolates of *Fusarium* spp. were grown on a PDB medium for 7 days. Round wooden toothpicks about 6cm long were boiled in distilled water for 1 hr to remove gum, resin, and other toxic particles which may inhibit the growth of fungus. The water was decanted and discarded and the procedure was repeated 3 more times. After boiling, toothpicks dried on sterile blotter paper. The toothpicks were bundled and packed in test tubes (10 toothpicks per each) and PDB (Hi-media) was added to each test tube to thoroughly moisten the toothpicks. Toothpick tips were kept in an upward direction with their pointed tips upwards. The amount of broth should be such that 1-1.5 cm tip of the toothpicks will remain above broth level in a test tube. The test tubes were autoclaved, cooled and inoculated with plugs of individual *Fusarium* isolate. The inoculated test tubes were incubated at 28±1 °C and incubated under 12-hour period of alternating light and dark for 10 days. Maximum mycelial growth would be multiplied on the toothpicks.

Artificial inoculation was done to the maize plants 45-50 days old, after the flowering stage. Mycelium was inoculated in the lower internodes preferably 2nd internode above the soil surface. The toothpicks were inoculated diagonally after stabbing/puncturing and making a 2cm hole by using jabber in internodes. Symptoms would have appeared after 20-25 days of inoculation. Disease intensity and severity (PDI) were calculated by following a 1-9 rating given by Payak & Sharma, [5]. Percentage disease index was calculated by using the formula (Horsefall-Barratt (H-B) scale) Herbert, T. T. [6].

Percent disease index=(sum of numerical rating)/(Total number of sample taken X Maximum grade) X 100

Table 1. List of maize inbreds used for resistance evaluation test against fusarium stalk rot.

SL. No	Inbreds	IC No.	Details
1.	2011077	IC0612735	DML-187-1
2.	2011204	IC0620972	DML-1441
3.	2011205	IC0620973	DML-1451
4.	2011212	IC0620984	DML-1497
5.	2011223	IC0620998	DML-1642
6.	2011229	IC0621011	DML-1802
7.	2011231	IC0621013	DML-1805
8.	2011236	IC0621020	DML-1818
9.	2011239	IC0621025	DML-1825
10.	2011247	IC0621034	DML-1841
11.	2011250	IC0621038	DML-1848
12.	2011274	IC0621069	DML-1924
13.	2011280	IC0621104	DQL-2048
14.	2011289	IC0621150	DQL-2028
15.	2011299	IC0621161	DQL783-31-15-10-1
16.	2011301	IC0621164	DQL-769-6-2-4-3
17.	2011319	IC0621564	A8-6
18.	2011320	IC0621565	A8-9
19.	2011344	IC0621583	Z172-221
20.	2011349	IC0621591	Z172-62
21.	2011355	IC0621594	NS-3
22.	2011368	IC0621605	NS-14
23.	2011370	IC0621609	NS-18
24.	2011382	IC0621625	DPcl-117
25.	2011385	IC0621629	IML-12-161

26.	2011405	IC0621656	IML-16-157
27.	2011429	IC 405280	HKI-1025
28.	2011447	IC 542344	VQL-2
29.	2011454	IC 553797	CML-32(PARENTAL-LINE
30.	2011461	IC 560488	NP-5088
31.	2011480	IC 571611	NK-125-(NECH-131-FP)
32.	2011542	IC0618733	FP:-QMISC-1407-for-IC618732
33.	2011585	IC0624636	KDM-895A
34.	2012129	IC0612799	IQL67
35.	2012131	IC-584601	HKI-6
36.	PFSR 5	-	-
37.	CM500	-	-
38.	CM600	-	-
39.	CM501	-	-
40.	CM 400	-	-

Table 2. Disease rating scale for maize fusarium stalk rot disease [5]

Rating scale	Degree of infection	Disease reaction
1	No discoloration or discoloration only at the point of inoculation	Highly resistant
2	<25% of the inoculated internode discoloured	Resistant
3	25 to <50 % of the inoculated internode discoloured	Moderately resistant
4	50 to <100 % of the inoculated internode discoloured	Moderately Susceptible
5	25% of adjacent internode discoloured	Susceptible
6	½ discoloration of the adjacent internode	Highly susceptible
7	Discoloration of three internodes	Highly susceptible
8	Discoloration of four internodes	Highly susceptible
9	Discolorations of five internodes or plants prematurely killed	Highly susceptible

3. RESULTS

In an effort to identify resistant source to utilize in resistance breeding programme we evaluated 40 inbred lines against five most virulent isolates (FUR 11, F59, F1, Raichur and Chokhla). Artificial inoculation of 40 inbred lines was done by toothpick inoculation during summer season of 2021. At the harvest, lesion length was measured and using the scale given by Payak and Sharma, [5] disease grades were given. At the first glance in field, few inoculated inbred lines were appearing green and healthy but when split open the lesion was showing the extended infection rate.

Some inbreds the whole plant was lodged. This may be due to the variation in maturity period of each inbred line. Another reason is that each inbred has different vegetation period. There was variation in internodal distance in all inbred lines. Some highly evolved inbreds were having longer intermodal length that the others which were having shorter intermodal length. The relative disease severity grades may vary on the basis of given scale which primarily based on the infection in intermodal space of plant (Fig. 1).

Out of 40 inbred lines 5 inbred lines were observed as highly resistant against FUR11 and Raichur isolates. 2 inbred lines (DML-1802 and NS-14) against FUR11 isolate showing average lesion length ranges from 0.5-1 cm, mean disease scores ranges from <2 with severity varies from 11.11-22.22%. 3 inbred lines (CML-32, NS-14, and DML-1805) against Raichur isolate caused lesion length varied from 0.7-1.2 cm with disease mean score <2 and severity varied from 11.11-22.22%. No resistance was observed in any inbred lines when inoculated with Chokhla, F1 and F59 isolates. 8 inbred lines were observed as resistant against virulent isolates of *Fusarium* spp. 2 inbred lines (IML-16-157 and DML-1451) against Chokhla isolate, one inbred line (DML-1642) against FUR11 and 5 inbred lines (NP-5088, DML-1441, DML-1802, KDM-895 A and CM500) against Raichur isolate were found resistant. The disease mean ranges between 2.1-2.4 with severity of 23-29 %. There were no inbred lines were found resistant against F1 and F59 virulent isolates.

11 inbred lines were observed as moderately resistant against virulent *Fusarium* spp. isolates. One isolate (NP-5088) against Chokhla isolate, one isolate (DML-1642) against F1 isolates, 04 inbred lines (DML-1848, A8-6, DML-187-1 and DML-1451) against FUR11, 03 inbreds (DML-1451, DML-187-1, CM501 against F59 isolate), 02 isolates (IML-12-161, DML-1841) against Raichur isolate were found moderately resistant (**Fig. 2, Table 3**). Average disease mean score of this inbreds were ranges from 3-3.6 with the severity of 30-39 %. 10 inbreds (IML-12-161, DPcl-117, QMISC-1407, 2011129, DML-1642, DML-1802, HKI-6, DML-1924,DML-1818 and CML-32) were showed moderately susceptible to Chokhla, 12 inbreds (DML-1451, DPcl-117, QMISC-1407, IML-12-161, NP-5088, DML-1802, DML-1441, CM600, DML-1497, DML-1841, A8-9 and DML-1805) were found moderately susceptible to F1, 15 inbred lines (DQL783-31-15-10-1, NS-18, DQL769-6-2-4-3, QMISC-1407, DML-1642, CM400, CM600, 2011129, NS-3, DQL-2028, A8-9, KDM-895 A, DML-1818 and DML-1805) were found moderately susceptible to FUR11, 14 inbreds (DQL783-31-15-10-1, NS-18, DQL769-6-2-4-3, QMISC-1407, DML-1642,

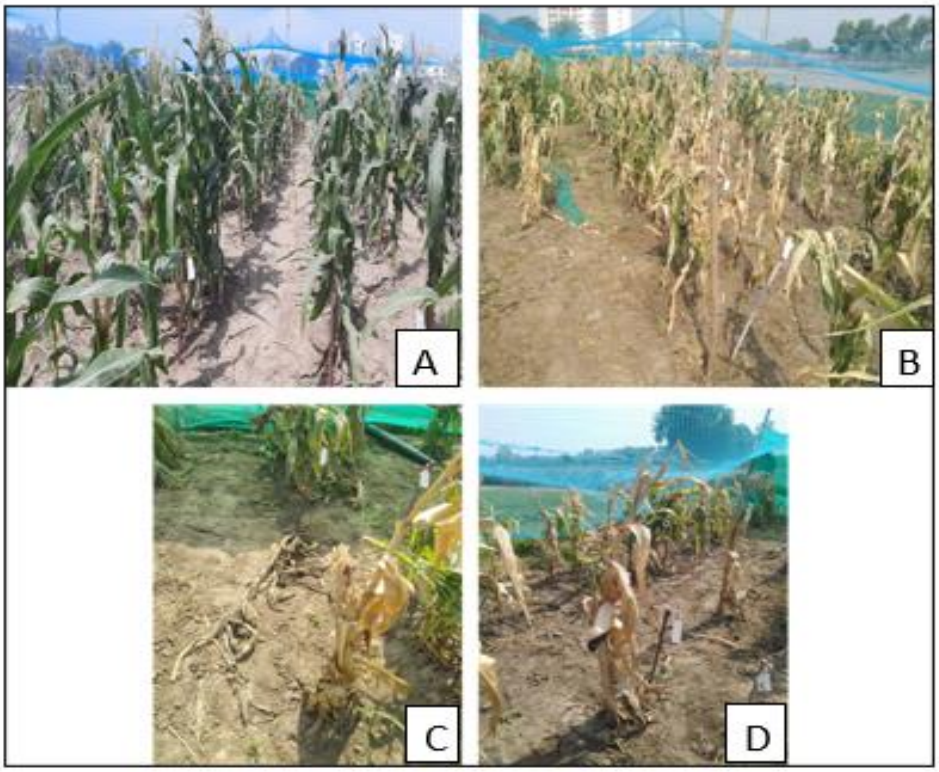

Fig 1: Experimental field view of resistance evaluation in Inbred line against virulent Fusarium isolates during summer 2021 **Fig A:** Maize inbreds at flowering stage, **Fig B:** Drying of plants due to pathogen infection **Fig C:** Lodging of whole plant, **Fig D:** Drooping of cobs/ angle between cob and stalk increases

CM400, CM600, 2011129, NS-3, DQL-2028, A8-9, KDM-895 A, DML-1818 and DML-1805) were found moderately susceptible to F59 isolate.

10 inbreds (IML-12-161, DPcl-117, QMISC-1407, 2011129, DML-1642, DML-1802, HKI-6, DML-1924,DML-1818 and CML-32) were showed moderately susceptible to Chokhla, 12 inbreds (DML-1451, DPcl-117, QMISC-1407, IML-12-161, NP-5088, DML-1802, DML-1441, CM600, DML-1497, DML-1841, A8-9 and DML-1805) were found moderately susceptible to F1, 15 inbred lines (DQL783-31-15-10-1, NS-18, DQL769-6-2-4-3, QMISC-1407, DML-1642, CM400, CM600, 2011129, NS-3, DQL-2028, A8-9, KDM-895 A, DML-1818 and DML-1805) were found moderately susceptible to FUR11, 14 inbreds (DQL783-31-15-10-1, NS-18, DQL769-6-2-4-3, QMISC-1407, DML-1642, CM400, CM600, 2011129, NS-3, DQL-2028, A8-9, KDM-895 A, DML-1818 and DML-1805) were found moderately susceptible to F59 isolate. 10 inbreds (DML-1451, DQL-2048, DML-1642, DML-1848,

PFSR-5, DML-1818, NS-18, NK-125-(NECH-131- FP), HKI- 6 and DML-1497) were found

moderately susceptible to Raichur. In all these moderately susceptible inbreds lines the disease mean score was ranges between 4-4.6 with a severity range from 40-49%. 3 inbred lines DQL783-31-15-10-1, DML-1441 and DML-1497 were found susceptible to Chokhla. 11 inbreds

(DQL769-6-2-4-3, HKI-16-157, IQL67, IML-16-157, NS-18, DML-1818, 2011131, DQL-2028, DQL-2048, DML-1848 and NS-14) were found susceptible to isolate F1. 2 inbred lines (IQL67 and NS-18) were found susceptible to FUR11. 6 inbreds lines (HKI-16-157, IML-12-161, DML-1441, DML-1848, DML-1802 and CML-32) were found susceptible to isolate to F59 isolate. 2 inbred lines (NS-3 and VQL-2) were found susceptible to Raichur isolate. The disease mean score of all inbred lines found susceptible were in a range of 5-5.8 with a severity of 50-76%. 4 inbred lines (NS-3, DML-1805, KDM-895 A and A8-6) were found highly susceptible to Chokhla isolate. 8 inbred lines (DQL783-31-15-10-1, CML-32, NS-3, KDM-895 A, VQL-2, A8-6, CM400 and CM500) were observed as highly susceptible to F1. 5 inbred lines (DML-1441, IML-16-157, DML-1805, HKI-6) and NK-125-(NECH-131- FP) were observed as highly susceptible to FUR11 isolate. 4 inbred lines (NP-5088, NS-18, A8-6 and HKI-6) were found highly susceptible against F59. 8 inbreds (DQL783-31-15-10-1, DQL769-6-2-4-3, HKI-16-157, 2011129, CML-32, IML-16-157, A8-6 and A8-9 were found highly susceptible to Raichur isolate. The disease mean score of all inbreds lines found highly susceptible were ranges from greater than 6 and severity shows greater than 77%. A8-6 line recorded as highly susceptible line against 4 virulent isolates Chokhla, F1, F59 and Raichur. The disease spreads more than 2 internodes (**Fig. 3, Table 3**).

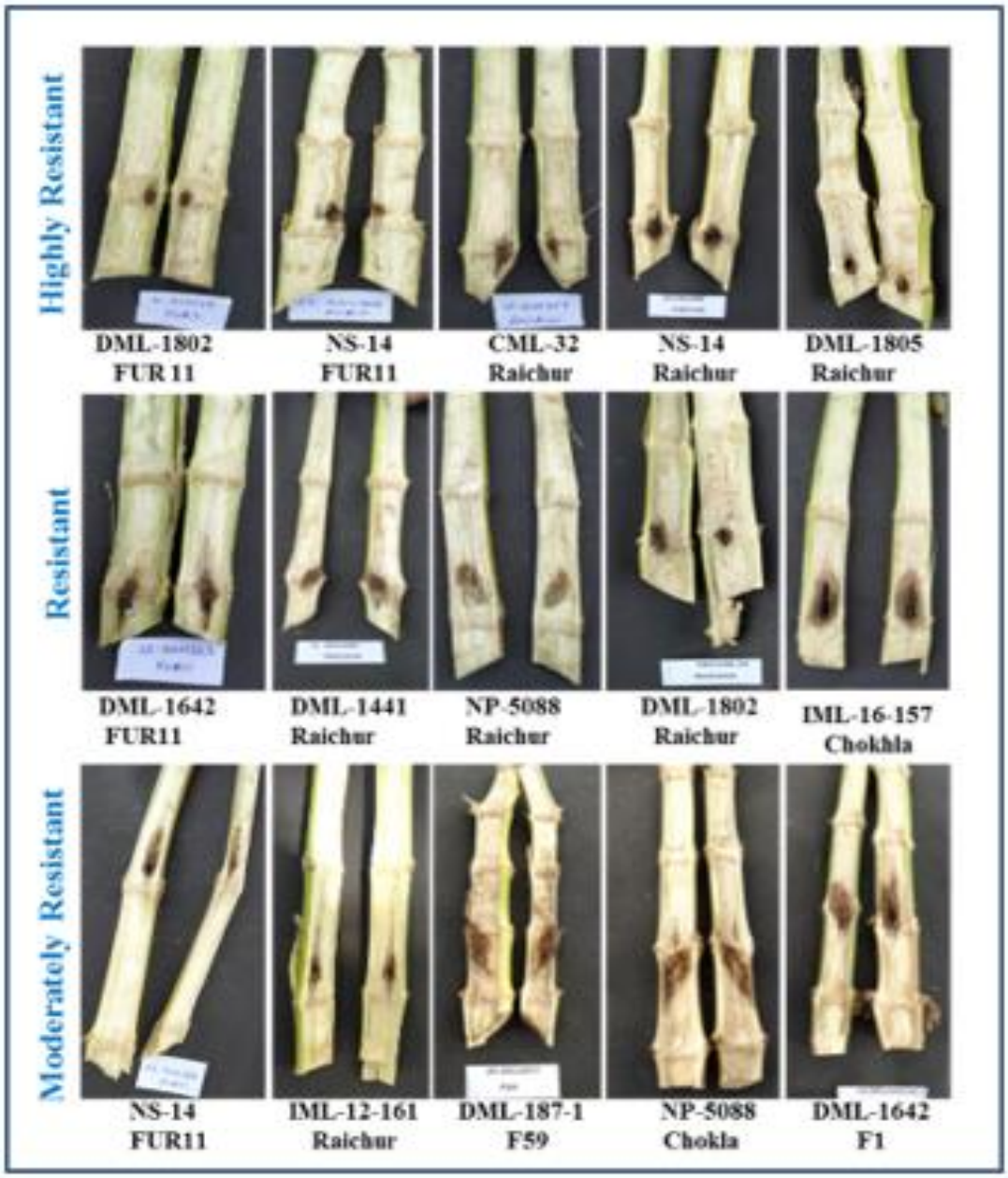

Fig 2: Lesion length in split open maize stalk of various inbred lines showing resistance reaction to the virulent isolates

Table 3. Inbreds showing disease reaction against 5 different virulent isolates of *Fusarium* stalk rot

Disease rating	Mean range	PDI range	Isolates									
			Chokhla	Lesion length(cm)	F1	Lesion length(cm)	FUR11	Lesion length(cm)	F59	Lesion length(cm)	Raichur	Lesion length(cm)
Highly resistant	<2	11.11-22.22					DML-1802 NS-14	0.5-1			CML-32 NS-14 DML-1805	0.7-1.2
Resistant	2.1-2.4	23-29	IML-16-157 DML-1451	1.83-2.83			DML-1642	1.5-2.66			NP-5088 DML-1441 DML-1802 CM500 KDM-895 A	2.5 – 3.33
Moderately resistant	3-3.6	30-39	NP-5088	4.16-6.83	DML-1642	4.33- 7.83	DML-1848 A8-6 DML-187-1 DML-1451	4.16-6	DML-1451 DML-187-1 CM501	4.83-7	IML-12-161 DML-1841	4.16-7
Moderately susceptible	4-4.6	40-49	IML-12-161, DPcl-117, QMISC-1407 2011129 DML-1642 DML-1802 HKI-6 DML-1924 DML-1818 CML-32	7.8-10.5	DML-1451 DPcl-117 QMISC-1407 IML-12-161 NP-5088 DML-1802 DML-1441 CM600 DML-1497 DML-1841 A8-9 DML-1805	7.6-10.83	DQL783-31-15-10-1 DPcl-117 DQL-2048 DML-1841 KDM-895 A DML-1818 NS-3 A8-9 DML-1497 DQL-2028 DQL769-6-2-4-3 HKI-16-157 IML-12-161 QMISC-1407	7.5-11	DQL783-31-15-10-1 NS-18 DQL769-6-2-4-3 QMISC-1407 DML-1642 CM400 CM600 2011129 NS-3 DQL-2028 A8-9 KDM-895 A DML-1818 DML-1805	7.16-10.66	DML-1451 DQL-2048 DML-1642 DML-1848 PFSR-5 DML-1818 NS-18 NK-125-(NECH-131-FP) 2011131 DML-1497	7.3-11.33
Susceptible	5-6	50-76%	DQL783-31-15-10-1 DML-1441 DML-1497	15.5-17	DQL769-6-2-4-3 HKI-16-157 IQL67 IML-16-157 NS-18 DML-1818 HKI- 6 DQL-2028 DQL-2048 DML-1848 NS-14	15.33-19	IQL67 NS-18	14.5-16.66	HKI-16-157 IML-12-161 DML-1441 DML-1848 DML-1802 CML-32	14-16.5	NS-3 VQL-2	14.83-17
Highly susceptible	>7	>77	NS-3 DML-1805 KDM-895 A A8-6	>17.5	DQL783-31-15-10-1 CML-32 NS-3 KDM-895 A VQL-2 A8-6 CM400 CM500	>20	DML-1441 IML-16-157 DML-1805 2011131 NK-125-(NECH-131- FP)	>17	NP-5088 NS-18 A8-6 HKI-6	>17	DQL783-31-15-10-1 DQL769-6-2-4-3 HKI-16-157 IQL67 CML-32 IML-16-157 A8-6 A8-9	17.5

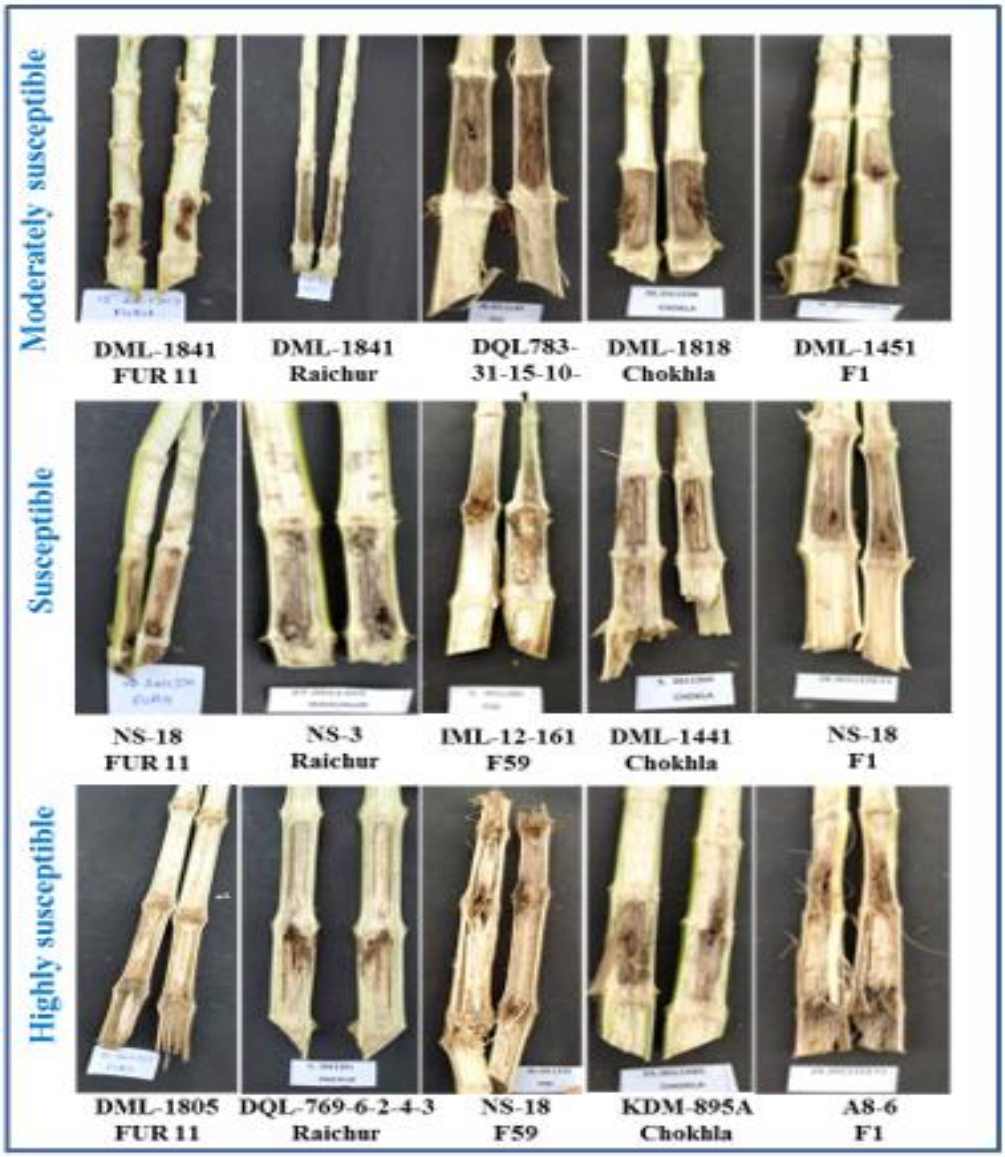

Fig. 3. Lesion length in split open maize stalk of various inbred lines showing susceptible reaction to the virulent isolates

4. DISCUSSION

FSR is one of the major diseases in maize which reduces yield directly by affecting the metabolic activities of the plants and leads to lodging, which is the major cause for economic yield loss. Resistance evaluation of this pathogen against inbreds/hybrids will play a very important role in breeding programmes for developing resistance against FSR of maize. Inbreds which were having longer lesion, meant that they were exposed to the effect of artificial inoculation for a longer period [7]. Khokhar and Sharma, [8] reported resistant sources against PFSR in normal and speciality corn incited by *F. verticillioides*. Szoke *et al*. [7] reported that hybrids were more susceptible than inbreds.

A set of 200 elite maize lines was screened against PFSR diseases at 9 different geographical locations of the country (Hooda *et al*., 2012). Three resistant lines, namely PFSR-13-5, JCY2-2-4-1-1-1-1 and JCY3-7-1-2-1-b-1 were identified to resistant PFSR (*Cephalosporium maydis* , *Fusarium moniliforme* and *Macrophomina phaseolina* (Shekhar et al.,2010). Out of 30 genotypes, six namely Rampur composite, Arun 2, Rampur 34, RamS03F08, TLBRS07F16 and Rampur 24 were found resistant against stalk rot complex with high yield at Rampur Chitwan in Nepal [9].

Harleen *et al*. (2016) identified 2 resistant lines PMH1 and LM-13 against stalk rot caused by Fusarium verticilliodes. Gopala *et al*. [10] screened 34 inbred lines and reported six lines viz. H 109, P 503, 15026, H37 P345 and 18758 as resistant against *F. verticillioides*. Jat *et al*. [11] screened 80 germplasm lines, out of which only two lines were found highly resistant reaction, 50 lines as resistant, 13 as moderately resistant, 10 as moderately susceptible and 3 as susceptible lines to PFSR. Jat *et al*. [11] reported speciality corn lines PMSQ5 and LQPMH 115 as highly resistant to PFSR. Lingaraju *et al*. [12-13] screened 5 resistant inbred lines (C298, SNL153296, VL1010764, VL05616 and VL1O36) and 10 hybrid resistant lines (VH152961, VH151797, 33554×104, VH152573, VH152809, VH152809, MAH 14-5, MAH 14-733, 267×40421, 10×729 and 40310×305) were found resistant against FSR. Kalpana et al. (2022), also reported that maize genotypes against *Fusarium verticiiloides* and identifies eight genotypes AH1625, BAU-MH-18-2, GGMH-114, GK3207, CMH-12-686, CAH1511, ADH1619 and FQH-148 with stable resistance [14-15].

5. CONCLUSION

Inbreds showing resistant reaction against *Fusarium* stalk rot were utilized in breeding programme for development of hybrids resistant against *Fusarium* stalk rot. All these inbreds were further examined in the field for their disease reaction assessment. The bring out information about the variability among *Fusarium* spp. causing *Fusarium* stalk rot was advantageous for advance research work, due to limited work done heterogeneity of *Fusarium* spp. in stalk rot of maize.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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