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### **Research Article**

# Identification of high yielding inbred lines resistant to late wilt disease caused by *Harpophora maydis* in maize

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#### Abstract

Diseases are the major constraints in realizing the yield potential of maize. Late wilt disease (LWD) caused by Harpophora maydisis one of the recently reported and widely spreading diseases across the world. Identification of LWD resistant source is an economical and eco-friendly approach. An experiment was conducted to identify LWD resistant inbred lines by subjecting 290 inbred lines to artificial screening. The same set of lines were evaluated for yield and yield attributing traits separately. Inbred lines were subjected to screening by inoculating Harpophora maydis spore suspension to stalks. Disease severity and intensity were recorded in split opened stalks using a 1 - 9 scale. Estimates of yield and yield attributing traits were also recorded and 14 inbred lines with the disease score ≤4 were identified as resistant/tolerant. Two inbred lines namely, 78 and 32589 are both tolerant to LWD and best yielding lines which can serve as potential parents for developing hybrids.

#### Kev words

Maize, inbred line, late wilt disease, artificial screening, disease score

#### INTRODUCTION

Maize stands third in production among cereal crops. Maize is grown in a wide range of agro-ecologies of the world. It has the highest genetic potential among the cereals. Biotic and abiotic stresses are the major constraints in realizing the yield potential in maize. About 9 per cent yield losses in maize are attributed to diseases alone (Oerke, 2006) which vary from 4 per cent in northern Europe to 14 per cent in West Africa and South Asia. Diseases cause severe yield loss in both quantity and quality of the grain and also increasing the cost of production. In Southeast Asia, hot, humid conditions have favoured disease development while economic constraints prevent the deployment of effective protective measures.

The post flowering stalk rot (PFSR) complex is one of the destructive and widespread groups of diseases in maize (Khokar *et al.*, 2014). The disease is known to be associated with many pathogens, majorly, *Fusarium* 

moniliforme, Macrophomin aphaseolina and Harpophora maydis (Shekhar et al., 2010). The disease causes internal decay and discoloration of stalk tissue, directly reducing yield by blocking translocation of water, nutrient and can result in death and lodging of the plant. PFSR is a complex disease and involves a number of fungi, bacteria and nematodes in decaying the pith (Cook, 1978). Harpophora maydis is one of the fungi involved in PFSR complex. When the maize crop is infected by H. maydis alone, it causes late wilt disease (LWD) which is seed-borne and soil-borne (Michail et al., 1999; Degani and Cernica, 2014) causing loss upto 51 per cent (Johal et al., 2004).

Late wilt disease is characterized by relatively rapid wilting of maize plants typically at the age of 70 to 80 days, before tasselling and until shortly before maturity (Chalkey, 2016). It is considered as endemic in major maize growing areas (Degani and Cernica, 2014). The LWD was first reported

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in Egypt in 1963 (Samra et al., 1963), subsequently it was reported from different maize growing countries such as Tanzania, Pakistan, Hungary and Kenya (Freeman and Ward, 2004), Egypt and India (Ward and Bateman, 1999), Portugal and Spain (Molinero-Ruiz et al., 2010), Romania (Bergstorm et al., 2008) and Israel (Drori et al., 2013). The disease is distributed widely in the Iberian Peninsula (Ortiz-Bustos et al., 2015).

This nature of disease misleads farmers from taking up plant protection measures. Later, the disease becomes severe leading to yield loss. Among the various methods to address the losses due to diseases, the use of resistant cultivars gains priority (El-shafey *et al.*, 1988). Hence, breeding for resistant cultivar is the need of the hour to combat the losses caused by LWD. In any breeding program, it is pre-requisite to identifying the resistant/ tolerant source of disease. With this background, research was conducted to identify inbred lines resistant/tolerant to LWD with high yield.

#### **MATERIALS AND METHODS**

The material for this study consisted of 290 inbred lines (**Table 1**) procured from CIMMYT, IIMR, Zonal Agricultural Research Station (ZARS), Mandya and University of Agricultural Sciences (UAS), Bengaluru along with a resistant check (DKC 9141) and a susceptible check (DKC 9081) procured from Monsanto India Ltd. The same set of 290 inbred lines were grown separately for recording yield and its attributes.

Inbred lines were grown along with a resistant check (DKC 9141) and a susceptible check (DKC 9081) for identifying LWD resistant/tolerant lines during *kharif-2016*. Whereas, SKV-50, MAI-105 and MAI-137 were used as a check for evaluating yield parameters. Separate

experiments were conducted for yield and disease screening. Each inbred line was planted in a single row of 3 m length, with a spacing of 0.6 m between the rows and 0.3 m between the plants within a row. The crop was raised by applying a recommended dose of nitrogen (two split doses) and phosphorous. Potassium was not applied in order to rule out the possibility of 'Potassium' conferred resistance of inbred lines. All other production practices were followed as per the recommended package of practice. However, all the recommended practices were followed for the experiment carried out to identify high yielding inbred lines.

Isolation and mass multiplication of H. maydis: Maize stalks showing symptoms typical of LWD were collected from the infected field and were split into small fibrous pieces and surface sterilized using 4 per cent sodium hypochloride solution. The stalks were then washed twice in sterile distilled water, air dried and plated on 39 per cent Potato Dextrose Agar (PDA) medium in petri plates. Petri plates were incubated for five days in Biological Oxygen Demand (BOD) incubator for the development of the pathogen. The pathogen colonies developed in petri plates were examined for morphology and fruiting body characteristics of H. maydis. Characteristics of typical mycelia of the late wilt pathogen are olivaceous brown with radiating hyphae at borders and the conidia are cylindrical, curved, borne in slimy heads (Gam, 2000). Once the characteristics were confirmed, the mycelia was then placed on PDA for pure culture and sub culturing. The mycelia were asceptically transferred to sterile 24 per cent Potato Dextrose Broth (PDB) in a conical flask for mass multiplication and incubated for 15 days for mycelial mat development. On the 15th day, the mycelial mat was ground and filtered to obtain a pathogen spore suspension.

Table 1. List of inbred lines, their pedigree and source of collection

SI No.	Inbred line	Pedigree	Source	SI No.	Inbred line	Pedigree	Source
1	33	MAI-415	IIMR	17	103b	MAI-429	IIMR
2	94b	MAI-416	IIMR	18	8b	MAI-430	IIMR
3	40424	MAI-417	IIMR	19	MAI204	1232-2	IIMR
4	40070	MAI-418	IIMR	20	40357	MAI-431	IIMR
5	MAI711	INDIMYT-345	Mandya	21	88	MAI-432	IIMR
6	40297	MAI-419	IIMR	22	MAI143	LM-13	Ludhiana
7	32561	MAI-420	IIMR	23	40061	MAI-433	IIMR
8	9	MAI-421	IIMR	24	10	MAI-434	IIMR
9	33189	MAI-422	IIMR	25	40423	MAI-435	IIMR
10	40022	MAI-423	IIMR	26	40089	MAI-436	IIMR
11	40003	MAI-424	IIMR	27	MAI308	2516-2	IIMR
12	76	MAI-425	IIMR	28	32589	MAI-437	IIMR
13	MAI728	INDIMYT-345	Mandya	29	32310	MAI-438	IIMR
14	40105	MAI-426	IIMR	30	MAI318	2354-1	IIMR
15	63	MAI-427	IIMR	31	5	MAI-439	IIMR
16	40496	MAI-428	IIMR	32	40085a	MAI-440	IIMR

33	40130	MAI-441	IIMR	86	13	MAI-556	IIMR
34	40085b	MAI-442	IIMR	87	MAI334	2570-4	IIMR
35	MAI7	INDIMYT-345	Mandya	88	29	MAI-557	IIMR
36	18715	MAI-443	IIMR	89	32076	MAI-558	IIMR
37	32702	MAI-444	IIMR	90	40	MAI-559	IIMR
38	32225	MAI-445	IIMR	91	MAI215	Z59-3	CIMMYT
39	40483	MAI-446	IIMR	92	40067	MAI-560	IIMR
40	79	MAI-447	IIMR	93	102	MAI-453	IIMR
41	40369	MAI-448	IIMR	94	40378	MAI-454	IIMR
42	MQ43	MAI-449	IIMR	95	1	MAI-455	IIMR
43	40490	MAI-450	IIMR	96	T20-45	MAI-456	IIMR
44	MAI712	INDIMYT-345	Mandya	97	MAI21	INDIMYT-345	Mandya
45	106b	MAI-451	IIMR	98	24	MAI-457	IIMR
46	40364	MAI-452	IIMR	99	MQPM2	MAI-458	IIMR
47	33018	MAI-531	IIMR	100	40058	MAI-459	IIMR
48	46	MAI-532	IIMR	101	62	MAI-460	IIMR
49	32850	MAI-533	IIMR	102	33154	MAI-461	IIMR
50	MAI380	2442-1	IIMR	103	MAI133	CML-172	IIMR
51	MAI319	2441-4	IIMR	104	40421	MAI-462	IIMR
52	40375	MAI-534	IIMR	105	15	MAI-463	IIMR
53	M20	MAI-535	IIMR	106	MAI168	SOOTLYQ-HG-B-B-B- 36-B-B	IIMR
54	72	MAI-536	IIMR	107	65	MAI-464	IIMR
55	MAI142(w)	CML-338	CIMMYT	108	103a	MAI-465	IIMR
56	MAI224	Z63-16	CIMMYT	109	106a	MAI-466	IIMR
57	18092	MAI-537	IIMR	110	MAI740	INDIMYT-345	Mandya
58	MAI725	INDIMYT-345	Mandya	111	40099	MAI-467	IIMR
59	MAI261	Z49-102	CIMMYT	112	20	MAI-468	IIMR
60	32871	MAI-538	IIMR	113	MAI760	INDIMYT-145	Mandya
61	82	MAI-539	IIMR	114	40073	MAI-469	IIMR
62	85	MAI-540	IIMR	115	31890	MAI-470	IIMR
63	51	MAI-541	IIMR	116	34	MAI-471	IIMR
64	MAI135	CML-41	CIMMYT	117	MAI214	249-87	IIMR
65	MAI298	1554	IIMR	118	40013	MAI-472	IIMR
66	31830	MAI-542	IIMR	119	94a	MAI-473	IIMR
67	31	MAI-543	IIMR	120	32084	MAI-474	IIMR
68	60	MAI-544	IIMR	121	40083	MAI-475	IIMR
69	10269	MAI-545	IIMR	122	MAI138	CML-326	IIMR
70	40058	MAI-546	IIMR	123	MAI170	(CML-165/ AMATLCOHS71-1- 1-1-2-1-1-1-B-B-BB) B-2-2-B-B	CIMMYT
71	MAI724	INDIMYT-345	Mandya	124	22	MAI-476	IIMR
72	97a	MAI-547	IIMR	125	40522	MAI-477	IIMR
73	31810	MAI-548	IIMR	126	26	MAI-478	IIMR
74	96	MAI-549	IIMR	127	2	MAI-479	IIMR
75	10235.27	MAI-550	IIMR	128	MAI726	INDIMYT-345	Mandya
76	32575	MAI-551	IIMR	129	40489	MAI-480	IIMR
77	104	MAI-552	IIMR	130	40376	MAI-481	IIMR
78	MAI13	INDIMYT-345	Mandya	131	67	MAI-482	IIMR
79	31734	MAI-553	IIMR	132	64	MAI-483	IIMR
80	MAI175	CM-132	IIMR	133	MAI250	Z50-3	CIMMYT
81	MAI262	Z49-49	CIMMYT	134	40155	MAI-484	IIMR
82	31956	MAI-554	IIMR	135	40480	MAI-485	IIMR
83	MAI755	INDIMYT-345	Mandya	136	40292	MAI-486	IIMR
84	MAI137	CML-359	IIMR	137	28	MAI-487	IIMR
85	12262	MAI-555	IIMR	138	MAI746	INDIMYT-345	Mandya

139	89	MAI-488	IIMR	195	66	MAI-602	IIMR
140	MAI754	INDIMYT-345	Mandya	196	MAI196	2268-1	IIMR
141	32785	MAI-489	IIMR	197	19104	MAI-493	IIMR
142	33174	MAI-490	IIMR	198	32541	MAI-494	IIMR
143	40310	MAI-491	IIMR	199	18	MAI-495	IIMR
144	47	MAI-492	IIMR	200	40065	MAI-496	IIMR
145	71	MAI-562	IIMR	201	MAI391	693-3	IIMR
146	40399	MAI-563	IIMR	202	40040	MAI-497	IIMR
147	3	MAI-564	IIMR	203	59	MAI-498	IIMR
148	40019	MAI-565	IIMR	204	32810	MAI-499	IIMR
149	40319	MAI-566	IIMR	205	40230	MAI-500	IIMR
150	MAI142	CML-338	CIMMYT	206	MAI389	2449-6-1	IIMR
151	MAI715	INDIMYT-345	Mandya	207	4	MAI-501	IIMR
152	30a	MAI-567	IIMR	208	MAI280	70-1	IIMR
153	42	MAI-568	IIMR	209	MQPM37	MAI-502	IIMR
154	40104	MAI-569	IIMR	210	MAI202	1204-1	IIMR
155	98	MAI-570	IIMR	211	MAI758	INDIMYT-345	Mandya
156	107	MAI-571	IIMR	212	93	MAI-503	IIMR
157	MAI322	2370-1	IIMR	213	32809	MAI-504	IIMR
158	99	MAI-572	IIMR	214	53	MAI-505	IIMR
159	40414	MAI-573	IIMR	215	MAI316	2270	IIMR
160	MAI338	2608-1	IIMR	216	75	MAI-506	IIMR
161	MAI764	INDIMYT-345	Mandya	217	MQ13	MAI-507	IIMR
162	12071	MAI-574	IIMR	218	31838	MAI-508	IIMR
163	MAI769	INDIMYT-345	Mandya	219	40402	MAI-509	IIMR
164	MAI211	Z49-57	CIMMYT	220	108	MAI-510	IIMR
165	16	MAI-575	IIMR	221	3b	MAI-511	IIMR
166	1092.79	MAI-576	IIMR	222	MAI230	Z52-3	CIMMYT
167	31837	MAI-577	IIMR	223	40396	MAI-512	IIMR
168	40224	MAI-578	IIMR	224	25	MAI-513	IIMR
169	MAI182	CML-238-B-B	IIMR	225	32931	MAI-514	IIMR
170	101	MAI-579	IIMR	226	MAI393	1506	IIMR
171	27	MAI-580	IIMR	227	32865	MAI-515	IIMR
172	6	MAI-581	IIMR	228	18005	MAI-516	IIMR
173	31792	MAI-582	IIMR	229	18758	MAI-517	IIMR
173	MAI267	Z57-28	CIMMYT	230	MAI729	INDIMYT-345	Mandya
175	19	MAI-583	IIMR	231	32645	MAI-518	IIMR
176	100			232	23		IIMR
177	48	MAI-584 MAI-585	IIMR IIMR	232	40458	MAI-519 MAI-520	IIMR
	40081		IIMR	234	MAI134		IIMR
178		MAI-586			MAI268	CML-304	
179 180	68	MAI-587	IIMR IIMR	235 236	M56	Z52-8	CIMMYT IIMR
181	10235	MAI-588	IIMR	237		MAI-521 Z56-5	CIMMYT
	10251	MAI-589			MAI275		
182	43	MAI-590	IIMR	238	57 MAI20	MAI-522	IIMR Mandya
183	40128	MAI-591	IIMR	239	MAI20	INDIMYT-345	Mandya
184	36	MAI-592	IIMR	240	MAI329	2422-4	IIMR
185	21	MAI-593	IIMR	241	31888	MAI-523	IIMR
186	70	MAI-594	IIMR	242	MAI751	INDIMYT-345	Mandya
187	MAI276	Z49-24	CIMMYT	244	40433	MAI-603	IIMR
188	33160	MAI-595	IIMR	245	77	MAI-604	IIMR
189	31708	MAI-596	IIMR	246	35	MAI-605	IIMR
190	97b	MAI-597	IIMR	247	40363	MAI-606	IIMR
191	17	MAI-598	IIMR	248	41	MAI-607	IIMR
192	84	MAI-599	IIMR	249	54	MAI-608	IIMR
193	18	MAI-600	IIMR	250	40250	MAI-609	IIMR
194	40377	MAI-601	IIMR	251	69	MAI-610	IIMR

252     49     MAI-611     IIMR     272     14b     MAI-631     IIMR       253     7     MAI-612     IIMR     273     30b     MAI-632     IIMR       254     73b     MAI-613     IIMR     274     32b     MAI-633     IIMR       255     73a     MAI-614     IIMR     275     40361     MAI-634     IIMR       256     12b     MAI-615     IIMR     276     56     MAI-635     IIMR       257     39     MAI-616     IIMR     277     74     MAI-636     IIMR       258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621 <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th>								
254     73b     MAI-613     IIMR     274     32b     MAI-633     IIMR       255     73a     MAI-614     IIMR     275     40361     MAI-634     IIMR       256     12b     MAI-615     IIMR     276     56     MAI-635     IIMR       257     39     MAI-616     IIMR     277     74     MAI-636     IIMR       258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623<	252	49	MAI-611	IIMR	272	14b	MAI-631	IIMR
255     73a     MAI-614     IIMR     275     40361     MAI-634     IIMR       256     12b     MAI-615     IIMR     276     56     MAI-635     IIMR       257     39     MAI-616     IIMR     277     74     MAI-636     IIMR       258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624 </td <td>253</td> <td>7</td> <td>MAI-612</td> <td>IIMR</td> <td>273</td> <td>30b</td> <td>MAI-632</td> <td>IIMR</td>	253	7	MAI-612	IIMR	273	30b	MAI-632	IIMR
256     12b     MAI-615     IIMR     276     56     MAI-635     IIMR       257     39     MAI-616     IIMR     277     74     MAI-636     IIMR       258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625	254	73b	MAI-613	IIMR	274	32b	MAI-633	IIMR
257     39     MAI-616     IIMR     277     74     MAI-636     IIMR       258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626 <td>255</td> <td>73a</td> <td>MAI-614</td> <td>IIMR</td> <td>275</td> <td>40361</td> <td>MAI-634</td> <td>IIMR</td>	255	73a	MAI-614	IIMR	275	40361	MAI-634	IIMR
258     40060     MAI-617     IIMR     278     58b     MAI-637     IIMR       259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       269     40080     MAI-	256	12b	MAI-615	IIMR	276	56	MAI-635	IIMR
259     83     MAI-618     IIMR     279     12a     MAI-638     IIMR       260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628<	257	39	MAI-616	IIMR	277	74	MAI-636	IIMR
260     32a     MAI-619     IIMR     280     38b     MAI-639     IIMR       261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-	258	40060	MAI-617	IIMR	278	58b	MAI-637	IIMR
261     32427     MAI-620     IIMR     281     40079     MAI-640     IIMR       262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	259	83	MAI-618	IIMR	279	12a	MAI-638	IIMR
262     80     MAI-621     IIMR     282     8a     MAI-524     IIMR       263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	260	32a	MAI-619	IIMR	280	38b	MAI-639	IIMR
263     58a     MAI-622     IIMR     283     50     MAI-525     IIMR       264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	261	32427	MAI-620	IIMR	281	40079	MAI-640	IIMR
264     40523     MAI-623     IIMR     284     55     MAI-526     IIMR       265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	262	80	MAI-621	IIMR	282	8a	MAI-524	IIMR
265     14a     MAI-624     IIMR     285     90     MAI-527     IIMR       266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	263	58a	MAI-622	IIMR	283	50	MAI-525	IIMR
266     11     MAI-625     IIMR     286     44     MAI-528     IIMR       267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	264	40523	MAI-623	IIMR	284	55	MAI-526	IIMR
267     40484     MAI-626     IIMR     287     MAI295     Z41-2     CIMMYT       268     37     MAI-627     IIMR     288     61     MAI-529     IIMR       269     40080     MAI-628     IIMR     289     MAI223     Z62-6     CIMMYT       270     38a     MAI-629     IIMR     290     32583     MAI-530     IIMR	265	14a	MAI-624	IIMR	285	90	MAI-527	IIMR
268   37   MAI-627   IIMR   288   61   MAI-529   IIMR     269   40080   MAI-628   IIMR   289   MAI223   Z62-6   CIMMYT     270   38a   MAI-629   IIMR   290   32583   MAI-530   IIMR	266	11	MAI-625	IIMR	286	44	MAI-528	IIMR
269 40080 MAI-628 IIMR 289 MAI223 Z62-6 CIMMYT   270 38a MAI-629 IIMR 290 32583 MAI-530 IIMR	267	40484	MAI-626	IIMR	287	MAI295	Z41-2	CIMMYT
270 38a MAI-629 IIMR 290 32583 MAI-530 IIMR	268	37	MAI-627	IIMR	288	61	MAI-529	IIMR
	269	40080	MAI-628	IIMR	289	MAI223	Z62-6	CIMMYT
271 78 MAI-630 IIMR	270	38a	MAI-629	IIMR	290	32583	MAI-530	IIMR
	271	78	MAI-630	IIMR				

Inoculum load and inoculation technique: Since the pathogen suspension is inoculated to stalks, the spore load plays a critical role in causing the disease. The spore suspension was observed under the microscope and the desired spore concentration of 4×106 spores ml-1 was adjusted using Haemocytometer. Whenever the concentration of spore was more, sterile distilled water was used for dilution to obtain the desired spore concentration. Spore concentration @ 4×106 spores ml-1 of H. maydis culture was injected in to the stalks at the second inter-nodal region from the base of the inbred lines using a medical syringe. Each inbred line was poked to hole and approximately 2 ml of spore suspension was dispensed to stalks of each inbred line at 55 days after sowing (1st inoculation) and 65 days after sowing (2<sup>nd</sup> inoculation). As a control, one row was injected with water blank and one row was left poked without injecting to have a comparative study.

Responses of inbred lines to LWD: 20-25 days after inoculation, LWD symptoms were observed on the inbred lines. For disease phenotyping, 30 days after inoculation, the stalks of the inbred lines were split opened and disease severity and intensity were recorded on an individual plant basis using 1-9 scale which takes into account both discoloration of tissues and disintegration of fibres (Rakesh *et al.*,2016 a). Further, inbred lines were categorized into different response groups (**Table 2**).

Observations on yield attributing characters *viz.*, days to 50% silking, days to 50% tasseling, anthesis-silking interval, plant height, cob length, cob diameter, kernel rows per cob, kernels per row, grain yield per plant, 100 seed weight and cob shelling per cent were recorded on five randomly selected plants of each inbred line based

on counting/measurement using appropriate scale depending on the traits.

Table 2. Classification of inbred lines into different response groups based on their scores of responses to late wilt disease

Response group of inbred lines
Highly Resistant
Resistant
Tolerant
Susceptible
Highly Susceptible

#### **RESULTS AND DISCUSSION**

The response scores of 290 inbred lines to LWD were subjected to ANOVA. Mean squares attributable to inbred lines, checks and inbred line vs. check were found significant. Out of 290 inbred lines, 7 lines were found resistant; 241 were tolerant; 30 were susceptible and 12 were found to be highly susceptible. However, none of the lines was found to be highly resistant. These inbred lines with the disease score of ≤4 are useful in the breeding programme, as they show lower infection (Mohamed et al., 1966; Rakesh et al., 2016 b). The lack of highly resistant sources among the inbred lines screened indicates the need for creating variability to identify inbred lines resistant to LWD for their commercial exploitation through heterosis breeding. Inbred lines with contrasting responses to LWD could be used to unravel the genetics of LWD resistance by classical phenotype-based and/ or marker assisted methods. Based on these results, 14 inbred lines with the ≤4 LWD response score were identified as a resistant source to LWD (Table 3).

Table 3. Late wilt disease resistant/tolerant maize inbred lines identified in preliminary screening

SI. No.	Identity of inbred lines	Late wilt disease Score	Response group	SI. No.	Identity of inbred lines	Late wilt disease Score	Response group
1	78	4.00	Tolerant	8	40376	4.00	Tolerant
2	40105	3.87	Tolerant	9	MAI-261	3.66	Tolerant
3	32589	2.75	Resistant	10	97b	3.00	Resistant
4	MAI-740	3.40	Tolerant	11	40423	4.00	Tolerant
5	8a	4.00	Tolerant	12	76	4.00	Tolerant
6	18092	4.00	Tolerant	13	32850	3.75	Tolerant
7	30a	3.80	Tolerant	14	40496	3.60	Tolerant

In the experiment conducted to evaluate yield and yield attributing traits, considerably good performance was recorded for 14 inbred lines identified as LWD resistant/ tolerant (**Table 4**). Anthesis-silking interval, one of the important traits ranged between -2.60 and 4. Inbred lines, 18092, 32850, 78, 32589 and 76 exhibited least ASI estimates of 0, -0.15, 0.6, 1.2 and 1.2, respectively. ASI is one of the major surrogate traits for drought resistance. Lower the magnitude ASI value irrespective of direction, genotype is said to be more resistant to drought as those types will surpass the flowering, one the critical stages quickly causing less damage to plant. Hence, inbred lines with resistance/tolerance to LWD and with lower ASI estimates are the valuable inbred lines that can be used in breeding programs.

Grain yield per plant, 100 seed weight and cob shelling per cent are the other important traits closely related to yield. Grain yield per plant ranged from 55.60 to 179 g. The highest estimate of 32.10 g and the lowest of 19.50 g was recorded for 100 seed weight (Prakash and Seetharam, 2012; Prakash et al., 2019). Whereas, cob shelling per cent showed a range of 75.54 to 87.0 per cent. Among the 14 inbred lines identified as resistant/tolerant to LWD, lines showing the highest grain yield per plant, 100 seed weight and cob shelling per cent can be used for developing hybrids resistant to LWD and high yielding. Inbred lines, 78 and 32589 with ASI value 0.6 and 1.2, the grain yield per plant 179 and 168.40 g, cob shelling per cent 82.30 and 86.98 per cent, respectively are the best lines identified from this study. These inbred lines can be further subjected to combined ability assessment and used in developing hybrids.

While Sabet *et al.* (1961) reported open-pollinated varieties as resistant to LWD over hybrid varieties, El-Morshidy *et al.* (1980) and Rao *et al.* (1990) reported resistance of hybrids evaluated. However, very few

Table 4. Estimates of grain yield and its component traits of LWD resistant/tolerant inbred lines

SI. No.	Genotype	DAS	DAT	ASI	PH	CL	CD	KRC	KR	GYP	100SW	CS%	LWD Score
1	78°	59.60	59.00	0.60	218.00	17.88	15.50	16.00	38.50	179.00	30.53	82.30	4.00
2	40105	68.00	64.00	4.00	205.00	16.60	13.90	14.00	31.20	95.00	25.50	78.13	3.87
3	32589°	57.60	56.40	1.20	216.00	18.32	15.04	14.80	41.80	168.40	29.10	86.98	2.75
4	MAI-740	55.20	53.60	1.60	174.00	16.00	14.83	16.00	31.67	108.67	24.10	86.93	3.40
5	8a	67.50	64.50	3.00	189.00	15.50	11.75	12.50	29.75	61.00	19.50	75.54	4.00
6	18092	57.00	57.00	0.00	195.00	15.84	13.40	14.40	30.00	98.20	20.61	79.07	4.00
7	30a	55.50	56.75	-1.25	150.60	14.40	12.00	15.20	27.20	75.20	22.40	87.04	3.80
8	40376	59.00	55.75	3.25	144.60	13.08	12.58	15.60	18.80	55.60	20.34	78.09	4.00
9	MAI-261	64.60	67.20	-2.60	179.20	15.90	12.00	10.80	25.40	75.00	29.21	79.20	3.66
10	97b	72.20	69.80	2.40	184.00	13.10	12.70	15.60	21.40	68.20	25.80	75.78	3.00
11	40423	60.75	58.75	2.00	161.20	15.04	14.02	15.60	31.40	122.80	26.30	85.52	4.00
12	76	60.00	58.80	1.20	215.50	14.63	11.50	12.50	30.25	62.75	20.60	79.43	4.00
13	32850	58.25	58.40	-0.15	176.20	19.67	13.83	11.33	41.00	142.67	32.10	82.31	3.75
14	40496	57.60	56.00	1.60	227.00	19.50	15.67	15.33	37.67	169.67	31.10	80.54	3.60

<sup>\*-</sup> High yielding and LWD resistant/tolerant; Inbred lines DAS- days to silking; DAT – days to tassel; ASI – anthesis-silking interval; PH – plant height; CL – cob length; CD – cob diameter; KRC – kernel rows cob<sup>-1</sup>; KR – kernels row<sup>-1</sup>; GYP – Grain yield plant<sup>-1</sup>; 100SW – seed weight; CS% - cob shelling %.

genotypes were reported to be resistant among the genotypes screened for LWD response (Sabet *et al.*, 1972; Singh *et al.*, 1986; Satyanarayana, 1995).

Inbred lines identified as resistant/tolerant with disease score ≤4 are the most promising source of resistance to LWD. Further, estimates of yield and yield attributing components of those inbred lines indicate that they are considerably good yielders. Two inbred lines namely, 78 and 32589 are both tolerant to LWD and best yielding lines serve as potential parents for developing hybrids.

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#### **REFERENCES**

- Bergstorm, G., Leslie, J., Lipps, H. D., Warden, H., Esker, P., Grau, C., Botratynski, T., Bulluck, R., Flyod, J., Bennett, R., Bonde, M., Dunkle, L., Smith, K., Zeller, K., Cardwell, K., Daberkow, S., Bell, D. and Chandgoyal, T., 2008, Recovery plan for late wilt of corn caused by *Harpophora maydissyn*. *Cephalosporium maydis*. United States Department of Agriculture, Agricultural Research Service, Office of Pest Management, National Plant Disease Recovery System, pp. 1-24.
- Chalkey, D., 2016. Invasive fungi, late wilt of maize-Harpophora maydis, systematic mycology and microbiology laboratory, Agricultural Research Service, United States Department of Agriculture.
- Cook, R. J., 1978. The incidence of stalk rot on maize hybrids and its effect of yield on yield of maize in Britain. Ann. Appl. Biol., 88(1): 23-30. [Cross Ref]
- Degani, O. and Cernica, G. 2014. Diagnosis and control of *Harpophor amaydis*, the cause maize.*Adv. Microbiol.*, **4**: 94–105. [Cross Ref]
- Drori, R., Sharon, A., Goldberg, D., Rabinovitz, O., Levy, M. and Degani, O., 2013, Molecular diagnosis for *Harpophora maydis*, the cause of maize late wilt in Israel. *Phytopath.Medi.*, **52**: 16-29.
- El-Morshidy, M. A., Hassaballa, E. S., Khalifa, M. A. and ABD El-Rahim, H. M., 1980. Yield and resistance to late-wilt disease of some newly developed maize varietal crosses. Research Bulletin, Faculty of Agriculture, Ain Shams University.
- El-Shafey, H. A., El-Shorbagy, F. A., Khalil, H. and El-Assiuty, E. M., 1988. Additional sources of resistance to the late-wilt disease of maize caused by Cephalosporium maydis. Agric. Biology, 6: 599-605.

- Freeman, J. and Ward, E., 2004, *Gaeumannomyces graminis*, the take-all fungus and its relatives. *Molecular Plant Path.*, **5**: 235-252. [Cross Ref]
- Gams, W. 2000. Phialophoraand some similar morphologically little-differentiated anamorphs of divergent ascomycetes. *Studies in Mycol.*, **45**: 18-720.
- Johal, L., Huber, D. M. and Martyn, R. 2004. Late wilt of corn (Maize) pathway analysis: Intentional introduction of *Cephalosporium maydis*, analysis for the introduction to the U.S. of Plant Pathogens of Economic Importance. United States Department of Agriculture, Animal and Plant Health Inspection Service. Technical Report No. 503025.
- Khokar, M. K., Hooda, K. S., Sharma, S. S. and Singh, V., 2014.Post flowering stalk rot complex of maize Present status and future prospects. *Maydica*, **59**: 226-242.
- Michail, S. H., Abou-Elseoud, M. S. and NourEldin, M. S. 1999. Seed health testing of corn for maydis. Acta *Phytopathologica et Entomologica Hungarica*, **34**: 35-42.
- Mohamed, H. A., Moneim, A. T. A. and Fathi, S. M. 1966. Reaction of corn inbred lines, varieties and hybrids to four fungi causing stalk rots. *Plant Dis. Rep.*, **50**: 401-402.
- Moline-Ro-Ruiz, M. L., Melero-Vara, J. M. and Mateos, A., 2010. Cephalosporium maydis, the cause of late wilt in maize, a pathogen new to Portugal and Spain. Pl. Dis., 94: 379. [Cross Ref]
- Oerke, 2006. Crop losses to pests. *The J. Agric. Sci.*, **144**(1): 31-43. [Cross Ref]
- Ortiz-Bustos, C. M., Testi, L., Garcia-Carneros, A. B. and Molinero-Ruiz, L., 2015. Geographic distribution and aggressiveness of *Harpophora maydis* in the Iberian peninsula and thermal detection of maize late wilt. *European J. Plant Patol.*, pp. 1-15. [Cross Ref]
- Prakash, M. and Seetharam, B. B. 2012. Variability studies of quantitative characters in Maize (*Zea mays* L.). *Electronic Journal of Plant Breeding*, **3**(4): 995-997.
- Prakash, R., Ravikesavan, R., Kumari Vinodhana, N. and Senthil, A. 2019. Genetic variability, character association and path analysis for yield and yield component traits in maize (Zea Mays L.). *Electronic Journal of Plant Breeding*, **10**(2): 518-524. [Cross Ref]
- Rakesh, B., Gangappa, E., Gandhi, S., Ramesh, S. and Nagaraju, N. 2016a.Identification of Maize (Zea mays L.) Inbred Lines Resistant for Late Wilt

- Disease caused by *Harpophora maydis*. *Mysore J. Agric*. *Sci.*, **50** (2): 366-368.
- Rakesh, B., Gangappa, E., Gandhi, S., VeereshGowda, R. P., Swamy, D. S., Ramesh, S., Hemareddy, H. B. and Nagaraju, N. 2016b. Modified Method of Screening Maize Inbred Lines to Late wilt Disease Caused by *Harpophora maydis*. *Mysore J. Agric. Sci.*, **50** (4): 684-690.
- Rao, G. K., Raj, R. B., Begum, H. and Mohammad, S., 1990. Genotypic variability on the incidence of late wilt and grain yield losses in maize. *J. Mycopathol.* Res., 28 (1): 33-38.
- Sabet, K. A., Sarma, A. S., Hingoran, M. K. and Mansour, I. M.. 1961. Stalk and root rots of maize in the United Arab Republic. FAO Plant Protectin Bulletin, 9: 121-125
- Sabet, K. A., Sharma, A. S. and Fangary, I. M., 1972. Mechanisms of resistance and susceptibility to infection with the late wilt disease of maize. *Phytopathol.Mediterr.*, 11: 10-18.

- Samra, A. S., Sabet K. A. and Hingorani, M. K., 1963. Late wilt disease of maize caused by *Cephalosporium* maydis. *Phytopathology*, **53**: 402-406.
- Satyanarayana, E<sub>.</sub>, 1995. Genetic studies of late wilt and tursicum leaf blight resistance in maize. *Madras Agric. J.*, **82**: 603-609.
- Shekhar, M. and Kumar, S., 2010. Potential biocontrol agents for the management of *Macrophomina phaseolina*, incitant of charcoal rot in maize. *Archives Phytopath*. *Pl. Prot.*, 43: 379-383. [Cross Ref]
- Singh, S. D. and Siradhana, B. S., 1986.Germplasm evaluation against *Cephalosporium maydis* incidence of late wilt of maize. *Indian J.Mycology Pl. Path.*, **16**: 295.
- Ward, E. and Bateman, G. I., 1999, Comparison of Gaeumannomycesad Phialophore-like fungal pathogens from maize and other plants using DNA methods. New Phytologist, 141: 323-331. [Cross Ref]