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IDENTIFICATION OF SUNFLOWER POWDERY MILDEW RESISTANT SOURCES UNDER ARTIFICIAL SCREENING

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SUMMARY

The powdery mildew disease caused by *Golovinomyces cichoracearum* is becoming serious constraint in cultivation of sunflower crop. Since decade, disease observed regularly during *rabi-summer* seasons and under severe conditions, disease is found infecting the cotyledonary leaves up to ray florets. The host plant resistance in released hybrids and parental lines is limited. Hence, screening of germplasm lines and promising CMS lines is pre-requisite to identify reliable source of resistance. Sunflower germplasm comprising of 120 accessions were screened under artificial inoculation conditions by spraying spore suspension with 1% sucrose solution. Among 120 accessions screened, only 2 restorer lines were found to be resistant and 48 accessions were categorised as medium resistant. The remaining 63 and 7 accessions screened were found to be susceptible and highly susceptible to powdery mildew, respectively. The microscopic observations of conidia stained with lactophenol blue were also in accordance with host reaction. Highly susceptible check 'Morden' showed 468 conidia/microscopic field where as resistant accessions like R-GM-41 and R-GM-49 recorded 52.2 and 56.8 conidia/microscopic field, respectively. The fungus conidial count ranged from 61.1 to 129 in medium resistant accessions whereas it was more than 367 conidia/microscopic field in all highly susceptible accessions. The identified source of resistance may serve as useful genetic material for breeding powdery mildew resistant sunflower hybrids.

Key words: Sunflower, powdery mildew, germplasm, conidia

Key findings : Identified resistant lines can act as good source of resistance for future sunflower breeding programmes.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in India, is cultivated in an area of 0.691 million ha with an average production and productivity of 0.546 million tonnes and 791 kg ha^{-1} respectively during the year 2013-14 (Annon., 2015). Presently Karnataka is the leading state in India contributing 64 and 54% of total area and production respectively. It is the second important oilseed crop after groundnut in the state having an area of 0.443 million hectares with production of 0.297 million tonnes. However, productivity (670 kg ha^{-1}) is lesser than the national average of 791 kg ha^{-1} (Anon., 2015).

The most serious diseases of sunflower are caused by fungi. The major diseases include *Alternaria* leaf spot, downy mildew, sunflower necrosis (caused by virus) and rust. Recently, the powdery mildew caused by *Golovinomyces*

cichoracearum (DC.) (formerly known as *Erysiphe cichoracearum*) is becoming major problem in sunflower growing regions in India specially during *rabi* season.

In India, the disease was first reported in Bombay (Patel *et al.*, 1949) later in Rajasthan (Prasada *et al.*, 1968), West Bengal (Goswami and Dasgupta, 1981). The disease originates as minute discoloured speck from which powdery mass radiates in all the sides of the leaves. Large area on the aerial parts of the host is covered with white powdery mass containing mycelia and conidia of the fungus (Singh, 1984). Since decade, disease observed regularly during *rabi-summer* seasons and under severe conditions disease is found infecting the cotyledonary leaves up to ray florets. Application of fungicides to manage the disease involves high cost, besides the environmental concern and the insensitivity built-up in the pathogen limit their usage (Gullino and Kuijpers, 1994). Hence, there is a need for identifying reliable sources of resistance to powdery mildew. The present investigation was taken up with an objective to identify source of resistance in sunflower germplasm by following artificial screening methodology.

MATERIALS AND METHODS

Experimental material

The plant material used in the study includes 120 sunflower germplasm maintained at AICRP on Sunflower centre, MARS, Raichur. This also includes some lines which are supplied by Indian Institute of Oilseeds Research, Hyderabad

Microscopic observation of pathogen

The microscopic observation of the fungus was carried out by staining with lacto phenol blue. The infected top leaves were scraped gently to dislodge the conidia, then these conidia were stained with lacto phenol blue and observed under Motic compound microscope at 10X.

Evaluation of germplasm lines

The 120 sunflower germplasm lines were sown in pots during *rabi* 2013-14 in greenhouse. Each germplasm was sown in 2 pots with 4 seedlings in each pot. The germplasm lines were screened for reaction to powdery mildew under controlled conditions following artificial inoculation. The powdery mildew infected leaves are collected from field and using camel hair brush powdery mass is discharged into 1% sucrose solution. This conidial suspension in 1% sucrose was sprayed on all the entries at 30 and 45 days after sowing. The powdery mildew disease incidence was recorded from each plant at 45, 60, 75 and 90 days after sowing (Figures 1 and 2).

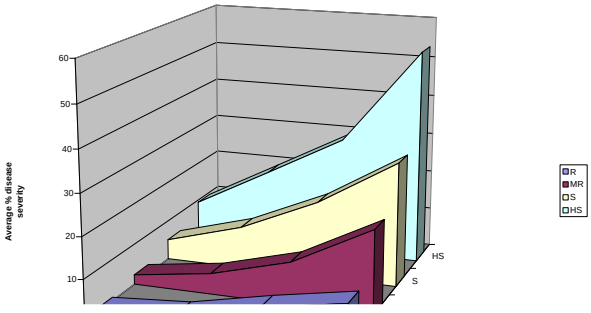

Figure 1. Average disease severity (%) in resistant, medium resistant, susceptible and highly susceptible genotypes.

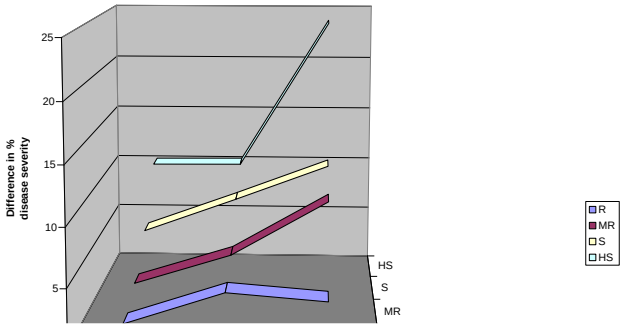

Figure 2. Difference in powdery mildew infection in resistant, medium resistant, susceptible and highly susceptible genotypes

Statistical analysis

The powdery mildew disease was scored in each entry according to 0-9 scale and observations were converted to percent disease index (PDI) using following formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of individual disease rating}}{\text{Number of plants rated X highest rating}}$$

The rate of development of disease (r) at different intervals was also calculated by following formula given by Van der plank (1963).

$$r = \frac{2.3}{t_2 - t_1}\left[\log\frac{X_2}{1-X_2} - \log\frac{X_1}{1-X_1}\right]$$

Where,

r = apparent rate of infection or spread
X_1 = percent disease index at time t_1
X_2 = percent disease index at time t_2
$t_2 - t_1$ = time interval in days between the 2 consecutive observations

RESULTS AND DISCUSSION

In this study, 120 sunflower germplasm lines along with 1 susceptible check 'Morden' were evaluated in green house condition by creating artificial epiphytotic condition. Conidial suspension prepared in 1% sucrose solution was sprayed on all the entries at 30 and 45 days after sowing. Later the powdery mildew incidence was scored at top, middle and bottom leaves at 15 days interval till plant maturity.

Out of 120 germplasm lines, none of them were immune. However, 2 lines were found to be resistant with 10% per cent disease severity. While 48 germplasm lines were found to be moderately resistant with less than 25% percent disease severity and remaining 70 lines showed susceptible/highly susceptible reaction to powdery mildew. While, the open pollinated variety 'Morden' registered highly susceptible disease reaction (59.1%) to powdery mildew.

The apparent rate of infection has been widely used in identification of genotypes with low rate of disease development. The low average 'r' values indicate less rate of infection compared to higher values. The apparent rate of infection 'r' values among 120 germplasm lines ranged from 0.001 to 0.137 across resistant to highly susceptible categories, indicating the importance of infection rate in spreading powdery mildew diseases.

The highly susceptible genotype like Morden having low apparent rate of infection (*r* = 0.003) actually recorded high disease infection at their early growth stage however further spread of disease is slow as indicated by low rate of infection. Whereas, resistant genotype R-GM-49 recorded both low level of infection (PDS = 10%) and apparent rate of infection (*r* = 0.004). The resistant and medium resistant genotypes like R-GM-41, GP6-917 and GP6-969 having high apparent rate of infection registered very low level of disease infection at their early crop growth stage, however once the disease infection occurs in these genotypes spread of the disease is fast. Whereas, in susceptible genotypes like GP6-952, GP6-961 and GP6-1001 higher rate of infection is coupled with high early stage disease infection makes them susceptible to powdery mildew. These results indicate the low apparent rate of infection only does not indicate the resistant level of the genotype. The calculated 'r' values varied and at times they did not remain consistent for given genotype and also did not show a particular trend in general. The apparent rate of infection only is not useful criteria for selecting the genotype. However, it can be used in studying the disease development in different genetic background Wilcoxson *et al.* (1975) and Nargund (1989).

The microscopic observation of the fungus was carried out on all 120 sunflower germplasm lines. For microscopic examination of pathogen, the infected top leaves were scraped gently to dislodge the conidia, then these conidia were stained with lacto phenol blue and observed under motic image capturing microscope at 10X. The numbers of conidia spores were counted in 5 different microscopic fields and the average number of conidia per microscopic field were analysed using DMRT (0.05). The resistant germplasm *viz.,* R-GM-41

(52.2) and R-GM-49 (56.8) recorded least number of conidia per microscopic field followed by medium resistant germplasm group with a range of 61.1 (RCR-1934/4-5-1-1) to 129 (R-GM-39).

The DMRT analysis categorised the average conidial count into 4 categories, indicating significant differences between sunflower germplasm for powdery mildew reaction (Table 1). The moderate resistant germplasm RCR-1934/4-5-1-1 recorded significantly low number of conidial spores (61.1) when compared to other medium resistant genotypes. However, the conidial spores for both resistant and medium resistant genotypes were significantly lower than susceptible check 'Morden' (468). We could also observe significant differences for number of conidial spores in susceptible genotypes as compared to highly susceptible check Morden. These microscopic observations are in line with Reddy *et al.* (2013) as they also reported less conidial spores and hyphal growth in resistant and moderately resistant sunflower genotypes compared to susceptible check.

CONCLUSION

To date, several studies have identified powdery mildew resistance in wild species of sunflower. However, transferring resistant genes from wild species to cultivated species requires special techniques like ovule/embryo culture and moreover resistant genes come with linkage drag. The identification of 2 sunflower germplasm lines (R-GM-41 & R-GM-49) as resistant to powdery mildew can serve for the immediate sunflower breeding programmes as availability of immune reaction for powdery mildew disease in cultivated species is absent (Table 2). Further resistant to powdery mildew is reported to exhibit differential reaction in different environmental conditions (Saliman, 1982) and hence screening under artificial epiphytotic condition is critical in identifying reliable source of resistance.

Table 1. Reaction of sunflower germplasm to *G. cichoracearum* under artificial conditions at 15 days interval.

No.	Accession	Per cent Disease Index (PDI) at				Host Reaction	Mean 'r' values	No. of Conidia per microscopic field
		45DAS	60DAS	75DAS	90DAS			
1	GP6-11	4.84	8.1	12.4	19.2	MR	0.004	80.2[b]
2	GP6-18	2.51	8.6	14.3	23.7	MR	0.010	80.4[b]
3	RCR-1934/4-5-1-1	4.73	8.6	13.2	23.4	MR	0.004	61.1[a]
4	RCR-1885/1-1	0.8	1.9	15.6	28.2	S	0.030	180.2[c]
5	RCR-1892/1-2	1.86	3.2	15.2	24.5	MR	0.016	112.3[b]
6	RCR-1901/1-1-1	1.07	2.8	10.9	25.6	S	0.060	175.1[c]
7	RCR-1892/1-3	1.92	3.6	9.5	18.1	MR	0.015	78.2[b]
8	RCR-1901/2-1-1	2.11	6.5	12.6	26.4	S	0.013	190.3[c]
9	RCR-1901/2-1-2	1.86	3.6	16.5	27.4	S	0.016	203.1[c]
10	RCR-1904/1-1-2	3.8	6.5	15.4	26.8	S	0.006	176.2[c]
11	RCR-1913/1-1-1	1.23	3.5	14.3	27.2	S	0.036	186.1[c]
12	RCR-1913/1-1-2	6.92	1.1	20.5	29.9	S	0.003	211.0[c]
13	RCR-1913/1-1-3	3.62	8.8	16.2	28.4	S	0.006	193.3[c]
14	RCR-1922/1-1-1	8.32	13.3	19.5	29.8	S	0.002	207.6[c]
15	RCR-1900/1-1-2	1.10	3.3	5.7	18.6	MR	0.052	80.1[b]
16	RCR-1892/1-3	1.67	2.6	7.9	22.1	MR	0.019	86.2[b]
17	RCR-1926/1-1	7.86	12.3	16.7	26.7	S	0.002	186.2[c]
18	RCR-1900/1-1-3	5.66	8.3	11.2	22.3	MR	0.003	88.0[b]
19	RCR-1951/2-1-1	1.66	1.9	10.3	25.1	S	0.020	173.0[c]
20	RCR-1971/2-1-1	2.33	6.9	12.5	29.6	S	0.012	222.0[c]
21	RCR-1945-2-3	1.67	2.2	9.8	22.6	MR	0.019	88.2[b]
22	RCR-1977-3-5-1	3.85	5.5	14.2	26.8	S	0.006	208.2[c]
23	RCR-1947/1-1-1	2.11	4.4	8.7	16.8	MR	0.013	79.4[b]
24	RCR-1947/2-1-1	7.21	9.5	13.3	21.5	MR	0.002	83.2[b]
25	RCR-1947/2-2-2	3.34	9.8	15.5	23.8	MR	0.007	88.6[b]
26	RCR-1947/2-2-1	1.95	3.9	14.6	24.5	MR	0.004	91.5[b]
27	RCR-1932-2-1-1	1.62	2.3	8.5	12.6	MR	0.004	76.2[b]
28	Morden ©	10.34	12.6	24.3	59.1	HS	0.003	468.0[d]
29	GP6-250	4.76	9.0	14.6	26.5	S	0.015	190.2[c]
30	GP6-263	5.41	13.2	22.6	32.3	S	0.006	231.0[c]
31	GP6-271	6.11	12.5	24.6	31.3	S	0.013	233.3[c]
32	GP6-282	3.65	6.8	12.9	26.8	S	0.006	185.0[c]
33	GP6-286	2.10	5.6	12.4	25.7	S	0.004	192.0[c]
34	GP6-297	3.80	6.5	15.5	26.9	S	0.013	187.6[c]
35	GP6-303	5.61	5.6	17.1	27.4	S	0.019	191.2[c]
36	GP6-305	2.11	6.5	13.2	26.4	S	0.050	182.2[c]
37	GP6-310	1.66	2.0	10.2	20.3	MR	0.002	86.3[b]
38	GP6-312	1.11	3.3	14.3	26.4	S	0.021	178.8[c]
39	GP6-313	8.62	20.5	23.6	52.5	HS	0.005	367.6[d]
40	GP6-317	1.58	2.2	9.9	23.7	MR	0.002	88.9[b]
41	GP6-324	4.62	10.2	20.1	48.2	S	0.001	248.1[c]
42	GP6-326	14.23	21.1	29.6	36.9	S	0.019	206.5[c]
43	GP6-331	6.94	16.3	28.4	46.2	S	0.003	266.0[c]
44	GP6-332	10.20	22.2	39.1	52.4	HS	0.002	389.4[d]
45	GP6-341	2.30	10.2	15.4	16.2	MR	0.011	78.2[b]
46	GP6-347	5.69	15.9	22.6	29.4	S	0.004	196.3[c]
47	GP6-357	8.20	28.1	30.2	36.4	S	0.002	208.0[c]
48	GP6-358	8.88	16.2	22.5	34.3	S	0.002	200.4[c]
49	GP6-366	7.60	15.7	29.4	39.4	S	0.003	233.5[c]
50	GP6-370	10.10	16.9	29.1	42.6	S	0.002	242.0[c]

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No.	Accession	Per cent Disease Index (PDI) at				Host Reaction	Mean 'r' values	No. of Conidia per microscopic field
51	GP6-371	7.23	9.6	13.3	16.4	MR	0.002	80.2 [b]
52	GP6-374	12.30	19.2	29.4	36.0	S	0.001	236.3 [c]
53	GP6-384	16.10	26.8	33.1	38.4	S	0.001	238.7 [c]
54	GP6-387	2.36	6.9	11.2	16.1	MR	0.011	81.0 [b]
55	GP6-400	11.50	29.4	41.0	53.3	HS	0.002	388.8 [d]
56	GP6-420	2.56	9.5	16.2	25.3	S	0.010	180.0 [c]
57	GP6-424	10.40	13.5	20.2	29.2	S	0.001	204.0 [c]
58	GP6-442	5.30	9.2	10.6	16.2	MR	0.003	80.6 [b]
59	GP6-445	8.20	16.5	20.5	28.3	S	0.002	204.2 [c]
60	GP6-451	4.30	12.4	29.0	33.1	S	0.005	216.3 [c]
61	GP6-459	0.59	5.5	11.1	24.5	MR	0.007	94.3 [b]
62	GP6-511	1.14	1.0	8.1	18.4	MR	0.045	89.2 [b]
63	GP6-517	1.41	2.3	12.1	24.5	MR	0.026	123.4 [b]
64	GP6-534	7.1	19.4	21.5	50.5	HS	0.003	391.0 [d]
65	GP6-561	1.06	1.1	7.8	21.7	MR	0.063	95.6 [b]
66	GP6-570	3.1	9.2	18.0	46.2	S	0.008	253.0 [c]
67	GP6-578	8.82	11.6	22.2	57.1	HS	0.002	401.0 [d]
68	GP6-579	12.71	20.1	27.5	34.9	S	0.001	195.6 [c]
69	R-GM-41	1.1	1.3	6.3	10.0	R	0.051	52.2[a]
70	R-GM-49	0.59	3.3	6.6	10.0	R	0.004	56.8[a]
71	R-GM-27	1.78	9.2	13.3	14.2	MR	0.002	86.3 [b]
72	R-GM-393	5.71	8.5	11.2	14.4	MR	0.017	87.1 [b]
73	R-GM-39	0.59	5.5	10.5	24.4	MR	0.005	129.0 [b]
74	GP6-589	5.42	15.3	26.3	44.2	S	0.003	222.0 [c]
75	GP6-614	8.68	21.2	37.0	50.4	HS	0.003	402.6 [d]
76	R-GM-69	4.17	14.9	20.5	27.4	S	0.003	180.5 [c]
77	GP6-657	6.68	27.1	28.1	34.4	S	0.002	189.6 [c]
78	GP6-699	7.36	15.2	20.4	32.3	S	0.003	201.2 [c]
79	GP6-714	6.08	14.7	27.3	37.4	S	0.001	218.3 [c]
80	GP6-734	8.58	15.9	27.0	40.6	S	0.018	246.6 [c]
81	GP6-792	10.78	18.2	27.3	34.0	S	0.041	225.5 [c]
82	GP6-794	1.72	8.9	13.5	26.2	S	0.025	180.2 [c]
83	GP6-819	0.34	2.2	13.1	22.5	MR	0.030	123.3 [b]
84	GP6-847	1.45	7.2	8.8	23.7	MR	0.007	125.2 [b]
85	GP6-854	0.4	2.5	7.3	16.1	MR	0.035	86.7 [b]
86	GP6-863	0.34	2.6	14.4	25.4	S	0.012	174.3 [c]
87	GP6-872	2.28	5.4	13.3	24.9	S	0.032	189.5 [c]
88	GP6-875	1.29	2.4	12.2	25.2	S	0.004	193.3 [c]
89	GP6-883	5.4	0.1	18.4	27.9	S	0.013	181.2 [c]
90	GP6-887	2.1	7.8	14.1	26.4	S	0.003	178.5 [c]
91	GP6-891	6.8	12.3	17.4	27.8	S	0.035	199.5 [c]
92	GP6-899	1.24	2.3	3.6	16.7	MR	0.086	91.0 [b]
93	GP6-906	0.15	1.5	5.8	20.1	MR	0.003	105.0 [b]
94	GP6-912	6.34	11.3	14.5	24.7	MR	0.005	119.0 [b]
95	GP6-917	4.14	7.3	9.1	20.4	MR	0.109	106.9 [b]
96	GP6-951	0.14	0.9	8.1	23.2	MR	0.031	120.5 [b]
97	GP6-952	0.81	5.8	10.4	27.7	S	0.137	200.1 [c]
98	GP6-953	0.15	1.1	7.6	20.7	MR	0.012	106.3 [b]
99	GP6-961	2.33	4.4	12.1	24.9	S	0.086	181.0 [c]
100	GP6-965	0.15	1.5	5.8	20.1	MR	0.003	93.4 [b]
101	GP6-967	6.34	11.3	14.5	24.7	MR	0.005	112.2 [b]
102	GP6-969	4.14	7.3	9.1	20.4	MR	0.068	106.1 [b]

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No.	Accession	Per cent Disease Index (PDI) at				Host Reaction	Mean 'r' values	No. of Conidia per microscopic field
103	GP6-990	0.14	0.9	8.1	23.2	MR	0.031	118.1 [b]
104	GP6-1001	0.81	5.8	10.4	27.7	S	0.137	195.6 [c]
105	GP6-1020	0.15	1.1	7.6	20.7	MR	0.012	88.6 [b]
106	GP6-1023	2.33	4.4	12.1	24.9	S	0.007	195.5 [c]
107	GP6-1037	5.69	8.4	11.2	19.5	MR	0.003	92.2 [b]
108	GP6-1047	1.82	8.8	13.4	21.8	MR	0.017	106.2 [b]
109	GP6-1060	3.24	7.9	12.4	24.6	MR	0.007	118.0 [b]
110	GP6-1063	3.89	12.2	20.5	30.4	S	0.006	203.3 [c]
111	GP6-1072	4.59	11.5	22.4	29.4	S	0.005	202.5 [c]
112	GP6-1075	0.43	2.8	12.4	22.6	MR	0.025	110.5 [b]
113	GP6-1089	2.13	5.7	10.7	24.9	S	0.013	174.5 [c]
114	GP6-1101	0.58	4.6	10.3	23.7	MR	0.006	108.8 [b]
115	GP6-1102	2.28	5.5	13.3	24.9	S	0.012	188.1 [c]
116	GP6-1114	2.51	3.6	6.5	21.4	MR	0.010	109.5 [b]
117	GP6-1117	1.93	2.5	11.1	19.6	MR	0.015	95.6 [b]
118	GP6-1127	1.96	4.6	16.8	29.5	S	0.015	210.2 [c]
119	GP6-1135	10.2	19.1	26.4	33.4	S	0.002	229.2 [c]
120	GP6-1150	2.34	12.4	16.2	28.1	S	0.012	195.6 [c]
	MEAN	4.2	8.8	16.1	27.7	--	--	--
	SEM	0.08	0.34	0.46	0.68	--	--	--
	CD @5%	0.22	0.98	1.08	1.92	--	--	--

Table 2. Sunflower germplasm categories based on powdery mildew incidence.

Disease Reaction	Disease Index Scale	No. of accessions	Range of 'r' values
Immune	0	Nil	--
Highly Resistant	1	Nil	--
Resistant	2	R-GM-41 & R-GM-49	0.004 - 0.051
Moderately Resistant	3-4	48	0.002 - 0.109
Susceptible / Highly Susceptible	5-9	70	0.001 - 0.137

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