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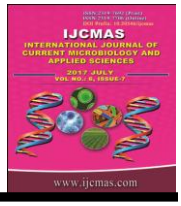

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Evaluation of Experimental Hybrids for Powdery Mildew Tolerance in Sunflower (*Helianthus annuus* L.)

P.G. Suresha[1*], Vikas V. Kulkarni[2], S.M. Supriya[1], and M.R. Govindappa[2]

[1]Department of Genetics and Plant Breeding, College of Agriculture, Raichur, Karnataka, India
[2]AICRP on sunflower, MARS, University of Agricultural Sciences, Raichur -584 104, Karnataka, India
**Corresponding author*

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A B S T R A C T

Powdery mildew caused by *Golovinomyces cichoracearum* (DC.) *V.P. Heluta* (formerly *Erysiphe cichoracearum*) is a common foliar disease on senescing leaves of cultivated sunflower in warmer regions of the world. In India, the powdery mildew disease is becoming major constraint in cultivation of sunflower crop especially during post rainy season. Screening of the sunflower germplasm lines against the disease would be of a great help to identify the resistance source. However, the development of resistant cultivars becomes very important if the crop is to expand into warmer regions where the disease may cause economic losses. Fifty six sunflower hybrids along with one susceptible check Morden were screened under artificial greenhouse conditions during the *rabi* season of 2013-14 by spraying spore suspension culture at 30 and 45 days after sowing. None of the hybrids screened were immune. However, 31 hybrids were found moderately resistant with less than 25% PDI while 25 hybrids registered susceptible reaction. The rate of apparent infection (r) in hybrids revealed a wide variation among the different hybrids at different intervals. The DMRT (Duncan's multiple Range Test) ranks were assigned to each genotypes based on 'r' value. The DMRT ranking categorized 57 sunflower genotypes into seven groups.

Introduction

Sunflower is an important source of nutritious edible oil and considered as good quality oil from health point of view due to presence of polyunsaturated fatty acids with linoleic acid (55-60%) and oleic acid (25-30%) which are known to reduce the risk of cardiac related problems. In India, Sunflower is being grown over an area of 0.55 million hectares with an average production and productivity of 0.42 million tonnes and 752 kg ha^{-1} respectively during the year 2014-15. Presently Karnataka is the leading state in the country contributing 64.35 and 51.08 per cent to total area and production respectively. It is the second important oilseed crop after groundnut in the state having an area of 0.36 million hectares with production of 0.21 million tonnes. However, productivity (597 kg ha^{-1}) is lesser than the national average of 752 kg ha^{-1} (Anon., 2016).

Powdery mildew caused by *Golovinomyces cichoracearum* (DC.) V.P. Heluta (formerly *Erysiphe cichoracearum*) is a common foliar

disease on senescing leaves of cultivated sunflower in warmer regions of the world. In India, the powdery mildew disease in sunflower has become a serious and major constraint in cultivation which was first reported in Bombay (Patel *et al.,* 1949), later in Rajastan (Prasada *et al.,* 1968) and West Bengal (Goswami and Dasgupta, 1981) causing a considerable reduction in yield. The disease originates as minute discoloured speck from which powdery mass radiates in all 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). Conidia (spores) are primarily dispersed by wind and will germinate on leaves within two to four hours under optimum conditions of high humidity (50-60%) and temperatures (20-25°C). A new crop of conidia can be produced within 5 to 7 days leading to rapid spread on the host canopy when conditions are favorable.

Since decade, disease was observed regularly during *rabi-summer* seasons and under severe conditions, the disease is found to be infecting the cotyledonary leaves up to ray florets. As powdery mildew of sunflower being an obligate pathogen needs a live host to survive. 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). Development of resistant cultivars is the ultimate option to overcome these constraints. The basic step in any successful disease resistance breeding programme is to identify genetic sources through screening a diverse set of germplasm lines for powdery mildew disease. Hence, there is a need for identification of reliable sources for resistance to powdery mildew. So that the resistant genotypes will serve as the potential donors of resistance. Wild *Helianthus* species represent a valuable reservoir of genes for several biotic stresses which have been successfully introgressed into cultivated sunflower (Seiler, 2008). The development of resistant cultivars will be of great importance to expand sunflower cultivation into warmer regions where the disease may cause economic losses. In this context an attempt was made to screening sunflower germplasm lines against the disease to identify the genetic resistance sources.

Materials and Methods

Experimental material

A set of 57 genotypes involving 49 hybrids and 8 checks were sown in pots at MARS, Raichur during the *rabi* 2013-14 under greenhouse condition. Each hybrid was sown in 2 pots with four seedlings in each pot. The hybrids were screened for reaction to powdery mildew under controlled conditions following artificial inoculation by spraying conidial suspension at 30 and 45 DAS. No crop protection was taken against powdery mildew disease. The disease incidence was recorded from five plants and each plant is divided into bottom, middle, top and observations were recorded as per the disease scale at 45, 60, 75 and 90 days after sowing.

Preparation of inoculum

The powdery mildew infected leaves were collected from field using camel hair brush. Powdery mass was discharged into 1 per cent sucrose solution and conidial suspension prepared was sprayed on all the entries at 30 and 45 days after sowing.

The genotypes were grouped into different categories using 0-9 disease index scale suggested by Mayee and Datar (1986) for sunflower powdery mildew.

Microscopic observation of pathogen

For microscopic examination of pathogen, the infected top leaves of medium resistant

genotypes 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 number of conidia spores was counted in ten different microscopic fields for four selected medium resistance genotypes, one susceptible genotype and highly susceptible check, Morden. The average number of conidia per microscopic field were analysed using DMRT (p=0.05).

Statistical analysis

The powdery mildew disease scored in each hybrid according to 0-9 scale (Table 1) and was converted to per cent disease incidence (PDI) using following formula given by Wheeler (1969). The rate of development of disease (r) at different intervals was also calculated by following formula given by Van der plank (1963).

Results and Discussion

The full potential of sunflower is far from being exploited due to several abiotic and biotic stresses. The crop suffers from many fungal diseases, among them foliar disease takes a heavy toll by reducing the yield to considerable extent. Among the foliar diseases, powdery mildew caused by *Golovinomyces cichoracearum* DC is a potential destructive disease in recent years causing severe yield loss. Therefore, resistant breeding appears to be the most important approach in disease management. Availability of resistance source and proper screening procedure is pre-requisite for development of high yielding and powdery mildew resistant hybrids of sunflower.

In the present study fifty six sunflower hybrids along with one susceptible check Morden were evaluated in green house condition under artificial epiphytotic condition. Conidial suspension prepared in one per cent 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 49 experimental hybrids screened under green house condition (Table 2), none were immune or resistant, however 26 hybrids were found to be moderate resistant while 23 hybrids were susceptible. All seven hybrid checks recorded moderate resistance to powdery mildew except KBSH-44 and RSFH-1887. While, the open pollinated variety Morden registered highly susceptible disease reaction (55.35%). These findings broadly agree with many of earlier reports by pathologists and breeders that no reliable source of resistance was identified (Karunanithi and Dinakaran, 1996). However few reports registered for existence of resistance sources (Hiremat, 1976, Shadakshari *et al.,* 1989, Suresh *et al.,* 1991).

These contradictory findings could be due to differences in scoring methodology, screening procedures and species or race spectrum prevalence. Only small number of accessions from *H. tuberosus, H. nutalii, H. maximilliani, H. grosseseratus,* (Dedic *et al.,* 2012) has been reported to be resistant. However, other researchers found *Helianthus nutalii* and *H. grosseseratus* to be highly susceptible to powdery mildew in both field and in green house conditions while *H. rigidus* registered moderate resistance to powdery mildew only after inoculation in green house condition (Saliman *et al.,* 1982).

The apparent rate of infection was calculated by using the formula given by Van der plank (1963). This formula is widely used in identification of genotypes with low rate of disease development. The range of ‘r’ values among 57 sunflower hybrids ranged from 0.025 to 0.1 indicating the importance of

infection rate in spreading powdery mildew diseases. The low average 'r' values indicate less rate of infection compared to higher values.

The average 'r' values were statistically analysed and compared by using DMRT (0.05). The DMRT ranking categorized 57 sunflower genotypes into seven groups indicating differential rate of infection among different genotypes. Based on apparent rate of infection, hybrids *viz*., CMS A10 x R2F01120 (B), CMS2A x R-GM-49, CMS A10 x R-GM-49, CMS A10 x 83-Br and CMS A10 x R-GM-39 and check hybrids RSFH-1887 and RSFH-10-600 recorded significantly lower 'r' values indicating the rate of infection in these genotypes is very slow.

Whereas, hybrids CMS 821A x R-GM-41, CMS A10 x R-GM-41 and check hybrid RSFH-130 recorded significantly higher 'r' values indicating fast spread of disease in these genotypes (Table 3).

Table.1 Disease scoring scale for powdery mildew

Rating	Description	Reaction
0	No powdery mildew on leaves	Immune
1	Powdery mildew specks covering 1 % or less area	Highly resistant
3	Powdery mildew lesions covering 1-10% of leaf area	Resistant
5	Enlarged powdery lesions covering 11-25% of leaf area	Moderately resistant/susceptible
7	Powdery lesions coalesce to form big patches covering 26-50% of leaf area	Susceptible
9	Powdery natches covering 51% or more of leaf area and defoliation occur	Highly susceptible

Table.2 Per cent disease severity of powdery mildew disease at 15 days interval in Sunflower hybrids and checks

Sl.No.	Genotypes	Percent disease severity at				Host reaction
		45 DAS	60 DAS	75 DAS	90 DAS	
1	CMS 2A x R-GM-49	4.83	8.00	12.33	19.00	MR
2	CMS 2A x R-GM-69	2.58	8.58	14.25	23.75	MR
3	CMS 2A x 83-Br	4.73	8.53	13.8	23.13	MR
4	CMS 2A x R-GM-41	3.13	5.86	14.4	25.60	S
5	CMS 2A x R-GM-39	3.93	12.33	21.93	32.20	S
6	CMS 2A x R-393	5.93	12.53	24.53	30.40	S
7	CMS 2A x R2F01120(B)	3.66	6.86	13.4	26.26	S
8	CMS 821A x R-GM-49	2.40	6.00	14.86	26.2	S
9	CMS 821A x R-GM-69	1.26	2.73	12.06	21.13	MR
10	CMS 821A x 83-Br	0.80	1.93	15.06	27.20	S
11	CMS 821A x R-GM-41	0.33	0.33	4	21.33	MR
12	CMS 821A x R-GM-39	1.53	3.33	14.73	24.33	MR
13	CMS 821A x R-393	2.66	3.66	6.33	20.00	MR

14	CMS 821A x R2F01120(B)	0.93	1.86	10.8	19.26	MR
15	CMS 850A x R-GM-49	1.66	4.80	14.86	27.20	S
16	CMS 850A x R-GM-69	3.60	12.80	18.00	29.20	S
17	CMS 850A x 83-Br	3.50	6.16	12.83	25.66	S
18	CMS 850A x R-GM-41	0.73	3.00	8.00	21.46	MR
19	CMS 850A x R-GM-39	0.60	2.46	9.00	24.86	MR
20	CMS 850A x R-393	0.58	1.16	8.66	23.16	MR
21	CMS 850A x R2F01120(B)	0.83	3.16	14.33	27.00	S
22	R-10-46-2A x R-GM-49	3.80	6.20	14.40	26.93	S
23	R-10-46-2A x R-GM-69	0.73	1.86	8.80	26.2	S
24	R-10-46-2A x 83-Br	1.58	3.66	16.5	27.5	S
25	R-10-46-2A x R-GM-41	2.11	6.33	12.5	25.44	S
26	R-10-46-2A x R-GM-39	0.6	2.06	9.73	20.13	MR
27	R-10-46-2A x R-393	1.00	3.53	9.40	18.4	MR
28	R-10-46-2A x R2F01120(B)	1.93	3.66	9.46	18.06	MR
29	CMS A4 x R-GM-49	1.00	2.46	11.00	23.53	MR
30	CMS A4 x R-GM-69	1.06	2.73	10.93	25.26	S
31	CMS A4 x 83-Br	3.86	6.13	13.00	25.86	S
32	CMS A4 x R-GM-41	1.33	2.83	12.13	27.00	S
33	CMS A4 x R-GM-39	1.66	1.73	9.73	22.6	MR
34	CMS A4 x R-393	2.30	6.80	12.50	24.30	MR
35	CMS A4 x R2F01120(B)	2.13	5.00	9.53	24.40	MR
36	CMS A6 x R-GM-49	0.66	1.93	9.20	25.13	S
37	CMS A6 x R-GM-69	0.66	2.73	10.00	18.66	MR
38	CMS A6 x 83-Br	0.66	3.06	10.16	22.60	MR
39	CMS A6 x R-GM-41	1.93	3.33	9.60	24.60	MR
40	CMS A6 x R-GM-39	4.73	7.40	10.00	24.33	MR
41	CMS A6 x R-393	1.86	4.00	16.46	27.80	S
42	CMS A6 x R2F01120(B)	4.33	7.00	12.73	24.60	MR
43	CMS A10 x R-GM-49	6.93	10.66	20.46	28.53	S
44	CMS A10 x R-GM-69	3.26	8.13	15.93	28.00	S
45	CMS A10 x 83-Br	8.20	13.00	19.40	29.80	S
46	CMS A10 x R-GM-41	0.00	0.33	1.66	22.66	MR
47	CMS A10 x R-GM-39	6.86	8.93	16.13	26.86	S
48	CMS A10 x R-393	0.86	2.46	8.73	16.73	MR
49	CMS A10 x R2F01120(B)	7.00	8.00	11.73	18.93	MR
50	Morden (c)	2.25	17.4	26.35	55.35	HS
51	RSFH-130 (c)	0.26	1.46	5.40	18.86	MR
52	KBSH-44 (c)	2.36	6.90	15.20	25.50	S
53	KBSH-53 (c)	1.46	3.80	5.20	20.00	MR
54	GK-202 (c)	1.66	2.53	7.86	21.00	MR
55	SB-207 (c)	0.93	2.53	5.86	19.93	MR
56	RSFH1887 (c)	7.53	11.33	15.66	26.26	S
57	RSFH-10-600 (c)	5.60	8.33	10.93	21.06	MR

Note: MR: moderate resistant; S: susceptible; HS: highly susceptible

Table.3 Apparent rate of infection 'r' of Powdery mildew at different stages of Crop growth in sunflower

Sl. No.	Crosses	Rate of spread 'r' at			Average 'r'	DMRT Ranks
		45-60 DAS	60-75 DAS	75-90 DAS		
1	CMS 2A x R-GM-49	0.036	0.032	0.034	0.034	CD
2	CMS 2A x R-GM-69	0.084	0.038	0.042	0.055	ABCD
3	CMS 2A x 83-Br	0.042	0.036	0.042	0.040	BCD
4	CMS 2A x R-GM-41	0.044	0.066	0.048	0.053	ABCD
5	CMS 2A x R-GM-39	0.082	0.046	0.035	0.054	ABCD
6	CMS 2A x R-393	0.055	0.055	0.020	0.043	ABCD
7	CMS 2A x R2F01120(B)	0.044	0.049	0.056	0.050	ABCD
8	CMS 821A x R-GM-49	0.064	0.067	0.047	0.059	ABCD
9	CMS 821A x R-GM-69	0.052	0.106	0.045	0.068	ABCD
10	CMS 821A x 83-Br	0.059	0.146	0.050	0.085	ABC
11	CMS 821A x R-GM-41	0.000	0.169	0.125	0.098	AB
12	CMS 821A x R-GM-39	0.053	0.107	0.041	0.067	ABCD
13	CMS 821A x R-393	0.022	0.038	0.087	0.049	ABCD
14	CMS 821A x R2F01120(B)	0.047	0.123	0.045	0.072	ABCD
15	CMS 850A x R-GM-49	0.073	0.083	0.051	0.069	ABCD
16	CMS 850A x R-GM-69	0.091	0.027	0.042	0.053	ABCD
17	CMS 850A x 83-Br	0.039	0.054	0.057	0.050	ABCD
18	CMS 850A x R-GM-41	0.096	0.069	0.076	0.080	ABCD
19	CMS 850A x R-GM-39	0.095	0.091	0.080	0.089	ABC
20	CMS 850A x R-393	0.047	0.139	0.077	0.088	ABC
21	CMS 850A x R2F01120(B)	0.091	0.109	0.053	0.084	ABCD
22	R-10-46-2A x R-GM-49	0.034	0.062	0.052	0.050	ABCD
23	R-10-46-2A x R-GM-69	0.063	0.108	0.087	0.086	ABC
24	R-10-46-2A x 83-Br	0.057	0.110	0.043	0.070	ABCD
25	R-10-46-2A x R-GM-41	0.076	0.050	0.058	0.061	ABCD
26	R-10-46-2A x R-GM-39	0.083	0.109	0.057	0.083	ABCD
27	R-10-46-2A x R-393	0.086	0.069	0.052	0.069	ABCD

28	R-10-46-2A x R2F01120(B)	0.044	0.067	0.050	0.054	ABCD
29	CMS A4 x R-GM-49	0.061	0.106	0.061	0.076	ABCD
30	CMS A4 x R-GM-69	0.064	0.098	0.067	0.077	ABCD
31	CMS A4 x 83-Br	0.032	0.055	0.056	0.048	ABCD
32	CMS A4 x R-GM-41	0.051	0.104	0.066	0.074	ABCD
33	CMS A4 x R-GM-39	0.003	0.121	0.066	0.063	ABCD
34	CMS A4 x R-393	0.075	0.044	0.053	0.057	ABCD
35	CMS A4 x R2F01120(B)	0.059	0.046	0.075	0.060	ABCD
36	CMS A6 x R-GM-49	0.072	0.109	0.080	0.087	ABC
37	CMS A6 x R-GM-69	0.096	0.092	0.048	0.079	ABCD
38	CMS A6 x 83-Br	0.104	0.085	0.063	0.084	ABCD
39	CMS A6 x R-GM-41	0.037	0.075	0.075	0.062	ABCD
40	CMS A6 x R-GM-39	0.032	0.022	0.071	0.041	ABCD
41	CMS A6 x R-393	0.052	0.103	0.045	0.067	ABCD
42	CMS A6 x R2F01120(B)	0.034	0.044	0.054	0.044	ABCD
43	CMS A10 x R-GM-49	0.031	0.051	0.029	0.037	CD
44	CMS A10 x R-GM-69	0.064	0.051	0.048	0.054	ABCD
45	CMS A10 x 83-Br	0.034	0.032	0.038	0.035	CD
46	CMS A10 x R-GM-41	0.000	0.108	0.190	0.100	A
47	CMS A10 x R-GM-39	0.019	0.045	0.043	0.036	CD
48	CMS A10 x R-393	0.071	0.089	0.049	0.070	ABCD
49	CMS A10 x R2F01120(B)	0.010	0.028	0.038	0.025	D
50	Morden	0.147	0.035	0.082	0.088	ABC
51	RSFH-130	0.116	0.090	0.094	0.100	A
52	KBSH-44	0.074	0.058	0.043	0.058	ABCD
53	KBSH-53	0.065	0.022	0.101	0.063	ABCD
54	GK-202	0.029	0.079	0.076	0.061	ABCD
55	SB-207	0.068	0.058	0.092	0.073	ABCD
56	RSFH1887	0.030	0.025	0.043	0.033	CD
57	RSFH-10-600	0.028	0.020	0.052	0.033	CD

Table.4 *Golovinomyces cichoracearum* conidial population on selected promising moderate resistant sunflower genotypes

Genotypes	PDS	Host reaction	No. of conidia spore/microscopic field										Mean	DMRT Ranks
			1	2	3	4	5	6	7	8	9	10		
R-10-46-2A x R-393	18.40	MR	21	32	39	46	17	49	53	58	17	14	34.6	E
R-10-46-2A x R2F01120(B)	18.06	MR	16	24	19	32	38	29	37	24	32	23	27.4	E
CMS A10 x R-393	16.73	MR	200	180	160	150	140	130	140	155	145	125	152.5	C
CMS A10 x R2F01120(B)	18.93	MR	64	102	117	103	109	74	132	114	126	88	102.9	D
CMS 850A x R-GM-69	29.20	S	215	220	210	180	160	150	180	205	225	190	193.5	B
MORDEN	55.35	HS	380	350	410	390	340	320	400	450	390	430	386.0	A

Fig.1 Microphotograph showing conidia and conidiophores of *G. cichoracearum* at 45X

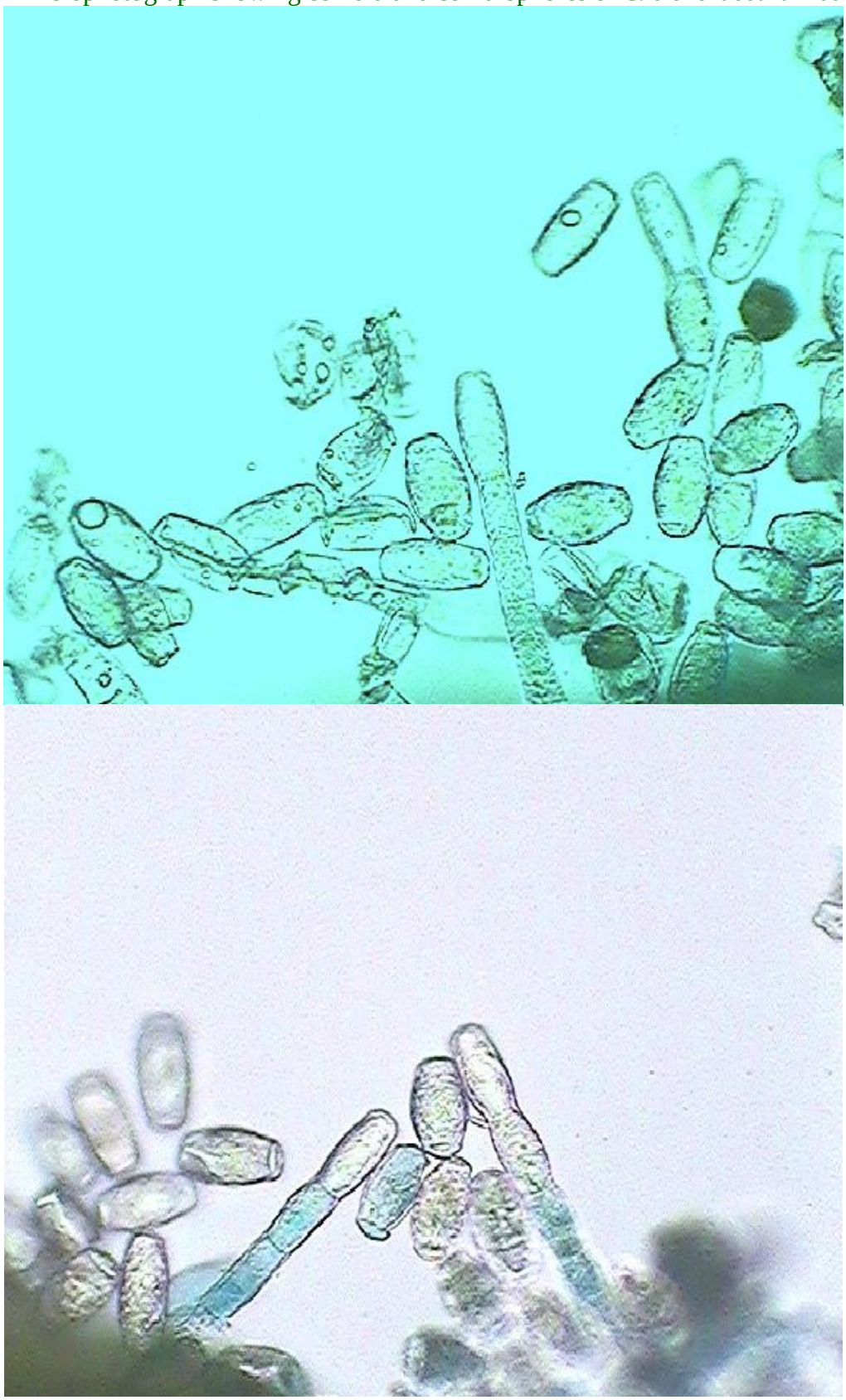
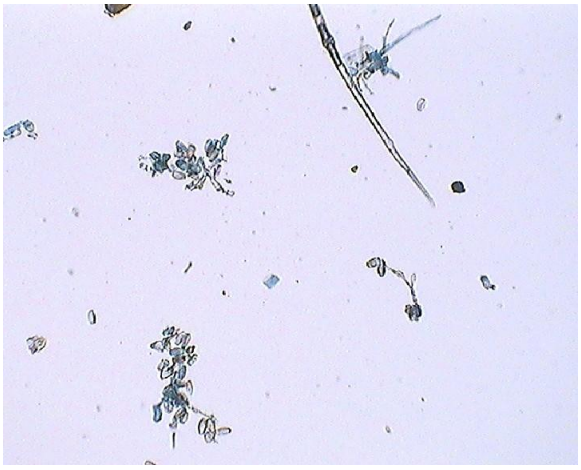
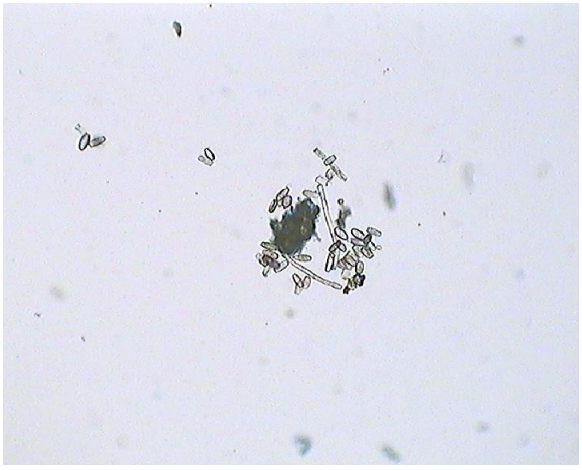


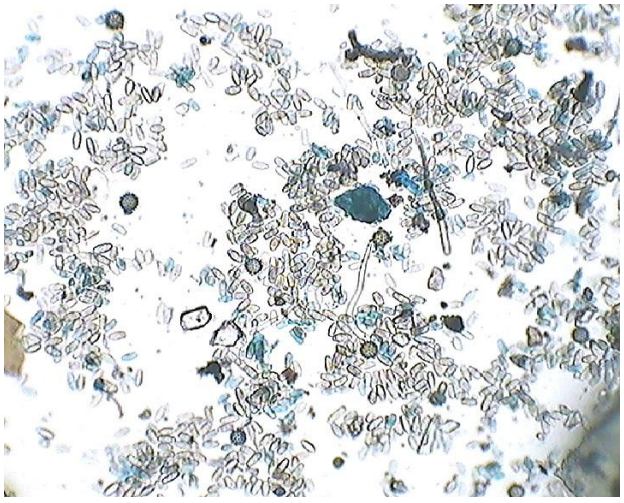
Fig.2 Microphotograph showing number of conidia spore per microscopic field of *G. cichoracearum* in moderate resistant hybrids and susceptible check Morden at 10X



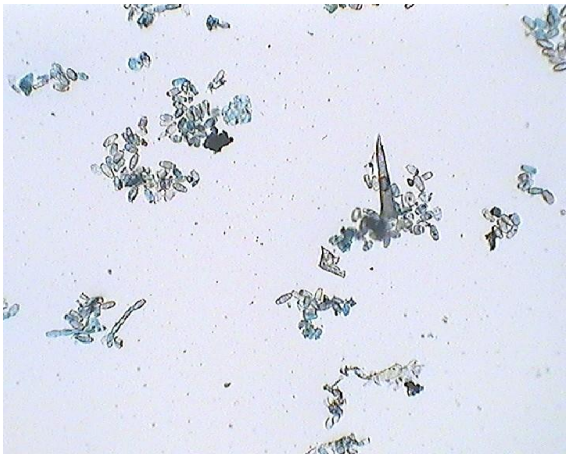
R-10-46-2A X R-393



R-10-46-2A X R2F01120



MORDEN



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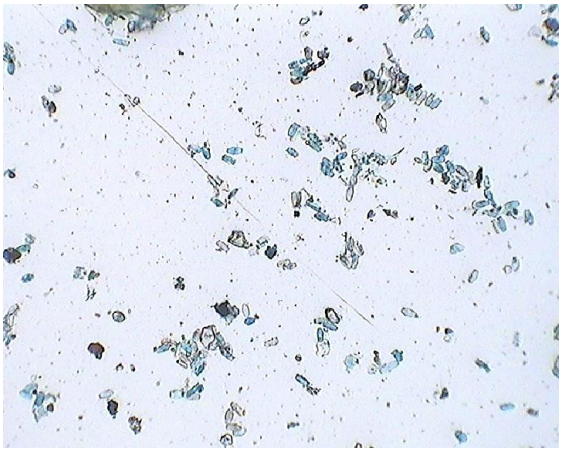


CMS A10 X R-393

Fig.3 Average per cent disease severity in moderate resistant, susceptible and highly susceptible sunflower genotypes

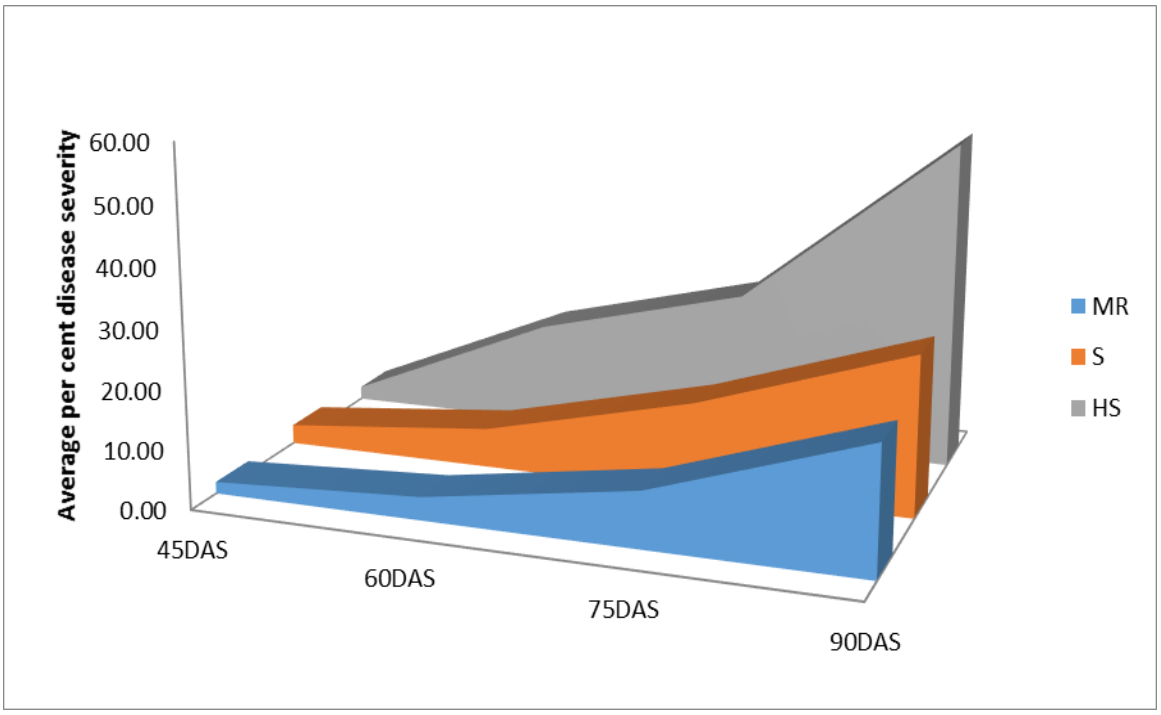
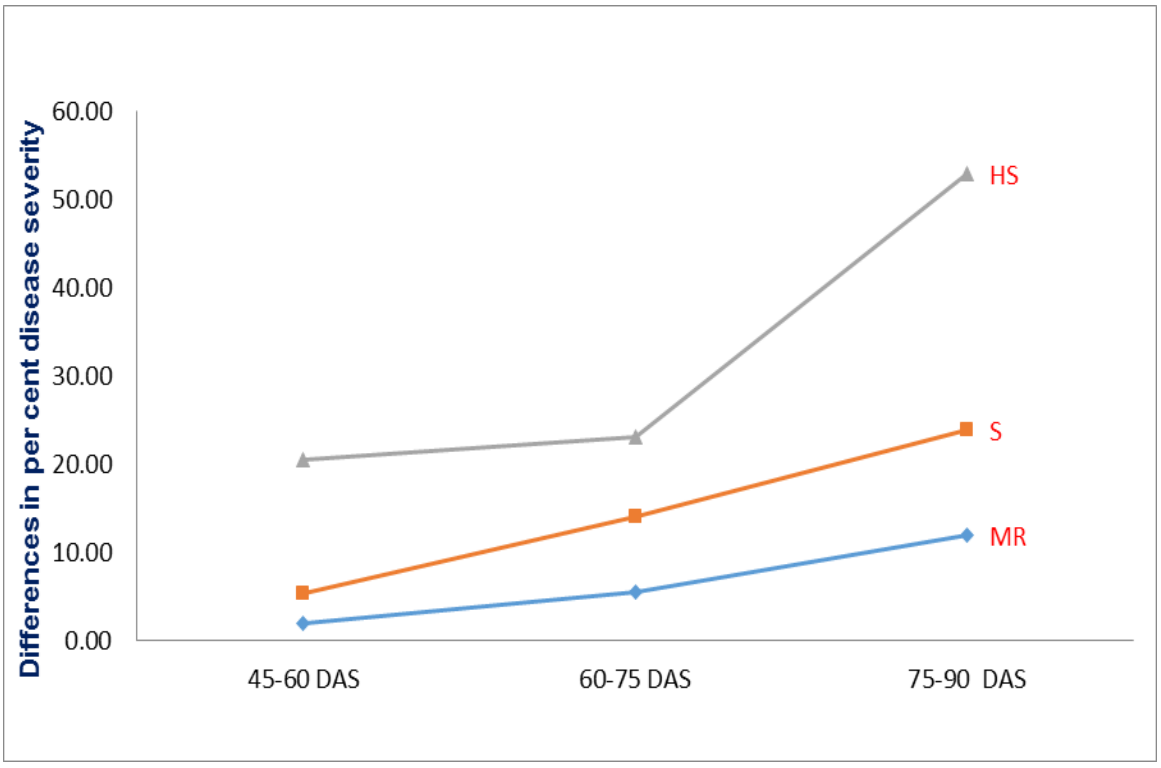


Fig.4 Differences in powdery mildew infection in moderate resistant, susceptible and highly susceptible sunflower genotypes



The genotypes *viz*., CMS A10 x R-GM-49, CMS A10 x 83-Br and CMS A10 x R-GM-39 having low apparent rate of infection actually recorded high disease infection at their early growth stage however infection rate was low. The genotypes CMS A10 x R-393, CMS A10 x R-GM-41 and RSFH-130 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. These results indicate the low apparent rate of infection 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.

This observation is in agreement with that of Wilcoxson *et al.,* (1975) and Nargund (1989) who pointed out that 'r' values are not useful criteria for selecting the genotype. However, it can be used in studying the disease development in different genetic background.

The microscopic observation of the fungus was carried out on selected moderate resistant genotypes which scored least per cent disease severity at 90 days after sowing (Fig. 3).

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 (Figs. 1 and 2).

The number of conidia spores was counted in ten different microscopic fields for four selected moderate resistant genotypes, one susceptible and highly susceptible check Morden. The average number of conidia per microscopic field were analysed using DMRT (0.05). The moderate resistant genotypes R-10-46-2A x R2F01120 (27.4) and R-10-46-2A x R-393 (34.6) recorded least number of conidia per microscopic field. The DMRT analysis categorised the average conidial count into 5 classes, indicating significant differences between 6 genotypes (Table 4). The other two moderate resistant hybrids CMS A10 x R-393 and CMS A10 x R2F01120 (B) recorded slightly higher number of conidial spores (152.5 and 102.9, respectively).

However, these conidial spores were significantly lower than susceptible hybrid CMS 850A x R-GM-69 (193.5) and highly susceptible check Morden (386). We could also observe significant differences for number of conidial spores in susceptible hybrid CMS 850A x R-GM-69 as compared to highly susceptible check Morden. These microscopic observations are in line with Reddy *et al.,* (2013) they also reported less conidial spores and hyphal growth in resistant and moderately resistant sunflower genotypes compared to susceptible check (Fig. 4).

In conclusion, the present study clearly indicates that it is possible to synthesize hybrids with reasonable degree of tolerance by involving moderate tolerant genotype as one of the parent.

Further, resistance to powdery mildew is reported to exhibit differential reaction in different environmental conditions (Saliman, 1982) and several studies have identified 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. Hence, in the absence of high level of resistance to powdery mildew, genes responsible for partial resistance are potentially useful for development of cultivars with durable resistance.

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