

Research Article

Evaluation of hybrids developed from diversified CMS lines for resistance to powdery mildew in sunflower (*Helianthus annuus* L.)

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Abstract

Two ninety two hybrids developed from crossing six diversified Cytoplasmic Male Sterile (CMS) lines viz., ARG-2-1-2, ARG 6-3-1-4, MUT2-8-3-2, ARG 3, PKUZ and PRUN 29 with 40 inbred lines. Hybrids were evaluated for powdery mildew resistance reaction. Among 292 hybrids screened, none of the hybrids were highly resistant to powdery mildew, 33 hybrids were resistant, 37 were moderately resistant, 95 were susceptible and 126 showed highly susceptible reaction. More number of hybrids developed from PRUN 29 line was found more resistant to powdery mildew. Out of 48 hybrids developed from PRUN 29 CMS line, 15 and 16 hybrids were found to be resistant and moderately resistant reaction. Similarly 12 and 5 hybrids developed from line ARG 6-3-1-4 showed resistant and moderately resistant reaction respectively. Hybrids developed from CMS lines ARG 2-1-2 and MUT2-8-3-2 were shown susceptible reaction. Hybrids which showed sterile reaction along with resistance to powdery mildew could be further used to develop inbreds which are resistant to powdery mildew. Hybrids which showed fertile reaction along with resistance to powdery mildew will be evaluated in multilocation and multiseason to identify hybrids which are high yielding coupled with resistance to powdery mildew disease.

Key words

Sunflower, Cytoplasmic Male Sterile (CMS) lines, Inbreds, Powdery mildew, Resistance

Introduction

Sunflower is an important oilseed crop of India, until recently the major diseases limiting sunflower cultivation in India were *Alternaria* leaf blight, downy mildew, necrosis virus disease and occasionally rust. From the last decade powdery mildew caused by *Golvinomyces cichoracearum* (DC) V.P.Heluta var. *cichoracearum* (1988) (formerly *Erysiphe cichoracearum* DC ex Meret, 1805) has become one of the major disease on sunflower in India. It is an economically important disease in the tropical regions and affects most of the commercial hybrids under present cultivation. In India powdery mildew was sporadically observed at a very low intensity till 2006. Severe foliar (80 %) infection of powdery mildew was observed during 2006 at Challakere and Chitradurga district of Karnataka (Annon, 2007). The disease is now seen regularly in almost all the sunflower growing areas of the country in moderate to severe form.

The disease causes severe economic loss in warmer regions of the world (Zimmer & Hoes, 1978), but is seldom severe enough to warrant the cost of fungicide applications in temperate climates (Gulyaet al., 1997). However, the development of resistance cultivars becomes very important if the crop is to expand into warmer regions where the disease may cause economic losses. (Zimmer and Hoes, 1978). The annual species *Helianthus debilis* ssp. *silvestris*, *H. praecox* sp. *praecox* and *H. bolanders* and the perennial species *H. californicus*, *H. ciliaris*, *H. decapetalus*, *H. lacinatus* and *H. rigidus* were

reported to be tolerant to powdery mildew in green house and field trials (Saliman et al., 1982).

Till now all the released hybrids in sunflower were developed from petiolaris (PET 1) male sterile source. PET 1 source was discovered by Leclereq (1969) in the progeny of cross between *Helianthus petiolaris* Nutt and cultivated sunflower and subsequent identification of genes for fertility restoration by Kinman (1970). Use of single cytoplasmic source for hybrid development has resulted in the genetic uniformity for cytoplasmic background in the crop. Prevalence of genetic uniformity of this kind over a large area could result in genetic vulnerability of hybrids if the cytoplasm becomes susceptible to a new strain of pathogen or pest similar to that happened in maize when texas cytoplasm became susceptible to *Helminthosporium maydis* in USA (Tatum, 1971). Among several strategies available to overcome this problem, diversification of CMS source itself is possibly the cheapest and most effective method.

In the present study we have evaluated the hybrids for powdery mildew which are developed from diversified Cytoplasmic male sterile (CMS) lines. These diverse cytoplasmic male sterile lines were developed from crossing cultivated sunflower with wild *H. agrophilus*, *H. preaux*, *H. annuus* lines. In earlier studies these lines were found to be resistant for powdery mildew (Saliman et al., 1982)

Materials and methods

Plant Material: The plant material used in the study included 292 hybrids developed by crossing

six diversified CMS lines with 49 CMS B lines (Table 1 & 2) at AICRP (Sunflower), UAS, GKVK, Bengaluru, Karnataka. Six diversified CMS lines obtained from Indian Institute of Oilseed Research and 49 CMS B lines developed at AICRP on Sunflower were used for crossing to develop hybrids.

Six diverse CMS lines having diversified cytoplasmic sources which are reported to have resistant to different diseases. Hence these diversified CMS lines used in the study. These lines were further used for development of CMS lines with diversified Cytoplasmic and further used for hybrid development. These hybrids were evaluated for powdery mildew resistance and to study the maintainer and restorer reaction of inbreds.

Collection of Pathogen: Two different genera of powdery mildew fungi *Sphaerotheca fuliginea* (Schlecht.Ex.Ft.)Pollacci and *Erysiphe cichoracearum* DC. f. sp. *helianthi* Jacz. (Patel *et al.*, 1949; Prasada *et al.*, 1968; Goswami and Dasgupta, 1981) are reported on sunflower in India. The fungus was collected from the affected sunflower plants in field, conidia from clear, separate and isolated colonies were picked up through the bristles of a brush and dusted onto glasshouse raised KBSH 44 plants which is highly susceptible to powdery mildew. Isolated colonies developing on these plants was further transferred to large number of KBSH 44 plants and a pure culture originating from a single, well developed isolated colony was established. The culture thereafter was maintained in the glass house to avoid contamination. The culture was transferred to new plants as and when required. This culture was sent to Herbarium Cryptogame India Orientalis (HCIO), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India for identification and the same was used in all further studies.

Etiology: The culture was sent to The Herbarium Cryptogame India Orientalis (HCIO), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India and identified as the oidium state of *Erysiphe cichoracearum* with the accession /Identification number HCIO 50056.

Screening of hybrids against powdery mildew: Screening of sunflower lines against powdery mildew caused by *Erysiphe cichoracearum* D.C. was carried out to identify the source or sources of resistance. A total of 291 hybrids developed were screened at Zonal Agricultural Research Station, Gandhi Krishi Vignana Kendra, UAS, Bengaluru during *Kharif* 2013 under natural conditions. The hybrids were sown in twin rows replicated twice (row length of 3.0 m each). KBSH 44 was used as susceptible check.

The disease severity was recorded using 0 to 9 scale (Table 3) given by Mayee and Datar (1986) on five marked plants of each germplasm at 50 per cent flowering stage of the crop.

The germplasm was further grouped into 6 categories based on reaction type as given by Khare and Lakpale (1997).

Results and discussion

Powdery mildew is a frequently found disease on sunflower in warmer regions of the world (Zimmer and Hoes, 1978). The disease usually appears at post flowering stage when senescence of lower leaves occur and is reported to cause limited damage in temperate climates. In India, the disease has become serious and occurring regularly since 2006. It is observed during different crop growing seasons and under severe conditions it is found infecting the cotyledonary leaves as well. Hence, identification of resistance source is necessary for future development of hybrids.

Till now all the hybrids developed and released for commercial cultivation in sunflower possess the PET 1 cytoplasm. Large scale cultivation of hybrids having single CMS source might pose a threat if it becomes susceptible to pests and diseases as was recorded in other crops like corn and pearl millet. Hence, in the present study we have evaluated 292 hybrids developed from different cytoplasmic source for powdery mildew.

Among 292 hybrids screened for powdery mildew none of the hybrids were highly resistant to powdery mildew, 33 hybrids were resistant, 37 hybrids were moderately resistant, 95 hybrids were susceptible and 126 hybrids showed highly susceptible reaction (Table 4). Hybrids developed from PRUN 29 lines were found to be resistant to powdery mildew. PRUN 29 line is a CMS source from *Helianthus precaux*, more number of hybrids found to be resistant from this cytoplasmic source. Out of 48 hybrids developed from PRUN 29 CMS line, 15 and 18 hybrids were found to be resistant and moderately resistant to powdery mildew and 12 and 3 hybrids were found to be susceptible and highly susceptible to powdery mildew. A total of 14 and 17 hybrids from ARG 6-3-1-4 showed susceptible and highly susceptible reaction respectively. Similarly 12 and 5 hybrids showed resistant and moderately resistant reaction respectively.

All hybrids developed from ARG-2-1-2 and MUT 2-8-3-2 CMS lines showed susceptible and highly susceptible reaction to powdery mildew. Hybrids developed from CMS lines ARG 3 and PKU 2 showed susceptible and highly susceptible reaction to powdery mildew. Only 4 hybrids developed from each source of ARG 3 and PKUZ

showed resistance reaction. Similarly 4 and 10 hybrids showed moderately resistant reaction to powdery mildew from ARG 3 and PKUZ CMS lines respectively. Results pertaining to this are presented in Table 5.

Out of 292 hybrids, 33 and 37 hybrids were found to be resistant and moderately resistant to powdery mildew respectively. A total of 95 and 126 hybrids showed susceptible and highly susceptible reaction respectively. Majority of hybrids developed from PRUN 29 (*Helianthus preaux*) CMS line were found to be resistant for powdery mildew followed by CMS lines ARG 6-3-1-4, PKU 2 and ARG 3. Hybrids developed from CMS lines ARG-2-1-2 and MUT 2-8-3-2 were found susceptible. Similarly hybrids developed from pollen parents CMS 335B, CMS 62B and CMS 122B were found resistant to powdery mildew. All 292 hybrids were evaluated to study the fertility restoration and sterility maintenance reaction. Hybrids which showed fertile reaction along with resistance to powdery mildew could be used to develop inbreds which are resistant to powdery mildew. Further these inbreds could be used for development of new hybrids with resistance to powdery mildew with diversified cytoplasmic background. Hybrids which showed fertile reaction along with resistance to powdery mildew will be evaluated further in multilocation and multiseason to identify hybrids which are high yielding coupled with resistance to powdery mildew disease. Further more emphasis has to be given to hybrids which are developed from the source PRUN 29 as it found to be more resistance to powdery mildew disease compared to other cytoplasmic sources.

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Table 1. Diversified CMS lines used to develop sunflower hybrids

S. No.	CMS designation	Origin
1	ARG-2-1-2	<i>H.agrophyllus</i>
2	ARG-6-3-1-4	<i>H.agrophyllus</i>
3	MUT-8-3-2	<i>H.annus</i>
4	ARG 3	<i>H.agrophyllus</i>
5	PKU 2	<i>H annus</i>
6	PRUN 29	<i>H praecox spprunyonni</i>

Table 2. Forty nine inbred lines used to develop sunflower hybrids

S. No	Inbred lines	S. No	Inbred lines	S. No	Inbred lines
1	CMS-335B	18	CMS-597B	35	CMS-300B
2	CMS-59B	19	CMS-62B	36	CMS-438B
3	CMS-852B	20	CMS-107B	37	CMS-607B
4	CMS-275B	21	CMS-112B	38	CMS-850B
5	CMS-336B	22	CMS-131B	39	CMS-122B
6	CMS-56B	23	CMS-243B	40	CMS-125B
7	CMS-207B	24	CMS-108B	41	CMS-127B
8	CMS-343B	25	CMS-110B	42	NDCMS-4B
9	CMS-17B	26	CMS-234B	43	CMS-851B
10	CMS-109B	27	CMS-101B	44	CMS-10B
11	CMS-102B	28	CMS-138B	45	CMS-47B
12	CMS-103B	29	CMS-55B	46	CMS-84B
13	CMS-111B	30	CMS-135B	47	CMS-124B
14	CMS-339B	31	CMS-89B	48	CMS-338B
15	CMS-54B	32	CMS-134B	49	CMS-338-1B
16	CMS-58B	33	CMS-148B		
17	CMS-60B	34	CMS-7-1B		

Table 3. Scale adopted for evaluating genotypes

Scale	Description	Reaction
0	No symptom of powdery mildew on leaves.	Immune (I)
1	Small scattered powdery mildew specks covering 1 % or less leaf area.	Highly resistance (HR)
3	Small powdery lesions covering 1-10 % of leaf area.	Resistance (R)
5	Powdery lesions enlarged covering 11-25% of leaf area.	Moderately resistant (MR)
7	Powdery lesions coalesce to form big patches covering 26-50% of leaf area.	Susceptible (S)
9	Big powdery patches covering: 51% or more of leaf area and defoliation occur	Highly susceptible (HS)

Table 4. Reaction of Sunflower hybrids to powdery mildew

Reaction	Disease severity	Hybrids	No.
0-Immune	0	-	0
1-Highly Resistant	1%	-	0
3-Resistant	1-10 %	2x19, 2x20, 2x21, 2x22, 2x27, 2x32, 2x33, 2x35, 2x37, 2x38, 2x39, 2x43, 6x3, 6x5, 6x6, 6x7, 6x10, 6x11, 6x13, 6x16, 6x17, 6x18, 6x19, 6x25, 6x29, 6x31, 6x47, 6x48, 4x1, 5x40, 5x42, 5x44, 5x46 and 3x1.	34
5-Moderately Resistant	11-25%	2x13, 2x23, 2x24, 2x25, 2x40, 6x4, 6x15, 6x20, 6x21, 6x22, 6x23, 6x24, 6x26, 6x27, 6x28, 6x31, 6x33, 6x34, 6x35, 6x36, 6x39, 6x42, 6x46, 4x2, 4x7, 4x8, 4x41, 5x2, 5x4, 5x6, 5x9, 5x10, 5x38, 5x39, 5x43, 5x45 and 5x48.	37
7-Susceptible	26-50 %	2x14, 2x15, 2x16, 2x17, 2x18, 2x31, 2x34, 2x36, 2x41, 2x42, 2x44, 2x45, 2x46, 2x47, 6x8, 6x9, 6x12, 6x14, 6x37, 6x38, 6x41, 6x43, 6x44, 6x49, 6x48, 4x3, 4x4, 4x5, 4x6, 4x13, 4x17, 4x18, 4x28, 4x35, 4x36, 4x37, 4x38, 4x39, 4x40, 4x42, 1x36, 1x37, 1x45, 1x46, 1x47, 1x48, 1x49, 5x3, 5x5, 5x7, 5x8, 5x11, 5x12, 5x13, 5x14, 5x15, 5x16, 5x17, 5x18, 5x19, 5x20, 5x21, 5x31, 5x32, 5x33, 5x34, 5x35, 5x36, 5x37, 5x41, 5x47, 5x49, 3x2, 3x9, 3x10, 3x11, 3x12, 3x13, 3x14, 3x15, 3x16, 3x17, 3x18, 3x19, 3x20, 3x23, 3x24, 3x25, 3x26, 3x28, 3x29, 3x30, 3x31 and 3x45.	95
9-Highly Susceptible	>50%	2x1, 2x2, 2x3, 2x4, 2x5, 2x6, 2x7, 2x8, 2x9, 2x10, 2x11, 2x12, 2x26, 2x28, 2x29, 2x30, 2x48, 6x1, 6x2, 6x45, 4x9, 4x10, 4x11, 4x13, 4x14, 4x15, 4x16, 4x19, 4x20, 4x21, 4x22, 4x23, 4x24, 4x25, 4x26, 4x27, 4x29, 4x30, 4x31, 4x32, 4x33, 4x34, 4x43, 4x44, 4x45, 4x46, 4x47, 4x48, 4x49, 1x1, 1x2, 1x3, 1x4, 1x5, 1x6, 1x7, 1x8, 1x9, 1x10, 1x11, 1x12, 1x13, 1x14, 1x15, 1x16, 1x17, 1x18, 1x19, 1x20, 1x21, 1x22, 1x23, 1x24, 1x25, 1x26, 1x27, 1x28, 1x29, 1x30, 1x31, 1x32, 1x33, 1x34, 1x35, 1x38, 1x39, 1x40, 1x41, 1x42, 1x43, 1x44, 5x22, 5x23, 5x24, 5x25, 5x26, 5x27, 5x28, 5x29, 5x30, 3x3, 3x4, 3x5, 3x6, 3x7, 3x8, 3x21, 3x22, 3x27, 3x32, 3x33, 3x34, 3x35, 3x36, 3x38, 3x39, 3x40, 3x41, 3x42, 3x43, 3x44, 3x46, 3x47, 2x48, 2x49 and 5x36.	126



Table 5. Hybrids from different diversified CMS lines showing disease severity to powdery mildew

Reaction	Disease severity	Number of hybrids from different diversified CMS lines showing disease severity to powdery mildew					
		ARG 6-3-1-4	PRUN 29	ARG 3	ARG 2-1-2	PKU Z	MUT 2-8-3-2
0-Immune	0	-	-	-	-	-	-
1-Highly Resistant	1%	-	-	-	-	-	-
3-Resistant	1-10 %	12	16	1	0	4	1
5-Moderately Resistant	11-25%	5	18	4	0	10	0
7-Susceptible	26-50 %	14	12	15	7	25	22
9-Highly Susceptible	>50%	17	3	29	42	10	25
	Total	48	49	49	49	49	48