Journal of Applied and Natural Science 9 (3): 1358 - 1364 (2017)

Inheritance of powdery mildew resistance in sunflower (*Helianthus annuss* L.)

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Received: May 9, 2016; Revised received: March 25, 2017; Accepted: July 5, 2017

Abstract: Powdery mildew caused by *Golovinomyces cichoracearum* is the most important disease on sunflower worldwide. The investigation was undertaken to determine the mode of genetic inheritance of powdery mildew resistance in five F_1 and F_2 populations of sunflower. The four hybrids were derived by crossing resistant with susceptible genotype and one hybrid between resistant and highly susceptible genotype. Out of 23 hybrids, five crosses *viz.*, PM-22x PM-36, PM-14xPM-36, PM-16xPM-38, PM-17xPM-35 and PM-34x PM-23 were resistant under natural as well as artificial epiphytotic screening conditions and the same five resistant hybrids segregated in 9R:7S ratio in F_2 population indicating involvement of two independent loci controlling powdery mildew resistance in sunflower.

Keywords: *Helianthus annuss* , Inheritance, Mildew, Sunflower

INTRODUCTION

Powdery mildew caused by *Golovinomyces cichoracearum* (DC.) V. P. Heluta (formerly *Erysiphe cichoracearum*) has become a serious problem in major sunflower growing regions of Southern India. Since decades, disease is being observed regularly during *rabi*-summer seasons and under severe conditions disease is found infecting whole plant starting from the cotyledonary leaves up to ray florets. The higher severity has been observed in tropical parts of the world, where it advances senescence of plant at the flowering or post flowering stages (Reddy *et al.,* 2013). In India, powdery mildew disease on sunflower was first reported in Bombay (Patel *et al*., 1949) later in Rajasthan (Prasada *et al*., 1968) and West Bengal (Goswami and Dasgupta, 1981) causing a considerable reduction in yield. Development of powdery mildew resistant cultivars is a promising solution that may allow production areas to be expanded. Sunflower cultivars differ in their reaction to powdery mildew, where resistance hybrids are not available and at present relatively little efforts have been devoted to develop resistant germplasm. However, wild sunflower species, *H. argophyllus*, *H. debilis*, *H. californicus, H. ciliaris*, *H. decapetalus* and *H. lacinatus* represent a valuable reservoir of genes for several biotic stresses which have been successfully introgressed into cultivated sunflower (Seiler, 2008).

Knowledge of genetic inheritance is very useful for formulation of breeding strategy to transfer genes into the agronomically superior cultivars.Although earlier studies reported digenic and quantitative genes governing resistant to powdery mildew (Seiler, 2008). However, the information on allelic relationship for powdery mildew resistance in sunflower is inadequate. The results from cross between *Helianthus argophyllus* x HA-89 and *Helianthus debilis* x HA-89 suggested that resistance in both the crosses is controlled by at least two genes. Therefore, objective of this study was to determine the inheritance of powdery mildew resistance in sunflower.

MATERIALS AND METHODS

The experiment material comprising of 23 F_1 and F_2 families along with susceptible check Morden were screened in natural as well as artificial epiphytotic conditions during 2013-14 and 2014-15 at MARS, Raichur.

Crossing programme: The material used for the study includes five resistant, susceptible and highly susceptible sunflower genotypes for powdery mildew. Individual plant heads were treated with 100 ppm GA_3 at button stage to induce male sterility. The pollen of the male lines was collected separately in petri-plate with the help of camel hairbrush during morning hours (9:00 to 11:00 AM) and dusted on to the stigma of female heads to derive desired crossed seeds. Immediately after hand pollination the heads were covered with cloth bags to avoid cross contamination. Pollination was done till all the florets in the capitulum showed sign of drying. The heads of all the resultant 23 hybrids were harvested, dried and threshed separately and stored with care to maintain viability. The well filled seeds from each cross were separated out for evaluation of hybrids for powdery mildew resistance during *kharif* 2014.

Screening of sunflower hybrids against powdery mildew in natural conditions: Twenty three hybrids were sown in RCBD design with three replications along with susceptible checks during late *kharif* 2014 and screening was carried out by leaf stapling method (Reddy *et al.*, 2013). The powdery mildew disease incidence was recorded at 15 days interval from 30 DAS upto 90 DAS by randomly selecting five plants in each cross and post harvest observations were also recorded after harvesting. The hybrid plants were bagged before flower opening to obtain the selfed seeds from each individual F_1 plants and forwarded to evaluate segregation analysis of F_2 generation.

The segregating population was planted along with susceptible checks for effective and uniform spreading of powdery mildew inoculum. Each F_2 population was grown in 20 rows plots of three meter length and susceptible check was sown at every 10 row interval. The plants were stapled with powdery mildew infected leaves at 30 DAS.

Screening of sunflower hybrids against powdery mildew in artificial conditions: Ten plants in each cross were sown in pots and screening was conducted by spraying conidial spore suspension (10^6 conidia/ml prepared in 1 per cent sucrose solution) at 30 and 45 DAS and powdery mildew disease incidence was recorded at 15 days interval from 30 DAS up to 90 DAS on individual plants of each cross.

Calculation of percent disease severity (PDS) and classification of parents and progenies: The powdery mildew disease incidence was recorded on five plants from bottom, middle and top portion of each plant and observations are made as per 0-9 (Mayee and Datar, 1986) scale at 30,45, 60, 75 and 90 days after sowing. Further, these observations were converted to percent disease severity (PDS) for every plant using following formula given by Wheeler (1969).

Sum of Disease Scores

PDS= ×100 Number of Plants Scored x Highest Rating

Chi-square test: Simple chi-square test was applied to test the goodness of fit for the genetic ratios.The segregation pattern was studied in F_2 population. The disease resistance was classified into two groups, those with less than 10 per cent PDS as resistant and those with more than 10 per cent PDS as susceptible. The reactions for disease were recorded as resistant and susceptible on all individual plants of F_2 generation of crosses.

RESULTS AND DISCUSSION

Sunflower seed yield and oil quality is being severely affected by incidence of powdery mildew disease at early stages of crop growth (Reddy *et al.*, 2013). The

Table 1. Reaction of sunflower hybrids to powdery mildew disease under natural conditions during *kharif*-2014.

S. N.	Cross	Parental reaction	Percent Disease Severity at (%)					Scale	Host reaction
			30 DAS	45 DAS	60 DAS	75 DAS	90 DAS		
1	PM-14x PM-36	R x S	0.3	1.2	2.8	4.3	8.5	3	R
2	PM-16 x PM-37	R x S	0.2	1.7	2.5	7.5	9.8	3	R
3	PM-16 x PM-38	R x S	0.5	1	2	4.7	7.1	3	R
4	PM-17 x PM-35	R x S	0.3	0.8	2.4	6	8.4	3	R
5	PM-17 x PM-36	R x S	0.8	1.4	4.2	7.5	9.4	3	R
6	PM-17 x PM-38	R x S	0.9	1.8	4.8	5.5	8.7	3	R
7	PM-22 x PM-36	R x S	0	0.3	1.5	2.9	4.7	3	R
8	PM-34 x PM-23	HS x R	0.0	0.5	1.0	2.3	3.6	3	R
9	PM-10 x PM-35	MRx S	0.1	1.2	10	17.8	21.4	5	MR
10	PM-18 x PM-34	MR xHS	0.4	1.5	3.4	9.6	12.2	5	MR
11	PM-20 x PM-38	S x S	1.2	3.7	9.6	16.9	20.4	5	MR
12	PM-21 x PM-37	MR x S	0.7	1.4	5.7	11.6	14.8	5	MR
13	PM-28 x PM-12	HS xMR	0.1	1.3	3.7	10.3	14.7	5	MR
14	PM-34 x PM-18	HS xMR	0.6	3.5	11.4	16.3	20.3	5	MR
15	PM-36 x PM-10	S x MR	2.0	3.7	10.2	17.2	23.3	5	MR
16	PM-38 x PM-20	S x S	0.4	1.2	7.4	16.4	18.0	5	MR
17	PM-40 x PM-19	HS xMR	1.4	3.5	5.0	13.0	15.6	5	MR
18	PM-20 x PM-35	S x S	0.3	1.9	8.4	20.8	27.5	7	S
19	PM-20 x PM-36	S x S	1.3	2.9	8.2	22.9	28.2	7	S
20	PM-28 x PM-4	HS x S	5.4	16.8	22.5	27.2	30	7	S
21	PM-28 x PM-19	HS xMR	1.0	3.4	12.1	21.2	28.9	7	S
22	PM-31 x PM-19	S x MR	0.5	1.3	11.7	21.2	26.2	7	S
23	PM-36 x PM-20	S x S	3.8	6.1	19	28.5	32.8	7	S

Table 2. Reaction of sunflower hybrids to powdery mildew disease under artificial conditions.

S. N.	Cross	Parental reaction	Per cent Disease Severity at (%) 30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Scale	Host reaction
1	PM-14x PM-36	R x S	0.8	1.7	4.5	7.4	8.7	3	R
2	PM-16 x PM-37	R x S	0.5	1.3	2.9	4.6	7.3	3	R
3	PM-16 x PM-38	R x S	0.6	1	2.1	5.5	8.7	3	R
4	PM-17 x PM-35	R x S	0.1	0.6	1.5	4.8	8.6	3	R
5	PM-17 x PM-36	R x S	0.5	1.4	3	5.3	8.5	3	R
6	PM-17 x PM-38	R x S	0.3	1.3	2.4	5	7.1	3	R
7	PM-22 x PM-36	R x S	0.5	1.4	2.6	6.1	9.7	3	R
8	PM-34 x PM-23	HS x R	0.3	0.8	2	5.3	8.9	3	R
9	PM-18 x PM-34	MR x HS	1.3	3	5.6	13.9	22.4	5	MR
10	PM-28 x PM-12	HS x MR	0.1	2	5.4	13.7	21.5	5	MR
11	PM-34 x PM-18	HS x MR	0.3	1.4	4	15.6	23	5	MR
12	PM-10 x PM-35	MRx S	4.6	11.5	21.5	36.9	42.7	7	S
13	PM-20 x PM-35	S x S	0.7	1.9	4.5	15.7	30.6	7	S
14	PM-20 x PM-36	S x S	1	2.8	15.2	30.6	32.6	7	S
15	PM-20 x PM-38	S x S	5.4	13.8	23.5	35.1	43.2	7	S
16	PM-21 x PM-37	MR x S	1	3	4.1	27.2	35	7	S
17	PM-28 x PM-4	HS x S	8.3	20.5	28.9	36.5	49.2	7	S
18	PM-28 x PM-19	HS x MR	4.5	8.8	14.1	33.4	39.4	7	S
19	PM-31 x PM-19	S x MR	5.2	12.7	17.3	25.2	29.7	7	S
20	PM-36 x PM-10	S x MR	7.7	12.7	22.2	34.1	42.3	7	S
21	PM-36 x PM-20	S x S	1.5	7.1	14	24.5	39.8	7	S
22	PM-38 x PM-20	S x S	3.8	8	15.5	24	45.3	7	S
23	PM-40 x PM-19	HS x MR	3.6	9	23.1	37.6	45.6	7	S

chemical based protection measures are uneconomical specially in rainfed ecosystem. Plant resistance is the most common and efficient method of tackling the powdery mildew disease. Therefore, resistant breeding appears to be the most important approach in disease management. For developing high yielding hybrids along powdery mildew resistant in sunflower, knowledge on inheritance pattern of resistance to powdery mildew is essential (Seiler, 2008). However, the reliable source of resistance has been identified, still there is a urgent need to screen the genotypes against powdery mildew and identify potential hybrids with genes for resistance to sunflower powdery mildew.

Results on reaction of twenty three crosses derived from the resistant x susceptible and resistant x highly susceptible crosses are given Table 1 and 2. Out of the 23 crosses evaluated under natural conditions, only eight crosses were found to be resistant *viz.,* PM-14 x PM-36, PM-16 x PM-37, PM-16 x PM-38, PM-17 x PM-35, PM-17 x PM-36, PM-17 x PM-38, PM-22 x PM-36 and PM-34 x PM-23 with PDS less than 10 per cent. The same resistant hybrids in field screening exhibited resistant reaction in artificial epiphytotic conditions also (Table 2). The F_1 plants of eight crosses both under field and artificial conditions are resistant inferring that resistance was governed by dominant gene(s). Out of eight crosses, five crosses were selected based on F_2 seed availability to represent both resistant x susceptible and resistant x highly susceptible crosses in F_2 segregating population. Each F_2 family was sown in 20 rows of three meter length along with susceptible check Mordent at every 10th row. Individual plants from each F_2 population were screened against powdery mildew by leaf stapling method under field conditions.

The five F_2 populations derived from the crosses PM-22(R) x PM-36(S), PM-14(R) x PM-36(S), PM-16(R) x PM-38(S), PM-17(R) x PM-35(S) and HS x R genotype PM-34 x PM-23 showed resistant reaction under both the conditions (Table 3 and 4). The segregation ratio was independently estimated for all five F_2 populations of resistant and susceptible/highly susceptible cross combinations. In each F_2 population, total number of plants were grouped into two categories viz., resistant with less than 10 per cent PDS at 90 DAS and susceptible with more than 10 per cent PDS at 90 DAS by pooling moderately resistant and susceptible plants

Table 3. Reaction of sunflower F_2 population to powdery mildew disease under natural field condition during *rabi* 2014-2015.

S. N.	Cross	Parental reaction	Per cent Disease Severity at (%)					Scale	Host reaction
			30 DAS	45 DAS	60 DAS	75 DAS	90 DAS		
1	PM-14x PM-36	R x S	0.5	1.2	2.0	5.4	6.8	3	R
2	PM-16 x PM-38	R x S	0.2	0.5	1.5	4.8	7.1	3	R
3	PM-17 x PM-35	R x S	0.3	0.9	2.9	6.5	8.0	3	R
4	PM-22 x PM-36	R x S	0.1	0.3	0.9	3.6	7.1	3	R
5	PM-34 x PM-23	HS x R	0.0	0.4	1.5	4.7	5.7	3	R
6	PM-16 x PM-37	R x S	0.7	1.8	2.5	10.5	16.8	5	MR
7	PM-17 x PM-36	R x S	0.1	1.3	3.7	10.1	14.9	5	MR
8	PM-17 x PM-38	R x S	0.4	1.5	3.4	9.4	12.5	5	MR
9	PM-20 x PM-36	S x S	0.7	1.6	3.4	9.3	16.8	5	MR
10	PM-21 x PM-37	MR x S	0.2	1.3	5.3	13.0	21.3	5	MR
11	PM-28 x PM-12	HS xMR	1.5	3.4	8.0	16.6	22.8	5	MR
12	PM-28 x PM-19	HS xMR	0.2	1.2	3.8	11.3	18.7	5	MR
13	PM-34 x PM-18	HS xMR	0.0	0.8	3.7	12.0	16.5	5	MR
14	PM-36 x PM-10	S x MR	1.3	3.6	10.5	20.3	23.8	5	MR
15	PM-10 x PM-35	MRx S	3.7	6.8	15.6	28.5	37.5	7	S
16	PM-18 x PM-34	MR xHS	3.7	5.4	12.5	23.6	30.3	7	S
17	PM-20 x PM-35	S x S	2.3	5.7	14.6	22.5	35.2	7	S
18	PM-20 x PM-38	S x S	1.8	15.0	21.5	24.1	29.6	7	S
19	PM-28 x PM-4	HS x S	3.0	5.7	15.8	22.4	27.8	7	S
20	PM-31 x PM-19	S x MR	1.3	3.5	8.4	19.8	25.7	7	S
21	PM-36 x PM-20	S x S	1.9	3.9	11.7	19.5	25.3	7	S
22	PM-38 x PM-20	S x S	2.5	5.6	18.0	31.0	39.1	7	S
23	PM-40 x PM-19	HS x MR	3.1	5.5	14.0	23.0	30.6	7	S

Table 4. Reaction of sunflower F_2 population to powdery mildew disease under artificial condition during *rabi* 2014-2015.

S. N.	Cross	Parental reaction	Per cent Disease Severity at (%)					Scale	Host reaction
			30 DAS	45 DAS	60 DAS	75 DAS	90 DAS		
1	PM-14x PM-36	R x S	0.5	0.8	2.9	5.8	9.5	3	R
2	PM-16 x PM-38	R x S	0.8	1.3	3.8	6.2	9.1	3	R
3	PM-17 x PM-35	R x S	0.6	1.4	2.3	5.4	8.5	3	R
4	PM-22 x PM-36	R x S	0.1	1.2	2.4	5.7	8.6	3	R
5	PM-34 x PM-23	HS x R	0.2	0.8	1.9	4.7	7.3	3	R
6	PM-16 x PM-37	R x S	0.6	1.6	3	10.1	20.4	5	MR
7	PM-17 x PM-36	R x S	0.8	2.6	4.8	14.8	18.7	5	MR
8	PM-17 x PM-38	R x S	2.6	5.6	7.9	13.9	21	5	MR
9	PM-28 x PM-19	HS xMR	0.9	3.5	7.5	14.6	20.4	5	MR
10	PM-34 x PM-18	HS xMR	2.2	6.1	10.9	15.9	23.8	5	MR
11	PM-10 x PM-35	MRx S	5.2	13.7	19.8	29.5	35.3	7	S
12	PM-18 x PM-34	MR xHS	4.9	15.2	25.5	34.5	39.6	7	S
13	PM-20 x PM-35	S x S	5	13.2	21	32.5	47	7	S
14	PM-20 x PM-36	S x S	2	8.6	12	20.2	28	7	S
15	PM-20 x PM-38	S x S	1.2	5.1	16	27.6	33.9	7	S
16	PM-21 x PM-37	MR x S	5	11	19.1	27.1	33.1	7	S
17	PM-28 x PM-4	HS x S	2.7	7.3	12.7	23.6	30.9	7	S
18	PM-28 x PM-12	HS xMR	5	9.7	16.7	26.5	33.2	7	S
19	PM-31 x PM-19	S x MR	3.6	8.1	18.5	27.9	34.5	7	S
20	PM-36 x PM-10	S x MR	2.3	8.5	15.7	27.1	33.1	7	S
21	PM-36 x PM-20	S x S	1.4	12.8	28.1	38.1	46.9	7	S
22	PM-38 x PM-20	S x S	2.6	9	20.4	30.5	36.9	7	S
23	PM-40 x PM-19	HS xMR	4	20	38	51.1	62.6	9	HS

Table 5. Chi- square analysis of segregation ratio of five F_2 population against sunflower powdery mildew.

Cross	F_1 reaction	Screening conditions	Total plants screened	Observed frequency: Resistant plants (<10% infection)	Observed frequency: Susceptible plants (>10% infection)	Ratio	Calculated Chi-square value	Table Chi-square value	Significance level
PM-22× PM-36 (R X S)	R	Natural	204	112	92	9:7	0.14	3.841	NS (P=0.05)
PM-14×PM-36 (R X S)	R	Natural	197	118	79	9:7	1.05	3.841	NS (P=0.05)
PM-16 ×PM-38 (R X S)	R	Natural	217	132	85	9:7	1.83	3.841	NS (P=0.05)
PM-17×PM-35 (R X S)	R	Natural	192	102	90	9:7	0.75	3.841	NS (P=0.05)
PM-34× PM-23 (HS X R)	R	Natural	211	128	83	9:7	1.66	3.841	NS (P=0.05)

Table 6. Chi- square analysis of segregation ratio across five F_2 population against sunflower powdery mildew.

Crosses	F_1's Reaction	Screening conditions	Total plants screened	Observed frequency: Resistant plants (<10% infection)	Observed frequency: Susceptible Plants (>10% infection)	Ratio	Calculated Chi-square value	Table Chi-square value	Significance level
Across five F_2 population	R	Natural	1021	592	429	9:7	1.23	3.841	NS (P=0.05)
		Artificial	60	41	19		3.55		
		pooled	1081	633	448		2.33		

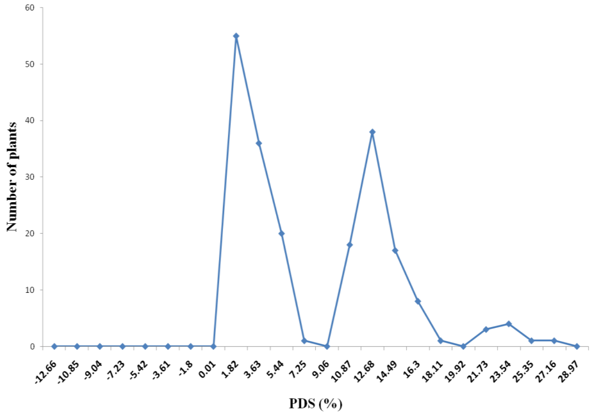

Fig. 1. *Bimodal distribution of F_2 plants of the cross PM-22xPM-36 for Powdery mildew disease severity .*

in susceptible class for segregation pattern analysis as followed by Rojas-Barros *et al.* (2005) to study the inheritance pattern in sunflower.

The chi-square analysis showed no significant difference between observed and expected ratio for 9 Resistant:7 Susceptible (Table 5 and 6).

Similarly, plants from artificial conditions were grouped into two classes *i.e.* resistant (<10 % PDS) and susceptible (>10 % PDS) and results of chi-square analysis revealed similar segregation pattern of 9 Resistant :7 Susceptible (Table 6). The crosswise pooled chi-square analysis of resistant (<10 % PDS) and susceptible (>10% PDS) plants also recorded 9 R: 7 S segregation ratio, indicating resistance to powdery mildew was governed by two genes with complementary gene action (Table 6). Rojas-Barros *et al.* (2004) reported two genes governing the powdery mildew resistance in sunflower based on segregation studies from *H. argophyllus* x HA 89 and *H. debilis* x HA 89 crosses and suggested that introgression of powdery mildew resistance easier using *H. argophyllus* and *H. debilis* ssp. *debilis*. Similar findings were also made by Ravindran and Devarathinam (1996); Raja Ravindran and Rathinam (1996); Ramana Rao *et al.* (2009); Rao *et al.*, 2012, Sravani *et al.* (2012) for powdery mildew resistance in sesame and Trobanco *et al.(*2012) for pea powdery mildew resistance.

Bimodal distribution is a type of continuous distribution with two distinct peaks. Similarly, segregation pattern of five F_2 populations when analysed for bimodal distribution showed two peaks indicating involvement of oligogenes controlling disease resistance for powdery mildew in sunflower (Fig.1-5). The segregation ratio was independently estimated for all five F_2 populations of resistant and susceptible/ highly susceptible cross combinations. Out of five F_2 population, total number of plants from each cross combinations viz., PM-22 × PM-36(RxS), PM-14 × PM-36 (RxS), PM-16 × PM-38 (RxS), PM-17 × PM-35 (RxS) and PM-34 × PM-23 (HS x R) under field condition were grouped into two categories viz., resistant (<10 % PDS) and susceptible (>10 % PDS) at 90 DAS for segregation pattern analysis. The segregation ratio of 9R:7S was analysed for all the five F_2 populations, did not show significant difference between observed ratio

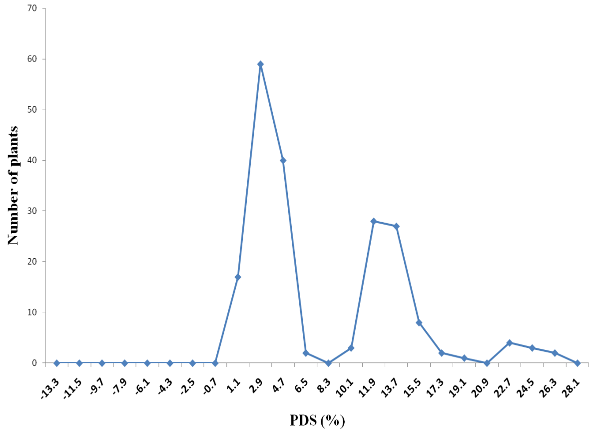

Fig. 2. *Bimodal distribution of F_2 plants of the cross PM-14xPM-36 for Powdery mildew disease severity .*

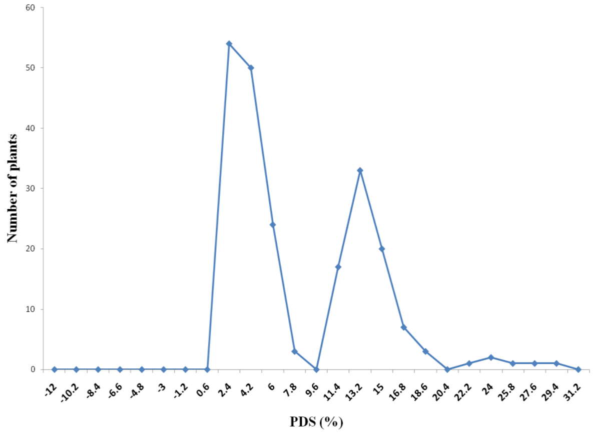

Fig. 3. *Bimodal distribution of F_2 plants of the cross PM-16xPM-38 for Powdery mildew disease severity.*

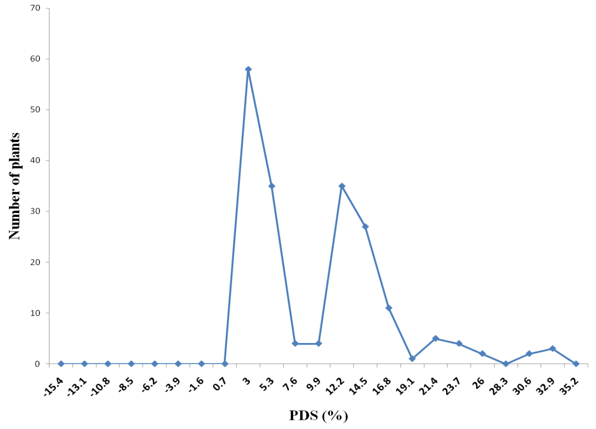

Fig. 4. *Bimodal distribution of F_2 plants of the cross PM-17xPM-35 for Powdery mildew disease severity.*

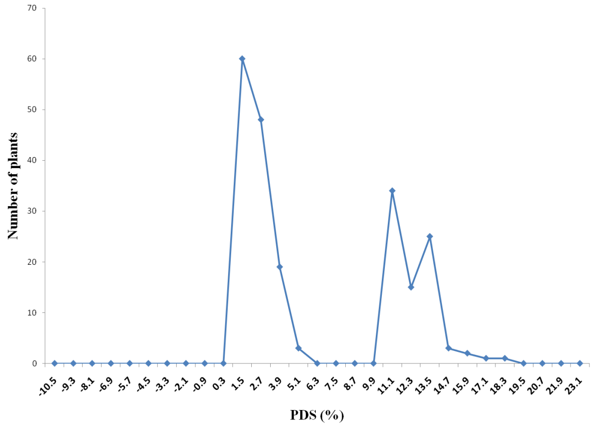

Fig. 5. *Bimodal distribution of F_2 plants of the cross PM-34xPM-23 for Powdery mildew disease severity.*

and expected ratio (9 R: 7 S). This revealed that resistance to powdery mildew in F_2 population of sunflower was governed by two dominant genes showing complementary gene action. As per the available literature this appears to be the first report of digenic genes controlling resistance to powdery mildew in cultivated sunflower.

Conclusion

The results of this study showed that the reactions of sunflower genotypes against powdery mildew both under field and artificial conditions can be classified into 0-9 scale. (i) It provides a clear evidence about digenic inheritance of powdery mildew resistance involving complementary gene interaction, and (ii) PM-22(R) x PM-36(S), PM-14(R)xPM-36(S), PM-16(R) xPM-38(S), PM-17(R)xPM-35(S) and HS x R genotype PM-34x PM-23 are identified source of resistance against the powdery mildew.This information can be relevant for plant pathologist or the implementation of breeding programs focused on development of new cultivar carrying genetic resistance to powdery mildew.

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