

## Inheritance of powdery mildew resistance and growth habit in fenugreek (*Trigonella foenum-graecum* L.)

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### Abstract

Inheritance of resistance to powdery mildew was studied by evaluating parents,  $F_1$  and  $F_2$  generations of cross UM-305  $\times$  UM-117 along with susceptible check varieties over the years for powdery mildew reaction. The  $F_3$  and  $F_4$  families of the cross were also evaluated along with the  $F_1$  parents and the check varieties for powdery mildew reaction to confirm the results.  $\chi^2$  test was applied to test the fitness of assumed ratio. The inheritance pattern suggested resistance to powdery mildew was monogenic recessive. Results indicated that gene for powdery mildew resistance and gene for determinate growth habit assort independent of each other.

**Key words:** disease resistance, growth habit, inheritance, powdery mildew, *Trigonella foenum-graecum*

### Introduction

Powdery mildew of fenugreek (*Trigonella foenum-graecum* L.) is caused by *Erysiphe polygoni* DC. This disease results in heavy loss to the crop. The existing high yielding varieties are not having the built-in resistance and thus there is a need to breed a resistant variety. Knowledge of genetic control of resistance is a prerequisite in any resistance breeding programme. In fenugreek, so far there has been only a single report about the inheritance of powdery mildew resistance (Raje *et al.* 2002). However, there are few reports of screening fenugreek germplasm lines for resistance to powdery mildew (Saxena *et al.* 1984; Mehta *et al.* 1994). Thus, in the present investigation an attempt has been made to determine the inheritance pattern of resistance to powdery mildew. In fenugreek, Raje *et al.* (2001) reported that a marker trait i.e. determinate growth habit

is under the control of a single recessive gene and indeterminate growth habit under its dominant allele. Thus, it was thought important to verify the linkage between the gene for this marker trait and the gene for resistance to powdery mildew in order to know about the possibility of indirect selection for powdery mildew resistance. Therefore, in the present investigation an attempt has been made to determine the inheritance of powdery mildew resistance and to test the possibility of linkage between the gene for powdery mildew resistance and the gene for growth habit.

### Materials and methods

The experimental material was obtained from A.I.C.R.P. on Spices, Department of Plant Breeding and Genetics, which consisted of two parents (UM-305 and UM-117),  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  generations of the cross (UM-305  $\times$  UM-117) and the check varieties (RMt-1 and local check).

The parent UM-305 is having determinate growth habit and is resistant to powdery mildew (Anonymous 2000). The other parent UM-117 is having indeterminate growth habit and is susceptible to powdery mildew (Anonymous 1995; 1996; 1997; 1998). Local check and RMt-1, which are susceptible to powdery mildew (Anonymous 1995; 1996; 1997; 1998) were grown as check varieties. The year-wise details of the experimental materials have been presented in Table 1. The experimental material was tested against powdery mildew during the *rabi* seasons in the years 1997-98, 1998-99, 1999-2000 and 2000-2001 under natural conditions. The susceptible check varieties also served as spreader rows and as a source of secondary infection.

The individual plants of parents, check varieties,  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  generations were evaluated for their reaction to powdery mildew and classified as either powdery mildew susceptible or powdery mildew resistant plants (based on 100% disease development). The plants in the resistant and susceptible categories were tagged individually for counting and were harvested individually for further advancement.

The cross UM-305 x UM-117 was attempted during *rabi* 1996-97.  $F_1$  seeds of the cross produced each year during *rabi* 1997-98, 1998-99 and 1999-2000 were used in subsequent experiments.

*Experiment I (rabi, 1997-98):* In this experiment  $F_1$ , parents and the check varieties (RMt-1 & local check) were grown in 2 m long single row plots and disease reaction to powdery mildew of individual plants was noted when the disease appeared in full expression (100%) on susceptible checks. The growth habit in  $F_1$  generation was also noted.

*Experiment II (rabi, 1998-99):* The parents,  $F_1$ ,  $F_2$  of the cross were grown with the check varieties in RBD with two replications in single row plots of 4 m length with row to row distance of 30 cm and plant to plant distance of 10 cm. All the individual plants were classified as resistant and susceptible following the procedure mentioned earlier. Each plant in  $F_2$  generation was also classified as having determinate or

indeterminate growth habit. Chi-square test was applied to test the goodness of fit for assumed segregation ratio.  $F_2$  plants were harvested separately to collect the seed of  $F_3$  families.

*Experiment III (rabi, 1999-2000):* Thirty  $F_3$  families of each cross were evaluated during this season. For this, only a part of the total seed of each  $F_3$  family was used for evaluation. The remaining seed of these  $F_3$  families was kept for evaluation during *rabi* 2000-2001 (experiment IV). Thus, in experiment III, 30  $F_3$  families of cross, parents,  $F_1$  and  $F_2$  of the cross and the check varieties were evaluated in RBD with three replications in single row plot of 3 m length. Since powdery mildew incidence was not observed during this year, only observation on growth habit was noted.

As the total number of  $F_2$  plants was less in the experiment II and also due to absence of powdery mildew incidence in the experiment III, it was thought that  $F_3$  and  $F_4$  families of the cross should be further evaluated in *rabi* 2000-2001 to confirm the results. Thus, 11  $F_3$  families were selected out of 30  $F_3$  families of the cross (UM-305 x UM-117) which were evaluated during experiment III. These 11  $F_3$  families were selected on the basis of reaction to powdery mildew of their progenitor  $F_2$  plants in experiment II. Out of them, the seven selected  $F_3$  families were progeny of seven susceptible  $F_2$  plants while the other four selected  $F_3$  families were progeny of four resistant  $F_2$  plants harvested individually during *rabi*, 1998-99. The remaining seed of these  $F_3$  families (kept in 1998-99) was used in the experiment IV during *rabi*, 2000-2001.  $F_4$  families were derived from the  $F_3$  families evaluated during the experiment III. From each of the eleven  $F_3$  families (grown during *rabi*, 1999-2000) 15 plants were randomly selected and harvested separately to get 15  $F_4$  families. One  $F_4$  family out of the 15  $F_4$  families derived from each of the  $F_3$  family was chosen for further evaluation. Thus, 11  $F_4$  families were chosen for further evaluation during *rabi*, 2000-2001.

*Experiment IV (rabi, 2000-2001):* In this experiment parents (UM-305 x UM-117),  $F_1$ , 11  $F_3$  fami-

**Table 1.** Experimental details

Experiment	Season and year	Sowing date	Experimental material
I	Rabi, 1997-98	16.11.1997	Parents (UM-305 and UM-117), F <sub>1</sub> (UM-305 x UM-117) and check varieties (RMt-1 and Local check )
II	Rabi, 1998-99	15.11.1998	Parents (UM-305 and UM-117 ), F <sub>1</sub> and F <sub>2</sub> of cross (UM-305 x UM-117), check varieties (RMt-1 and Local check )
III	Rabi, 1999-2000	23.11.1999	Parents (UM-305 and UM-117), F <sub>1</sub> and F <sub>2</sub> of the cross (UM-305 x UM-117), 30 F <sub>3</sub> families of the cross (UM-305 x UM-117) and the check varieties (RMt-1 and Local check)
IV	Rabi, 2000-2001	25.12.2000	Parents (UM-305 and UM-117), F <sub>1</sub> (UM-305 x UM-117), 11 F <sub>3</sub> families (remnant seeds of 1998-99) (7 F <sub>3</sub> families derived from 7 susceptible F <sub>2</sub> plants and 4 F <sub>3</sub> families derived from 4 resistant F <sub>2</sub> plants of 1998-99, respectively), 11 F <sub>4</sub> progeny families (derived from 11 F <sub>3</sub> families evaluated in 1999-2000) and check varieties (RMt-1 and Local check)

lies and their 11 F<sub>4</sub> progeny families and check varieties were evaluated in RBD with two replications in single row plot of 2 m length. The row to row distance was 30 cm. This experiment was sown one month late in order to ensure the incidence of powdery mildew. Observation on reaction to powdery mildew of individual plant was recorded. Growth habit of individual plant was also recorded.

In all the four experiments recommended package of practices was followed but no disease control measure was followed.

### Results and discussion

Disease reaction of the parents, check varieties, F<sub>1</sub>s and F<sub>2</sub>s have been presented in Table 2 and Table 3. In the experiment I the plants of UM-305 showed resistant reaction whereas plants of UM-117 showed susceptible reaction. All the F<sub>1</sub>s showed susceptible reaction. There was heavy incidence of powdery mildew as

evident from the occurrence of the disease in check varieties. Thus, in experiment I reaction of parents and F<sub>1</sub>s indicated that susceptibility was a dominant character and resistance was the recessive character. Similar findings for powdery mildew resistance have been reported in pea (Narsinghani 1979; Singh *et al.* 1983; Janila *et al.* 2001).

In experiment II the plants of parents, F<sub>1</sub>s and F<sub>2</sub>s were evaluated along with check varieties for powdery mildew reaction (Tables 2 & 3). Again, the parent UM-305 showed resistance whereas, UM-117 showed susceptible reaction. The F<sub>1</sub>s showed susceptible reaction, which again indicated that resistance was recessive and susceptibility was dominant. F<sub>2</sub> plants showed segregation for susceptible and resistance reaction (Table 3). In F<sub>2</sub> generation, there were in total 41 susceptible and 12 resistant plants. This observed ratio of 41 susceptible : 12 resistant plants showed good fit to a 3:1

**Table 2.** Reaction of parental strains, F<sub>1</sub> and checks to powdery mildew

Genotype	Experiment-I (Rabi, 1997-98)	Experiment-II (Rabi, 1998-99)	Experiment-III (Rabi, 1999-2000)	Experiment-IV (Rabi, 2000-2001)
Parents				
UM-305	R	R	Disease did not occur	R
UM-117	S	S	Disease did not occur	S
Checks				
RMt-1	S	S	Disease did not occur	S
Local check	S	S	Disease did not occur	S
F <sub>1</sub>				
UM-305x UM-117	S	S	Disease did not occur	S

R : Resistant; S : Susceptible

**Table 3.** F<sub>2</sub> segregation ratio for reaction to powdery mildew and growth habit in fenugreek (experiment II, Rabi, 1998-99)

Cross	Segregation ratio for reaction to powdery mildew	X <sup>2</sup> (3:1)	Segregation ratio for growth habit	X <sup>2</sup> (3:1)	Segregation ratio for reaction to powdery mildew & growth habit				X <sup>2</sup> (9:3:3:1)
					Susceptible & indeterminate	Susceptible & determinate	Resistant & indeterminate	Resistant & determinate	
UM-305 × UM-117	41 S : 12R	0.0565	39 Indeterminate: 14 Determinate	0.0063	29	12	10	2	0.4675

R : Resistant; S : Susceptible

monohybrid ratio. The calculated value of X<sup>2</sup> was non-significant which indicated that the data fit to a monohybrid ratio of 3 susceptible : 1 resistant. Thus, on the basis of resistant reaction of the parent UM-305, susceptible reaction of the parent UM-117, susceptible reaction of F<sub>1</sub>s and a perfect fit to a 3 susceptible : 1 resistant ratio in F<sub>2</sub> (Tables 2 & 3) it can be concluded that resistance to powdery mildew in UM-305 is governed by a single recessive gene and the susceptibility in UM-117 by its dominant allele. The gene symbols *pmr* and *Pmr* are assigned for resistance and susceptibility to powdery mildew, respectively. Similar findings of monogenic control of powdery mildew resistance have been reported in peas (Narsinghani 1979; Sokhi *et al.* 1979; Singh *et al.* 1983; Janila *et al.* 2001).

In the experiment III there was no powdery mildew incidence hence, the investigation was carried out in the experiment IV.

In the experiment IV there was heavy incidence of powdery mildew as evident from 100% disease development in the both the checks. This may be due to delayed sowing by one month. In Table 4, reaction of eleven F<sub>3</sub> families and their respective F<sub>4</sub> progeny families during *rabi*, 2000-2001 has been shown along with the reaction of parental F<sub>2</sub> plants (during experiment II i.e., in *rabi*, 1998-99) from which these F<sub>3</sub> and F<sub>4</sub> families were derived. Out of the total F<sub>2</sub> parental plants used for getting F<sub>3</sub> and F<sub>4</sub> families, seven F<sub>2</sub> plants had susceptible and four F<sub>2</sub> plants had resistant reaction in 1998-99 experiment (experiment II). Thus, if the resistant reaction is governed by a single recessive gene and susceptibility by its dominant allele as indicated by a perfect fit of F<sub>2</sub> data (in 1998-

99 experiment) to a 3 susceptible : 1 resistant plants, the genotypes of these F<sub>2</sub> plants could be derived on the basis of segregation ratios in F<sub>3</sub> and F<sub>4</sub> families. Therefore, plant numbers 1-7 could have either SS (homozygous dominant for susceptible reaction) or Ss (heterozygous for susceptible reaction) genotype while the resistant F<sub>2</sub> plants must have ss (i.e. recessive homozygous) genotype (Table 4). To confirm these results the disease reaction of F<sub>3</sub> progeny families, F<sub>4</sub> progeny families along with those of parents, F<sub>1</sub>s and check varieties were recorded in experiment IV (Table 4). The parent UM-305 showed resistance whereas, other parent UM-117 showed susceptible reaction. All the F<sub>3</sub>s showed susceptible reaction, which again confirmed that susceptibility was dominant and resistance was recessive. The susceptible check varieties (Rmt-1 and local check) showed susceptible reaction. Reaction of the two check varieties and the parental genotypes over the years (1997-98, 1998-99 and 2000-2001) indicated that race flora was same over the years. The reaction types of F<sub>3</sub> families and their respective F<sub>4</sub> families derived from different susceptible and resistant F<sub>2</sub> plants were analyzed with respect to reaction type of the F<sub>2</sub> plants in 1998-99. In the F<sub>3</sub> progeny of the F<sub>2</sub> plant No. 1 all the plants showed susceptible reaction, which indicated that the genotype of its progenitor F<sub>2</sub> plant was SS. This was again confirmed by the presence of all susceptible plants and by the absence of any resistant plant in the F<sub>4</sub> progeny. Thus plant No. 1 had genotype SS.

In the F<sub>3</sub> progeny of F<sub>2</sub> plant No. 2 there were 12 resistant and 43 susceptible plants while in F<sub>4</sub> progeny there were 11 resistant and 39 susceptible plants. The segregation pattern in F<sub>3</sub> & F<sub>4</sub> progenies was a perfect fit to a 3 susceptible

Table 4. Reaction of F<sub>2</sub> genotypes of cross UM-305 x UM-117 to powdery mildew and segregation ratios in their F<sub>3</sub> and F<sub>4</sub> progenies in fenugreek

F <sub>2</sub> genotype	Reaction of F <sub>2</sub> genotypes (1998-99) used to get F <sub>3</sub> families		Predicted genotypes of F <sub>3</sub> segregation (2000-2001)			F <sub>3</sub> plants used to get F <sub>4</sub> families			Predicted genotypes of F <sub>4</sub> segregation (2000-2001)					
	S	S	R	Number of plants		R	S	S	Number of plants		R	S	S	X <sup>2</sup>
				S	S				S	R				
1	S	S	0	49	-	-	SS	0	48	-	-	-	-	-
2	S	S	12	43	3:1	0.1514	Ss	11	39	3:1	0.1066	-	-	-
3	S	S	16	54	3:1	0.0761	Ss	15	56	3:1	0.3802	-	-	-
4	S	S	15	38	3:1	0.1572	Ss	13	46	3:1	0.1412	-	-	-
5	S	S	10	39	3:1	0.3333	Ss	0	44	-	-	-	-	-
6	S	S	9	37	3:1	0.4637	Ss	0	53	-	-	-	-	-
7	S	S	11	41	3:1	0.2306	Ss	56	0	-	-	-	-	-
8	R	R	54	0	-	-	ss	48	0	-	-	-	-	-
9	R	R	52	0	-	-	ss	49	0	-	-	-	-	-
10	R	R	55	0	-	-	ss	56	0	-	-	-	-	-
11	R	R	54	0	-	-	ss	69	0	-	-	-	-	-

R : Resistant; S : Susceptible

: 1 resistant ratio. This indicated that the F<sub>2</sub> plant must have been heterozygous i.e., Ss and also that the F<sub>3</sub> plant in 1999-2000 from which F<sub>4</sub> progeny family was derived was also heterozygous i.e. Ss. Similar results were obtained in the F<sub>3</sub> progeny family and F<sub>4</sub> progeny family of the F<sub>2</sub> plant No. 3 and 4, and the segregation ratios were perfect fit to 3 susceptible : 1 resistant ratio. Thus, the F<sub>2</sub> plant No. 2, 3 and 4 were having genotype Ss and the F<sub>3</sub> plants from which the F<sub>4</sub> progeny families were derived had the same heterozygous genotype i.e. Ss. Thus, on the basis of reaction of parents, F<sub>1</sub> and segregation ratios in F<sub>3</sub> and F<sub>4</sub> it can be concluded that the resistance is governed by a single recessive gene in UM-305 while susceptibility is governed by its dominant allele in UM-117.

The F<sub>2</sub> plant No. 5 and 6 (Table 2) had susceptible reaction in 1998-99. The F<sub>3</sub> progeny family of these two plants showed segregation for susceptible and resistant plants. The F<sub>3</sub> progeny family of F<sub>2</sub> plant No. 5 segregated into 39 susceptible : 10 resistant plants while the F<sub>3</sub> progeny family of plant No. 6 also segregated into 37 susceptible : 9 resistant plants. These ratios were tested by Chi-square test (Table 4), which indicated that these ratios were perfect fit to the 3 susceptible : 1 resistant ratio. This again confirmed that resistance is governed by a single recessive gene and susceptible reaction by its dominant allele. In the F<sub>4</sub> progeny families of these two F<sub>3</sub> families (derived from F<sub>2</sub> plants no 5 and 6) (Table 4) all the plants were susceptible which indicated that the F<sub>3</sub> plants used to derive F<sub>4</sub> progeny families were homozygous dominant (SS).

Another F<sub>2</sub> plant No. 7 had susceptible reaction in 1998-99. The F<sub>3</sub> progeny family derived from this plant showed segregation for susceptible and resistant plants. The ratio of susceptible to resistant plants in this F<sub>3</sub> progeny was 41:11 which was good fit to 3:1 ratio with X<sup>2</sup> value of 0.2306, which was non-significant (Table 4). This again indicated that resistance was governed by a single recessive gene and susceptibility by its dominant allele. The segregation in this F<sub>3</sub> family indicated that the parental F<sub>2</sub> plant of this progeny i.e. F<sub>2</sub> plant No. 7 was heterozygous and had the genotype Ss.

Interestingly, the  $F_4$  family derived from the  $F_2$  plant No.7 had only resistant plants and there was not a single susceptible plant. This indicated that the  $F_4$  family was derived from the recessive homozygous (ss) plant of  $F_3$  progeny in the year 1999-2000. This again confirmed that the resistance is governed by single recessive gene.

The resistant  $F_2$  plants that were used to derive  $F_3$  and  $F_4$  progeny families were the  $F_2$  plant No. 8, 9, 10 and 11 (Table 4). All the  $F_3$  progeny families derived from these  $F_2$  plants did not show segregation for susceptibility and resistance. All the plants in these  $F_3$  families were resistant. This indicated that all the four parental  $F_2$  plants of these  $F_3$  families i.e.  $F_2$  plant No. 8, 9, 10 and 11 were recessive homozygous for the resistance gene. Moreover, the  $F_4$  families derived from the resistant  $F_2$  plant No. 8, 9, 10 and 11 had only resistant plants which indicated that these  $F_4$  families were derived from the recessive homozygous  $F_3$  plants of  $F_3$  generation of the year 1999-2000. The recessive homozygous nature of the four  $F_2$  plants and their  $F_3$  and  $F_4$  progeny plants alongwith the susceptible reaction type of the  $F_1$  (Table 2 and 4) again confirmed that the resistance is governed by single recessive gene.

On the basis of reaction type of parents,  $F_1$ s, the segregation ratios in  $F_2$  and segregation ratios in  $F_3$  and  $F_4$  families in the cross (UM-305 x UM-117) it can be concluded that resistance to powdery mildew in UM-305 is governed by a single recessive gene and the susceptible reaction type in UM-117 is governed by its dominant allele. Similar findings of monogenic recessive control of powdery mildew resistance were reported in garden peas (Narsinghani 1979; Sokhi *et al.* 1979; Singh *et al.* 1983; Janila *et al.* 2001). The high yielding resistant  $F_5$  lines identified in the experiment IV will be useful in further breeding programme.

In the present investigation inheritance pattern of growth habit was also considered along with the inheritance pattern of powdery mildew resistance in order to verify the possibility of linkage between the genes governing these two characters. In fenugreek, indeterminate growth

habit is under the control of single dominant gene and the determinate growth habit under its recessive allele (Raje *et al.* 2001). In experiment I and II all  $F_1$ s showed indeterminate growth habit, which indicated that indeterminate growth habit was dominant to determinate growth habit.

In the experiment II (*rabi*, 1998-99),  $F_2$  generation showed segregation ratio of 3 indeterminate : 1 determinate which was a good fit to 3:1 monohybrid ratio (Table 3). On the basis of dominance of indeterminate growth habit in  $F_1$  generation and a good fit to a 3 indeterminate : 1 determinate ratio in  $F_2$  generation of both the crosses it was again concluded that indeterminate growth habit was under the control of single dominant gene and determinate growth habit by its recessive allele as earlier reported by Raje *et al.* (2001) based on the same genetic material used in the experiment II of present investigation. The gene symbols *Dgh* and *dgh* are assigned for indeterminate and determinate growth habit, respectively. Thus, both resistance to powdery mildew and determinate growth habit were under the control of single recessive gene. It was thought to verify whether the genes for resistance to powdery mildew and determinate growth habit are linked. For this, in the  $F_2$  generation segregation for both the characters i.e. powdery mildew resistance and growth habit were considered simultaneously and the data was tested by Chi-square test for independent assortment. For this  $F_2$  plants were classified into four categories i.e. susceptible with indeterminate growth habit, susceptible with determinate growth habit, resistant with indeterminate growth habit and resistant with determinate growth habit. The  $F_2$  data has been presented in Table 3. Chi-square test was applied to test the goodness of fit to the classical dihybrid  $F_2$  phenotypic ratio of 9:3:3:1. The observed 9 susceptible indeterminate : 3 susceptible determinate : 3 resistant indeterminate : 1 resistant determinate ratio was fitting at a very high level to a 9:3:3:1 dihybrid classical ratio. Thus, it was concluded that the gene for powdery mildew resistance and gene for determinate growth habit assort independently of each

other and are not linked. Moreover, the  $F_3$  progeny families of the  $F_2$  plants having determinate growth habit bred true while the  $F_3$  families derived from  $F_2$  plants having indeterminate growth habit segregated for indeterminate and determinate growth habit or bred true. This again confirmed that the indeterminate growth habit was under the control of single dominant gene and determinate growth habit under its recessive allele. In the experiment IV (Table 4) out of 11  $F_3$  families, in three  $F_3$  families i.e. family No. 2, 6 and 8, all the plants had determinate growth habit. Moreover, all the three-progeny  $F_4$  families derived from these three  $F_3$  families were also having all the plants with determinate growth habit. This confirms that determinate growth habit was under the control of single recessive gene. On considering both the characters simultaneously it was observed that all the four categories of plants i.e. indeterminate susceptible, indeterminate resistant, determinate susceptible and determinate resistant were present in the  $F_3$  and  $F_4$  family plants. This confirmed again that these two characters assort independently of each other.

On the basis of present investigation it can be concluded that the resistance to powdery mildew in UM-305 is governed by single recessive gene and susceptible reaction to powdery mildew in UM-117 is governed by its dominant allele. It is also concluded that gene for resistance to powdery mildew and gene for determinate growth habit assort independent of each other and are not linked.

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