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Genetics of downy mildew resistance in indigenous cucumber germplasm

Vishwanath Bidaramali, T. L. Bhutia, A. K. Sureja, A. D. Munshi, Amrita Das[1], Boopalakrishnan, G., Gopalakrishnan, S.[2], T. K. Behera[3] and S. S. Dey*

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi-110012, Delhi, India.

ABSTRACT

Cucumber (*Cucumis sativus* L.) is a major Cucurbitaceous vegetable crop widely grown in tropical and subtropical regions worldwide. Downy mildew is the most dangerous disease in cucumbers worldwide, causing significant yield loss. The vast diversity of economically important traits, including severity to downy mildew infestation, is available in Indian-originated germplasm. In order to investigate the genetic inheritance of cucumber downy mildew disease resistance, one resistant genotype (DC-70) from India was crossed with a contrasting susceptible genotype (DC-773). The parents and their progenies were evaluated in field and net house conditions for downy mildew disease resistance. Field condition data were recorded 4 times from 30 days until the plants died. The lower leaf surface was inoculated 20-25 days after sowing at the seedling stage by spraying the inoculum. The proportion of infection was recorded 4 times from 8 days to 65 days after inoculation, and the per cent disease index and area under the disease progress curve (AUDPC) were calculated from these proportions. Based on the PDI and AUDPC, it was established that a major gene controlled resistance to downy mildew in the genotype DC-70. Additive-dominance model fits the segregation data, and the additive effect was significant. Finally, the downy mildew disease inheritance pattern indicated that it is controlled by a single recessive gene in DC-70, which can be successfully introgressed into desired elite genotypes to facilitate the downy mildew resistance breeding programme.

Keywords: *Cucumis sativus*, Downy mildew, Genetics, Inheritance, Resistance breeding.

INTRODUCTION

Cucumber is one of the most important vegetable crops cultivated throughout the world. It is a popular summer vegetable crop mostly for its edible immature fruits, which are used in salads, pickles, desserts, and as a cooked vegetable. Downy mildew (DM) caused by the obligate biotrophic oomycete, *Pseudoperonospora cubensis* is the most dangerous disease in cucumber causing significant yield loss and has been reported in over 80 countries around the world (Lebeda and Cohen, 9). The DM is a major problem that occurs during cool, moist weather resulting in a drastic reduction in leaf chlorophyll concentration because of multiple changes in the key proteins with a knock-on effect on photosynthesis and carbon fixation. Symptoms of cucumber DM occur first on the lower side of the leaves, with chlorotic (yellow) lesions not occurring in major leaf veins, within days of infection, chlorotic lesions develop necrotic and sporulation emerges on the lower surface of leaves. As the disease progress, infected leaves gradually collapse, leading to a loss in cucumber production due to a lack of photosynthesis in leaves (Liu *et al.,* 13). Infection with DM reduces cucumber yield and in severe cases results in crop loss of up to 50% to 70% due to this disease (Palti and Cohen, 11). Cucumber DM is currently controlled by the use of fungicides. However, ineffectiveness was caused by the frequent appearance of isolates that were insensitive to conventional chemicals. Besides, excessive fungicide application is harmful to the environment and human health (Palti and Cohen, 11). As a result, developing resistant cultivars, which is a major objective of the cucumber breeding program, is the most effective strategy for the management of this important disease.

Identification of genotype with effective resistance to this disease is the most important task before undertaking a disease-resistance breeding programme. India is endowed with huge diversity for several economically important traits in cucumber being the centre of origin. Therefore, it is important to collect and evaluate the indigenous cucumber lines and accessions to strengthen the on-going DM-resistance breeding programme throughout the world. Besides, it is essential to have a better understanding of the genetic mechanisms of DM resistance in the indigenous cucumber genotypes. Identification of DM resistant lines and understanding the nature of inheritance are pre-requisite for a successful breeding programme. For the past 70 years, scientists have been trying to figure out

*Corresponding author: shyam.iari@gmail.com
[1]Division of Plant Pathology, ICAR-IARI, New Delhi-110012, India
[2]Division of Genetics, ICAR-IARI, New Delhi-110012, India
[3]ICAR-IIVR, Varanasi- 221305, Uttar Pradesh, India

the nature of the inheritance of DM in cucumber (Criswell, 5). However, the inheritance pattern is so complex that there have been conflicting reports about whether it is monogenic or polygenic. A single recessive gene for resistance to DM in different genotypes have been reported (Van Vliet and Meysing, 15). Three recessive resistance genes were reported for the first time by Shimizu (14) and were later reported by a few other workers. As a result, many different methods of detecting and evaluating resistance may exhibit different inheritance patterns. The purpose of this study was to understand the nature of the inheritance of DM resistance identified in the genotype, DC-70. This information is important for designing a breeding program to develop downy mildew-resistant varieties.

MATERIALS AND METHODS

The research was carried out at the Division of Vegetable Science Research Farm, ICAR-Indian agricultural research institute, New Delhi. The genotype, DC-70 (P_1, Resistant) was crossed with downy mildew susceptible DC-773 (P_2, susceptible) to derive for F_1s (DC-773 × DC-70). These F_1s crossed to their parents P_1 (Resistant) and P_2 (susceptible) to get BC_1P_1 and BC_1P_2 generations. At the same time, the F_1s were selfed to develop F_2 seeds. The experimental materials of six generations (P_1, P_2 F_1, F_2, BC_1P_1, and BC_2P_2) derived from the cross were screened in both the field and net house conditions for resistance to downy mildew. DM disease screening under field conditions (Table 3) (*Kharif*) and the disease incidence was recorded using a 0-9 scale (Fig. 1) to determine the degree of disease until plant growth stopped, Scoring were done as per Jenkins and Wehner, (8). Artificial Screening for resistance to downy mildew in net house conditions (Table 3) was also conducted in September 2021. Using a 1 lit spryer, 20-25-day-old seedlings were inoculated with a sporangium (Fig. 3) suspension containing 10,000/ml sporangia on the abaxial leaf surfaces. After the 8th-day of spraying disease incidence was recorded by using a linear 0 to 4 scale (Fig. 2) suggested by Cohen *et al*., (4). The formula for calculating the percent disease index (PDI,) was used, PDI= (Sum of numerical values/ Number of leaves graded × Maximum ratings) × 100. The PDI was calculated using symptomatic leaf area data and the plants were characterised into four groups, resistant (0–20%), moderately resistant (21–40%), susceptible (41–60%) and highly susceptible (>60%) based on the calculated PDI. Estimated PDI thorough analysis to generation mean analysis, the means and standard error were calculated (Hayman and Mather, 6). The A, B, C, and D scaling tests were used to detect the presence or absence of epistasis (Hayman and Mather, 6). The insufficiency of a basic additive-dominance model is demonstrated by the significance of either one or two scaling tests. Mather and Jinks, (10) proposed a model to estimate gene effects, this provides information about the genetic components of variances (*m, d, h, i, j, l*) and epistasis and broad-sense heritability also calculated. The area under disease progress curve (AUDPC) was calculated using the following formula based on the percent disease index, AUDPC=∑ [{Xi+1+ Xi+1/ 2} *x*

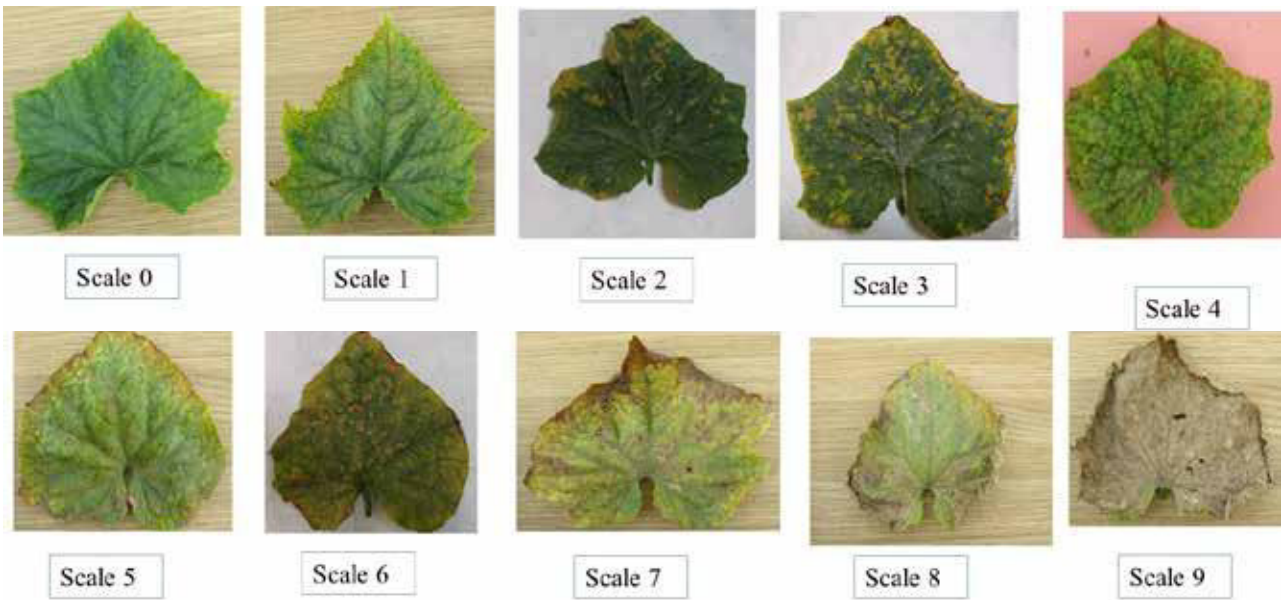

Fig. 1. The disease incidence will be scored in field condition by using a 0-9 scale

Fig. 2. The disease incidence will be scored in net house condition by using a 0-4 scale

{ti+1 − ti}] (Jeger and Viljanen-Rollinson, 7). Castle Wright Estimation is used to find out the minimum number of effective genes contributing to the disease resistance between two inbred lines, N= D^2 / 8(VF_2-VF_1) (Castle and Wright, 3). Chi-Square () Test**,** the observed segregation ratio for the downy mildew disease in cucumber expected for a single gene's goodness of fit was tested using the classical chi-square (χ2) test. chi-square (χ2) = (Observed number – Expected number)^2 / Expected number suggested by Panse and Sukhatme, (12).

RESULTS AND DISCUSSION

One of the genetic approaches for estimating various genetic effects is generation mean analysis. The means and standard errors of the parents, F_1, F_2, and backcross generations were calculated and presented in Table 1. In this study, the F_1 generation's mean was greater than the mid-parent value in net house conditions, but in field conditions, the F_1 generation's mean was lower than the mid-parent value due to environmental effects. The mean of the F_2 generation was higher than the BC_1P_1 generation in both screening studies and cross, but it was lower than the F_1 and BC_2P_2 generations in both screening experiments. This means F_2 and BC_1P_1 are towards the resistant parents and F_1 and BC_2P_2 are towards a susceptible parent (Shashikumar *et al.,* 13). In field conditions, the mean of BC_2P_2 generations was higher than the mean of F_1 generations, but in artificial conditions vice versa. In both screening experiments, individual F_2, BC_1P_1, and BC_2P_2 progeny were observed to be transgressive with extreme mean PDI values than either parent. This could be attributed to different parental means, for generation mean analysis, however, contrasting parents are required (Mather and Jinks, 10).

In this study, four scaling tests (A, B, C, and D) for scale effects were used, When the scale is adequate, the values of A, B, C, and D are non-significant, and the cross is referred to as a non-interacting gene and fit for the additive –dominance model, indicating the absence of epispastic. Out of six genetic parameters, only one parameter was significant in both screening experiments (Table 1). This cross expressed significant and negative additive (*d*) gene effects in both experiments, in the six-parameter model, this cross showed a non-significant except the main gene components contributed towards susceptibility rather than resistance with a high additive gene in field condition (-23.12*) and net house condition (-27.03*), However, a negative indication associated with gene effects suggested that the disease level in this cross might be lower than in the mid parent. Mid-parent heterosis in the field (40.24) experiment and in the net house (55.17) experiment (Table 1). According to Castle Wright's estimation, the number of effective gene-controlling resistance was 1.03-1.05 and broad-sense heritability (Table 1) from both field

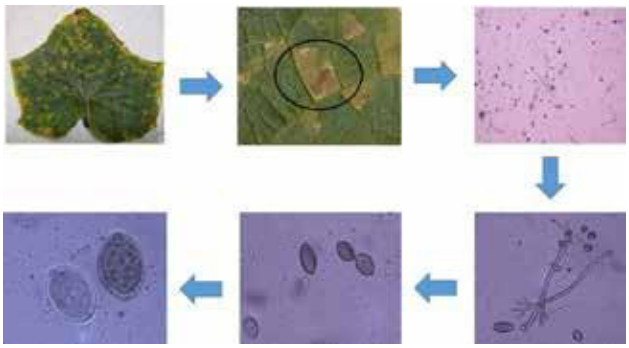

Fig. 3. Sporangia of *Pseudoperonospora cubensis* (Downy mildew in cucumber)

Table 1. Means, standard errors, Scaling Test and gene effects of six generations of cucumber cross screened for resistance to downy mildew in the field and net house condition.

Means and standard errors			Scaling Test			Gene Effects		
Generation	Field condition	Net house condition		Field condition	Net house condition		Field condition	Net house condition
	DC- 70	DC- 773		DC- 70	DC- 773		DC- 70	DC- 773
P1	17.08±1.72	7.96±0.73	A	14.30 NS	21.15 NS	[m]	31.50NS	30.01 NS
P2	63.33±1.16	62.03±1.94	B	3.05 NS	-9.14 NS	[*d*]	-23.12*	-27.03*
F1	56.38±2.41	54.31±2.50	C	8.65 NS	7.03 NS	[*h*]	50.95 NS	41.28 NS
F2	50.46±1.39	46.41±1.49	D	-4.35 NS	-2.49 NS	[*i*]	8.70 NS	4.98 NS
BC1P1	43.88±5.22	41.71±5.17				[*j*]	5.62 NS	15.15NS
BC2P2	61.38±1.74	53.59±2.00				[*l*]	-26.06 NS	-16.99 NS
						MPH	40.24	55.17
						h^2	0.743	

*, Significant at 0.05, i (Add * Add), j (Add * Dom), l (Dom * Dom), MPH mid-parent heterosis, h² (Broad Sense heritability), and NS, Non-significant.

and net house experiments is 0.74. The generation mean analysis implies that gene is distributed in a unidirectional manner between the two parents. Evidence of transgressive segregants in F_2 and BC_1P_1 generations in this cross would be due to the presence of at least one factor for resistance in the resistant parent. Epistatic interaction is absent in this cross so fit for additive–dominance model. In both screening studies, broad-sense heritability estimates were high, implying that donor parents could transfer resistance character to recipient parents to easily. In this cross, fixable gene effects (*d*) were higher than non-fixable (*h*) and (*l*) gene effects in two different screening trails, demonstrating that additive effects play a larger role in the inheritance of downy mildew resistance. A trait with a high heritability can increase the prevalence of a trait under selection (Mather and Jinks, (10).

The Area Under Disease Progress Curve (AUDPC shows how far the disease has progressed throughout a specific crop growth season (Table 2). The resistant parent DC-70 (P_1) showed less AUDPC values of 83.45 and 60.48 respectively, compared with populations (F_1, F_2, BC_1P_1, and BC_2P_2) under-screened for resistance in both field and net house conditions (Table 2: Fig. 4 and 5). The highest AUDPC value was recorded in the susceptible parent (P_2) (360.63) and (345.54) respectively. The disease progress curve depicts the large range in the population's (P_1, P_2 F_1, F_2, BC_1P_1, and BC_2P_2) average percent disease index screened under field conditions but in net house conditions, the disease progression curve shows less compared to the field condition (Fig. 5). Under high infection pressure, differences in field, and resistance were characterized by a delay at the beginning of infection and a slower rate of disease progress (Lebeda and Cohen, 9). In the resistant genotype, pathogen development is usually slower, which delays and limits pathogen colonization and disease symptoms.

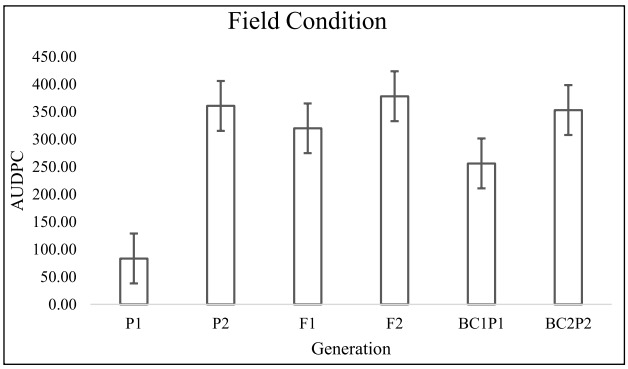

Fig. 4. Distribution of the area under the disease progress curve (AUDPC) for cross DC-70 × DC- 773 in field condition

Table 2. The area under the disease progress curve (AUDPC) for the cross of DC-70 × DC-773.

Cross		P_1	P_2	F_1	F_2	BC_1P_1	BC_2P_2
DC-70 × DC-773	Field condition	83.45	360.63	320.02	378.22	256.14	353.063
	Artificial condition	60.48	345.54	291.20	250.48	241.92	315.32

Table 3. Frequency distributions and segregations for leaf reaction to downy mildew in parents, F_1, F_2, BC_1P_1, and BC_2P_2 generations in both field and artificial conditions.

Field Conditions									
Generation	Total No. of Plants	No. of plants in disease reaction classes				Observed No. of plants		Ratio	X^2 0.05
		R	MR	S	HS	R	S		
DC-70(R)	20	20	-	-	-	20			
DC-773(S)	20	-	-	-	20	20			
DC-70 × DC-773 (F_1)	20	-	-	-	20		20		
DC-70 × DC-773 (F_2)	159	19	26	34	80	45	114	1:3	0.92^{NS}
BC_1 (F_1 × P_1)	50	11	13	10	16	24	26	1:1	0.08^{NS}
BC_2 (F_1 × P_2)	50	-	-	18	32	-	50	-	
Artificial Conditions									
Generation	Total No. of Plants	No. of plants in disease reaction classes				Observed No. of plants		Ratio	X^2 0.05
		R	MR	S	HS	R	S		
DC-70(R)	20	20				20	-		
DC-773(S)	20				20	-	20		
DC- 30 × DC-773 (F_1)	20				20		20		
DC- 30 × DC-773 (F_2)	145	16	21	41	67	37	108	1:3	0.02^{NS}
BC_1 (F_1 × P_1)	50	10	12	13	15	22	28		0.72^{NS}
BC_2 (F_1 × P_2)	50	-	-	23	27	-	50		

* R, resistant, MR, Moderately resistant, S susceptible, HS, highly susceptible and NS, non-significant

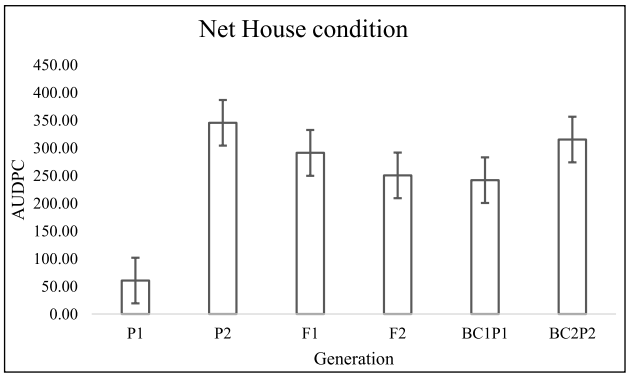

Fig. 5. Distribution of the area under the disease progress curve (AUDPC) for cross DC-70 × DC- 773 in Net house condition.

Low temperatures in the leaf tissues can cause symptom development and colonization to be delayed, whereas, at higher temperatures, lesion formation and chlorosis will be more rapid, perhaps inhibiting pathogen growth. (Cohen *et al.,* 4).

Distribution of the Measured Percent Disease Index via. Box Plots, Box plots based on percent disease index values (S_1 and S_2) for the downy mildew of generations i.e., P_1, P_2, F_1, F_2, BC_1P_1, and BC_2P_2 are presented in Fig. 6. The plots depicted the mean PDI differences under two conditions (i.e., Field and Net house condition) and the performance of populations varied significantly except for F_1, F_2, and BC_1P_1 (Fig. 6). Overall, the PDI range was higher in Field conditions compared to Net house conditions. The generation P_1, P_2, F_1, F_2, and BC_2P_2 exhibited symmetric distribution while the population BC_1P_1 exhibited skewed distributions under Field conditions (Shashikumar *et al.,* 13). Under Net house conditions, all the PDI values decreased in comparison to Field conditions except BC_1P_1 exhibited symmetric distribution under Net house conditions also (Fig. 6). The PDI means the consistent performance of populations under field and net house conditions is due to the presence of downy mildew gene/QTLs in this cross.

The genetics of downy mildew resistance were tried to fit into a classical monogenic recessive of Mendelian ratio, by categorizing disease reactions into two groups: resistant and susceptible. The inheritance of resistance against downy mildew resistance is mainly based on field and net house tests, which is allow a clear difference between susceptible and resistant. The Resistant × susceptible

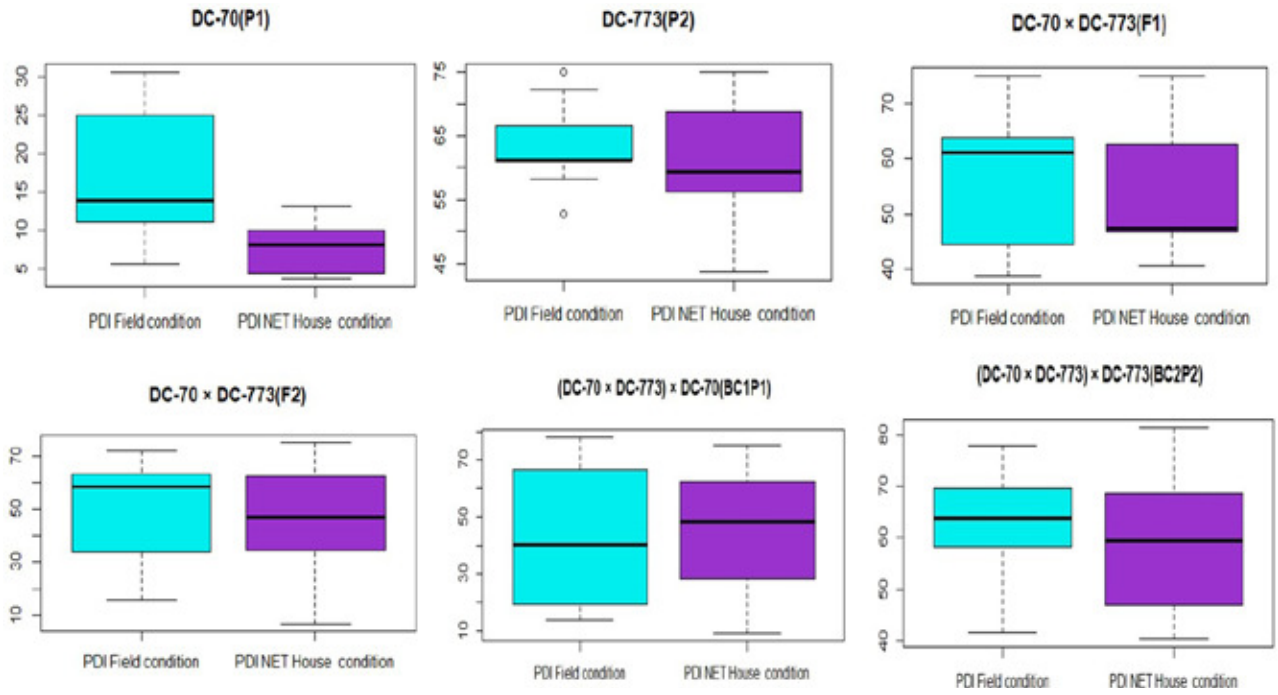

Fig. 6. Box plot of percent disease index for downy mildew disease both field and net house condition

parents were used to develop the F_2 and back cross generations. The F_1 plants had a susceptible reaction to downy mildew infection in both conditions (field and net house). The segregation of the F_2 populations to a ratio of 1 Resistant: 3 Susceptible. When backcrosses to the resistant parent were performed, the segregation ratio was 1 resistant: 1 susceptible, indicating that the monogenic recessive model was best suited with a high probability with the resistant parent. However, all plants in the backcrosses made to the susceptible parent were susceptible to downy mildew disease, For the backcross with the susceptible parent, there is no fit for either 3:1 or 1:1. As a result, revealed that cucumber downy mildew resistance is governed by a single recessive gene. For the goodness of fit to Mendelian ratios, the data presented for these crosses were subjected to a chi-square test (Table 3). Some earlier studies provided evidence for the single recessive gene control of resistance (Van Vliet and Meysing, 15: Bhutia, 2), but some scientists are reported that three recessive genes (Shimizu *et al.,* 14). Susceptibility to downy mildew disease was completely dominant over resistance while resistance was a recessive character in this cross (Criswell *et al.,* 5). The resistant parent's gene was given the name RRss, whereas the susceptible parent's gene was given the name rrSS. The dominant gene "S," which expresses susceptibility, inhibited the function of the dominant gene "R," which expresses resistance, however recessive gene "r" is responsible for resistance genes expression (Badra and Mohamed, 1).

AUTHORS' CONTRIBUTION

Conducting experiment, draft preparation (VB, TLB); Supervision (ADM, AKS): Supervision, Editing (AD): Disease inoculation, Scoring (BG): Analysis (GG): Planning, Analysis (TKB, SSD): Conceptualization, Supervision, Editing, Draft preparation (SSD)

DECLARATION

The Authors have no conflict of interest

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