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RESEARCH ARTICLE

Inheritance of Resistance to Downy Mildew [*Pseudoperono sporacubensis* (Berk. and Curt.) Rostovzev.] in Ridge Gourd [*Luffa acutangula* (Roxb.) L.]

B Varalakshmi*, M Pitchaimuthu, E Sreenivasa Rao and P Chowdappa[1]

**Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India*
[1]Retired Director, ICAR-CPCRI, Kasaragod-671124, Kerala, India

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Ridge gourd [*Luffa acutangula* (Roxb.) L.] is one of the important cucurbit vegetable cultivated widely in India and other Asian countries. Downy mildew caused by *Pseudoperono sporacubensis* (Berk. and Curt.) Rostovzev severely affects the ridge gourd production during rainy season. Therefore, in the current study 12 genotypes including some promising inbred lines were screened for downy mildew resistance which resulted in the selection of three moderately resistant advanced selections *viz*., IIHR-17-2-1 (PDI -16.25), IIHR-7-5-1 (PDI -21.40) and IIHR-17-1-7-3 (PDI -21.60) with low AUDPC values (467.59-634.26).Genetics of downy mildew resistance in two different F_2 populations derived from IIHR-52-1-30 × IIHR-17-1-7-3 and IIHR-23-8-10 × IIHR-7-5-1 respectively revealed that two major genes are interactive in dominant suppression epistasis and complementary epistasis. These results will aid ridge gourd breeder to develop a strategic downy mildew disease resistant breeding program.

Key Words: Downy mildew screening, Inheritance, Resistance, Ridge gourd

Introduction

Ridge gourd [*Luffa acutangula* (L.)Roxb.] is one of the important cucurbits grown as a cash crop in many tropical and sub-tropical regions of the India and abroad. It is enriched with vital elements such as vitamin C, zinc, iron, riboflavin, magnesium, thiamine and dietary fibre and therefore, contribute to human nutrition. It is traditionally described as a medicinal plant which used to cure a number of ailments. In India, it is commercially grown in Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Maharashtra, Assam and West Bengal during spring-summer and rainy season and provides a livelihood for resource-poor farmers.

However, the rainy season crop is found to be severely affected by downy mildew disease caused by *Pseudoperono sporacubensis* (Berk. and Curt.) Rostovzev. It is one of the important foliar disease, causing significant yield losses in region with high humidity and rainfall. The pathogen survives on live plants for reproduction and sporangia produced are dispersed through air, rain splash or physically through equipments (Lange *et al*., 1989). Survival of sporangia is highly depended on the prevailing environmental conditions (Thomas,1996). Since the identification of *P. cubensis* in Cuba by Berkeley and Curtis (1868), this pathogen has been reported from 70 countries worldwide infecting more than 20 genera of cucurbits (Lebeda and Urban, 2007). Downy mildew management relies on aggressive spray programme in conjunction with resistant varieties and cultural techniques to reduce the losses. However, efficacy of fungicides control has been diminished with increasing insensitivity of *Ps. cubensis* population towards it (Reuveni *et al*., 1980; Thomas and Jourdain, 1992; Heaney *et al*., 2000). Resistant varieties, an integral component of disease management programme, are economically viable and environmentally safe solution to manage this devastating disease. Host resistance has been identified in cucumber (Barnes and Epps, 1954; Wehner and Shetty, 1997; Shetty *et al*., 2002) and melon (Lebeda and Widrlechner, 2003; Taler *et al*., 2004). However, there is no report on downy mildew disease resistant sources and inheritance pattern in ridge gourd. Efforts are underway to identify ridge gourd resistance source(s) against downy mildew disease at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru since 2010. The present research work was taken up to confirm the downy mildew resistance

*Author for Correspondence: Email- Varalakshmi.B@icar.gov.in

in advanced inbred lines of ridge gourdand further studying its inheritance so that resistance loci could be incorporated into a commercial ridge gourd cultivar.

Materials and Methods

Natural Screening and Disease Assessment

Ridge gourd germplasm screening trials for downy mildew screening was initiated at ICAR-IIHR, Bengaluru since 2010. Resistant selections were advanced through selfing for 3-4 generations. Finally, four advanced promising inbred selections (IIHR-17-1-7-4, IIHR-7-5-1, IIHR-17-2-1, IIHR-17-1-7-3), two advanced breeding lines (IIHR-53-1-3, IIHR-6-1-1), two released varieties (Arka Sujat, Arka Sumeet), two popular hybrids (Naga, NS-03) along with two highly susceptible checks (IIHR-52-1-30, IIHR-23-8-10) were screened against downy mildew resistance during *kharif* season. All these genotypes were true-to-type with highly uniform plant type due to continuous selfing for 3-4 generations.

Screening experiment was conducted in randomized block design with three replications (six plants per replication) by repeating susceptible checks between the test rows and around the field to facilitate the disease infection. Each genotype was sown in a single row-to-row and plant-to-plant spacing of 150 cm and 50 cm respectively. Recommended cultural practices except the application of fungicides were followed throughout the growing period. Plants were scored based on the 0-9-point interaction phenotype scale as suggested by Jenkins and Wehner (1983) where 0=No disease, 1=Few small leaf lesions, 2=Few lesions on few leaves with no stem lesions, 3=Few lesions on few leaves or with superficial stem lesions, 4= Few well-formed leaf lesions or superficial stem lesions, 5=Few well-formed leaf lesions or enlarging stem lesions, 6=Many large leaf lesions or deep stem lesions with abundant sporulation or plant more than 50% defoliated, 7= Many large coalescing leaf or stem lesions, over 75% of plant area affected or defoliated, 8=Plants largely defoliated, leaf or stem with abundant sporulating lesions and 9= Plants dead. Downy mildew incidence was recorded after appearance of several disease symptoms on the susceptible checks (45 days after sowing) and continued throughout the growing period at 30 days interval. Total three scoring were used to calculate percent disease index (PDI) as per the formula followed by Jamadar and Desai (9) and Santhosh (17). Average PDI was used to calculate apparent infection rate (r) and area under and disease progress curve (AUDPC) for each genotype (Vander Plank, 1963; Wilcoxen *et al.* (1975).

Artificial Screening and Disease Assessment

Artificial screening by leaf disc assay method of the four advanced selections along with a susceptible check (IIHR-52-1-30) was conducted during *kharif* as per method described earlier (Salati *et al*., 2010). Seven leaf discs per plant (three plants per genotype) placed abaxially onto 0.4 per cent agar medium in petri-plates were inoculated with three droplets (10 µl) of inoculum suspension and incubated for 5 days at 20 ºC temperature, 80 per cent relative humidity and photoperiod of 16 h. One petri-plate with seven leaf discs without inoculation were kept as control. Petri dishes were stored under dark condition for sporulation at 20 ºC. Test plants were scored for disease development after 7 days post inoculation (dpi) using a binocular magnifier. Plants were rated on 0-4 phenotypic scale where 0= no sporulation; 1= light sporulation (difficult to see with naked eye) 2 = sporulation area inferior to the diameter of the deposited inoculum droplet; 3 = sporulation area corresponding to the diameter of the deposited inoculum droplet; 4 = sporulation area superior to the diameter of the deposited inoculum droplet. Percent disease index (PDI) was calculated as per the formula followed by Salati *et al*. (2010).

Inheritance of Downy Mildew Resistance

This experiment was conducted in two different crosses. In the first cross, highly susceptible ridge gourd advanced line 'IIHR-52-1-30' (female parent) was crossed with the promising line IIHR-17-1-7-3 (male parent) whereas in the second cross, highly susceptible line IIHR-23-8-10 (female parents) was crossed with the advanced inbred lines IIHR-7-5-1 (male parent) to get two F_1 populations. The F_1 plants were selfed to get F_2 seeds and back crosses (BC1; F_1 × susceptible parent, BC_2; F_1 × resistant parent) were also performed to evaluate the various inheritance patterns determined from analyzing the F_2 segregation data. In the field, 30 plants of susceptible parent (IIHR-52-1-30), 30 plants of resistant parent (IIHR-17-1-7-3), 30 F_1 (IIHR-52-1-30 × IIHR-17-1-7-3) plants, 178 F_2 individuals, 29 susceptible back cross progenies (F1 × IIHR-52-1-30) and 30 resistant back cross progenies (F1 × IIHR-17-1-7-3) were evaluated against the downy mildew disease under high disease pressure conditions during *kharif* season.

Another F_2 population with 45 plants obtained by crossing IIHR-23-8-10 × IIHR-7-5-1 was also evaluated to study the genotypic effect, if any, in the inheritance pattern of downy mildew resistance in ridge gourd. Twenty-nine plants of susceptible parent (IIHR-23-8-10), 30 plants of resistant parent (IIHR-7-5-1), 7 F_1 (IIHR-23-8-10 × IIHR-7-5-1) plants, 45 F_2 individuals, 13 susceptible back cross progenies (F_1 × IIHR-23-8-10) and 30 resistant back cross progenies (F_1 × IIHR-7-5-1) were evaluated against the downy mildew disease under similar epiphytotic conditions.

Plants were rated on 0-9 phenotypic scale as suggested by Jenkins and Wehner (1983). PDI was calculated as per the formula followed by Jamadar and Desai (1999) and Santhosh (2011). Mean PDI data was used for the calculations.

PDI	Reaction categories
1-10	Resistant (R)
11-25	Moderately resistant (MR)
26-50	Moderately susceptible (MS)
51-75	Susceptible (S)
>75	Highly susceptible (HS)

Results and Discussion

The reaction of test genotypes of ridge gourd to downy mildew disease under field and laboratory conditions is summarized in Table 1 and 2. Three advanced lines have showed promising response towards downy mildew resistance as there were mild symptoms of downy mildew throughout growing period. The susceptible lines (IIHR-52-1-30 and IIHR-23-8-10) were highly infected with symptoms of leaf lesions, stem lesions, intensive defoliation with abundant sporulation. The disease development was very slow and delayed in four advanced inbred selections when compared with highly susceptible lines (Fig. 1). Among twelve genotypes at advanced stage of screening, three genotypes viz., IIHR-7-5-1, IIHR-17-2-1, IIHR-17-1-7-3 (PDI= 11.00-25.00) (Table 1, Fig.1) were found to be moderately resistant, IIHR-17-1-7-4 and Arka Sumeet were found to be moderately susceptible (PDI=26.00-50.00). Rest of the selections and commercial varieties and hybrids were found to be susceptible and mildew growth was observed on leaves (Table 1).

AUDPC values widely varied from 467.59 (IIHR-17-2-1) to 2819.00 (IIHR-23-8-10) under different screening conditions (Table 1). Three advanced selections with lower PDI also had lower AUDPC values which were ranging between 629.00-810.00 (Table 1). Most susceptible genotypes showed higher PDI and AUDPC values which indicated the significance of disease parameters used in the study. Apparent infection rate of advanced selections was slightly higher because of some infection in the later stages of the crop growth (105 DAS) (Table 1). PDI values of test genotypes in natural and controlled screening were positively co-related and correlation coefficient values under natural epiphytotic condition was 0.97 (Table 2, Fig. 2, 3). These moderately resistant sources against downy mildew along with alreadyidentified sources in melon (Goswami *et al*., 2011; Lebeda and Widrlechner, 2003; Taler *et al*., 2004) and cucumber (Shetty *et al*., 2002; Wehner and Shetty, 1997) remain useful in integrated disease management programme. These identified lines of ridge gourd can

Table 1. PDI, AUDPC and apparent rate of infection against downy mildew in ridge gourd advanced inbred selections under field conditions

S. No.	Genotype	PDI			Mean PDI	Disease Reaction	AUDPC	Apparent rate of infection (r)
		45 DAS	75 DAS	105 DAS				
1	IIHR-17-1-7-4	0.62	20.74	65.92	29.09	MS	810.19	0.15
2	IIHR-7-5-1	1.85	19.75	42.59	21.40	MR	629.63	0.11
3	IIHR-17-2-1	0.00	13.58	35.18	16.25	MR	467.59	0.14
4	IIHR-17-1-7-3	0.62	19.75	44.44	21.60	MR	634.26	0.14
5	IIHR-52-1-30	77.16	99.38	100.00	92.18	HS	2819.44	0.01
6	IIHR-23-8-10	76.67	98.89	100.00	91.85	HS	2808.33	0.01
7	IIHR-6-1-1	69.14	91.36	99.26	86.58	HS	2633.33	0.01
8	IIHR-53-1-3	52.22	74.44	96.67	74.44	S	2233.33	0.02
9	ArkaSumeet	24.20	46.42	64.07	44.90	MS	1358.33	0.03
10	ArkaSujat	61.11	83.33	93.83	79.42	HS	2412.04	0.01
11	Naga	55.56	77.78	96.91	76.75	HS	2310.19	0.02
12	NS-03	35.19	57.41	77.78	56.79	S	1708.33	0.03

PDI-Per cent disease index; AUDPC-Area under disease progress curve; DAS-Days after sowing; r-mean apparent rate of infection; MR-Moderately resistant; MS-Moderately susceptible; HS-Highly susceptible; S-Susceptible

Fig. 1. ***Field screening*****: IIHR-7-5-1, moderately resistant inbred selection (*****left*****) and highly susceptible inbred selection, IIHR-52-1-30 (*****right*****) against downy mildew in ridge gourd**

Fig. 2. ***Artificial screening*** **(left control, right inoculated leaf discs in each photo): IIHR-7-5-1, moderately resistant inbred selection (*****left*****) and highly susceptible inbred selection, IIHR-52-1-30 (*****right*****) against downy mildew in ridge gourd**

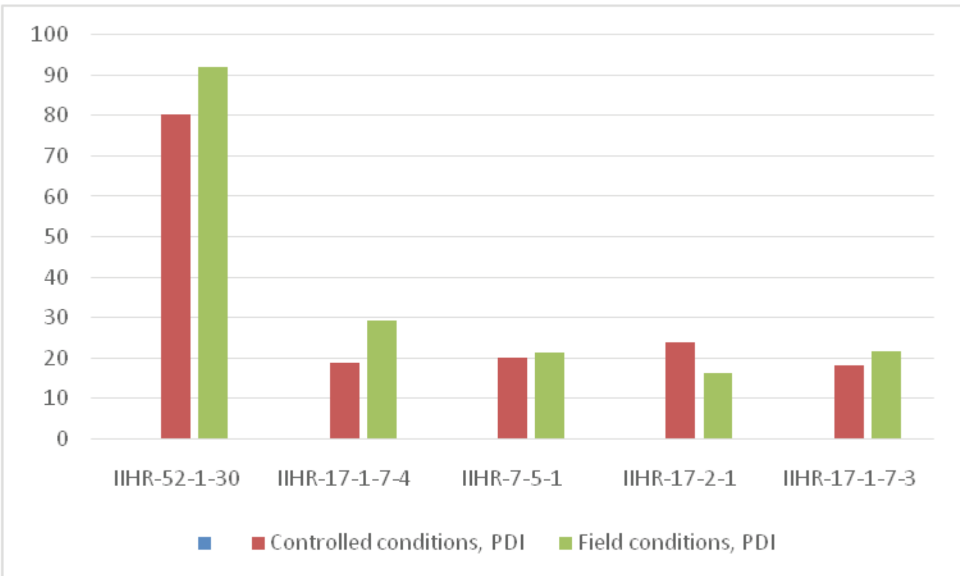

Fig. 3. Comparison of inbred selections PDI under controlled conditions and field conditions

Table 2. Disease response of selected ridge gourd advanced selections along with susceptible checks under field and laboratory (Controlled) conditions

Genotype	Controlled conditions		Field conditions
	Sporulation Rank	PDI	Mean PDI
IIHR-52-1-30	3.21	80.16	92.18
IIHR-17-1-7-4	0.75	18.65	29.09
IIHR-7-5-1	0.80	20.11	21.40
IIHR-17-2-1	0.95	23.81	16.25
IIHR-17-1-7-3	0.72	17.99	21.60
Correlation coefficient			0.977**

** Significant at 1% level
PDI-Per cent disease index

be advanced further for improvement in downy mildew resistance.

The segregation in F_2 and backcross progeny of the two crosses was subjected to chi-square analysis for assessing the goodness of fit to various classical Mendelian ratios (Table 3). Data represented in Table 3 showed that all the plants of inbred lines used as female parent, i.e. IIHR-52-1-30 were susceptible while those of the male parent IIHR-17-1-7-3 were resistant to downy mildew disease. The F_1 plants showed susceptible reaction against downy mildew infection. The results may indicate the dominance of susceptibility over resistance. The number of segregants into resistant and susceptible classes in the F_2 generation was 33 and 145 respectively. Out of the various Mendelian ratios tested, the chi-square values were significant for all ratios except 3 (resistant): 13 (susceptible) with a chi square value of 0.005 and probability of 0.90-0.95 which suggested that two pairs of genes were responsible for resistance to downy mildew disease in IIHR-17-1-7-3. In the test cross with resistant parent, the segregation ratio had the best fit with 1:1 (χ^2= 0.533; P = 0.40 – 0.50). However, as in the test cross with susceptible parent, the segregation ratio had goodness of fit with 1:0 (χ2 = 0.00; p = 1.00) *i.e*., all the plants were susceptible. These results showed that the parents were different in pairs of epistatic interaction and expressed dominant and recessive interaction. Thus, two pairs of genes of dominant and recessive interaction governed the inheritance of downy mildew disease in ridge gourd. Similarly, El-Hafez *et al*. (1990) mentioned that resistance to downy mildew in cucumber plants is controlled by two pairs of dominant and recessive interaction genes (13 susceptible: 3 resistant). Badr and Mohamed (1998) also reported similar results for downy mildew resistance in cucumber. However, so far, reports on genetic analysis of downy mildew resistance in ridge gourd are not available.

In the second cross IIHR-23-8-10 × IIHR-7-5-1, the number of segregants into resistant and susceptible classes in the F_2 generation was 26 and 19 respectively. Out of the various mendelian ratios tested, the chi square values were significant for all ratios except 9:7 (resistant: susceptible) (χ^2 = 0.0.043; P = 0.80 – 0.90). In the test cross with susceptible parent, the segregation ratio had the best fit with 1:3 (χ^2= 0.026; P = 0.80 – 0.90). While in the test cross with resistant parent all the plants were resistant, hence the segregation ratio had goodness of fit with 1:0 (χ2 = 0.00; p = 1.00). The available evidence supported the presence of complementary gene action for the downy mildew resistance. This segregation pattern was not reported by any workers for downy mildew resistance in cucurbits. However multiple genes for downy mildew resistance have been reported in melon and cucumber (Epinat, 1994; Ren *et al*., 2009; Criswell

Table 3. Estimates of chi square values and their probability for classical Mendelian ratios for downy mildew resistance in the F_2 and test cross population of ridge gourd

Cross		Generations	Number of Plants			Genetic ratio	Chi square value	P value
			Resistant (R)	Susceptible (S)	Total	R:S		
I.	IIHR-52-1-30 x IIHR-17-1-7-4 (S × R)	P1	0	30	30	-	-	-
		P2	25	5	30	-	-	-
		F1	0	30	30	-	-	-
		F2	33	145	178	3:13	0.005	0.942
		BC1	0	29	29	0:1	-	-
		BC2	17	13	30	1:1	0.533	0.465
II.	IIHR-23-8-10 x IIHR-7-5-1 (S × R)	P1	0	29	29	-	-	-
		P2	30	0	30	-	-	-
		F1	7	0	7	-	-	-
		F2	26	19	45	9:7	0.043	0.836
		BC1	3	10	13	1:3	0.026	0.873
		BC2	28	2	30	1:0	-	-

et al., 2011; Zhang *et. al*., 2013; Yoshioka *et. al*., 2014; Wang *et. al*., 2016).

The difference in gene interaction in the two crosses studied,may be attributed to the presence of set of non-allelic genes in male and female parents. Therefore, it has been concluded that the complicated nature of digenic dominant interaction restricts the understanding on nature of inheritance and steps forwards to improve the crop to sustain downy mildew resistance. Therefore because of the complex nature of inheritance, the breeding programshould be accompanying with crossing followed by recurrent selection which may be effective to get desirable recombinants with downy mildew resistance.

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