



Genetic analysis of yellow mosaic disease resistance in loofah

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Abstract

Yellow mosaic disease caused by tomato leaf curl New Delhi virus is very severe and causing 100 per cent yield loss under epiphytotic conditions. Chemical control of this disease is neither economical nor eco-friendly and genetic resistance is an efficient means for its management. Resistance to yellow mosaic disease in advanced inbred line IIHR-Sel-1 and IIHR-137 have been confirmed during screening experiment. A crossing programme was planned using Arka Prasan as susceptible and IIHR-Sel-1 and IIHR-137 as resistant parents. We used the F₁, F₂, BC₁ and BC₂ generations derived from the two crosses viz. Arka Prasan × IIHR-Sel-1 and Arka Prasan × IIHR 137 to study the inheritance of resistance to yellow mosaic disease. Results indicated monogenic recessive resistance to yellow mosaic disease in the genetic background of both parents which need to be confirmed through repeated experiments with large population size. These results are imperative for breeding yellow mosaic disease resistant cultivars in *Luffa* species.

Keywords Inheritance · *Luffa* · Monogenic · Tomato leaf curl New Delhi virus · Mosaic · Recessive · Resistant

Introduction

Ridge gourd [*Luffa acutangula* (L.) Roxb.] and Sponge gourd [*L. cylindrica* (L.) Roem.] are two important cucurbits grown as a cash crops in many tropical and sub-tropical regions of the world. In India, the average productivity of gourds is 9.59 t/ha (FAO 2018) which is very low and biotic and abiotic stresses are the major limiting factors in its production. Among various factors, viral diseases such as yellow mosaic disease caused by tomato leaf curl New Delhi virus (ToLCNDV) is one of the most important constraints in ridge gourd production. Reported yield reduction caused by yellow mosaic disease is as high as 100 per cent under high disease pressure conditions (Patil et al. 2017). It also has been noticed that mosaic disease severely affects the flowering and subsequent fruit quality. It is mainly transmitted by an insect vector, *Bemisia tabaci* (Gennadius 1889) (*Hemiptera: Aleyrodidae*) in circulative persistent manner in dicotyledons crops (Castillo et al. 2011). Symptoms of yellow mosaic disease consists of yellow mosaic on young

leaves, chlorosis, upward curling, blistering, cupping of leaves, reduction in leaf area and thickening of leaves, flower abortion altogether with stunting of infected plants (Vasudeva and Samraj 1948; Singh et al. 2001; Patil et al. 2017). Cultural, physical, chemical, and biological methods are often used as part of vector management strategies. However, the continuous changes in whitefly epidemics and insensitivity of insect vectors towards different pesticides are the new challenges in management of viral diseases (Caballero et al. 2013). Besides this, numbers of health and environmental risks are associated with pesticide use and therefore discourage the continuous use of pesticides in viral disease protection. Use of resistant varieties is preferred method to reduce yield losses under epiphytotic conditions.

Very limited information is available on the inheritance pattern of leaf curl diseases caused by begomovirus, in particular, ToLCNDV. Reports suggesting one dominant gene conferring resistance in 'DSG-6' and 'DSG-7', sponge gourd advanced inbred lines (Islam et al. 2011), a single recessive gene in 'IC-274014' melon accession (Romay et al. 2019) and one major gene with two minor genes in wild *Cucumis melo* var. *Agrestis* group (WM-7) (Saez et al. 2017) are available. One dominant gene has been reported for the resistance to chilli leaf curl virus in chilli pepper (Thakur et al. 2019; Yadav et al. 2020) and one recessive gene with

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one additional allele for tomato yellow leaf curl virus in tomato (Hutton et al. 2012) was reported. Polygenic nature of inheritance was also involved in leaf curl virus resistance in tomato with additive or epistatic effects among genes (Anbinder et al. 2009; Prasanna et al. 2015). The possible explanation of perplexity in inheritance pattern might be varied with experimental conditions, use of different genetic materials, virus isolates, inoculation time and screening methods. Hence, understanding the inheritance of resistance to such complicated pathogen are important to design appropriate breeding strategies.

Ridge gourd yellow mosaic disease caused by ToLC-NDV is a recently reported disease in ridge gourd. So far, no reports are available regarding genetics of inheritance for yellow mosaic resistance in ridge gourd. Therefore, the main objective of this study was to understand the inheritance pattern of yellow mosaic disease resistance in ridge gourd.

Materials and methods

Plant material

One commercial cultivated variety Arka Prasan and two advanced inbred lines IIHR-Sel-1 and IIHR-137 belonged to *Luffa* genus were used in the current study. Arka Prasan is a commercial high yielding ridge gourd variety with long and tender fruits, however susceptible to yellow mosaic disease. *Luffa acutangula* genotype IIHR-Sel-1 is yellow mosaic disease resistant advanced breeding line maintained as inbred after at least six generation of self-pollination at Division of Vegetable Crops, ICAR-IIHR, Bengaluru. *L. cylindrica* genotype IIHR-137, an advanced inbred line earlier has been reported resistant to yellow mosaic disease (Islam et al. 2010; 2011) and it was kindly made available from ICAR-Indian Agricultural Research Institute, New Delhi, India. All three genotypes were true-to-type with highly uniform plant type due to continuous self-pollination.

Disease hotspot and experimental layout

ICAR-IIHR, Bengaluru with its unique location and climatic conditions (latitude 13°58' North; longitude 78°45' East; altitude 890 m above mean sea level; average temperature 25–30 °C, annual rainfall 890 mm) is a hot spot for yellow mosaic disease of cucurbits. The major pathogen ToLCNDV is prevalent throughout the year and transmitted through insect vector whitefly under natural conditions. Therefore, the infection occurs in the susceptible genotypes once the insects visit the plants. The experiment was laid out in a randomized complete block design with three replications. Susceptible checks were planted at regular interval to facilitate the infection and to avoid any chance of disease

escape. Seedlings of all the six populations were transplanted 14 days after sowing on raised bed covered with plastic mulch at a spacing of 150 cm between the beds and 50 cm between the plants. All the recommended cultural practices were followed to raise a healthy crop except the use of pesticides.

Inheritance pattern of yellow mosaic disease resistance

Controlled crosses were made between the susceptible *L. acutangula* cultivar Arka Prasan (used as female parent; P₁) and the resistant *L. acutangula* genotype IIHR-Sel-1 and *L. cylindrica* genotype IIHR-137 (used as male parents; P₂) to develop F₁ generations. Two F₁ populations were self-pollinated to produce subsequent F₂ populations and back crosses were made to develop BC₁ (backcross with susceptible parent) and BC₂ (backcross with resistant parent) populations in spring summer season. In the field, 30 plants of each P₁, P₂ and F₁ (Arka Prasan × IIHR-Sel-1) whereas 276 plants of F₂ population and 57 plants each of BC₁ (F₁ × Arka Prasan) and BC₂ (Arka Prasan × IIHR-Sel-1) populations were used for screening, whereas in the interspecific cross of Arka Prasan × IIHR-137, 30 plants of susceptible parents (P₁), 27 plants of resistant parent (P₂), 21 F₁ plants (Arka Prasan × IIHR-137), 111 F₂ individuals, 48 susceptible back cross progenies (F₁ × Arka Prasan) and 42 resistant back cross progenies (F₁ × IIHR-137) were evaluated under high disease pressure conditions with high epidemics of whitefly, the insect vector for disease transmission during spring–summer season under field conditions.

Plants were evaluated on standardized 6-point interaction phenotype scale (Sohrab 2005) (Fig. 1), where 0 = No symptoms, 1 = mild mosaic pattern in young leaves covering > 10% area, 2 = mosaic pattern in young leaves covering > 25% area, 3 = mosaic pattern in young leaves covering > 50% area, blistering and puckering of leaves, 4 = widespread mosaic pattern in young leaves covering > 75% area, distortion of leaves and 5 = widespread mosaic pattern in young leaves covering > 75% area, distortion of leaves and stunting of the plants. Total eight scorings were taken at weekly interval after the appearance of first disease symptoms on susceptible line (18 days after transplanting). Plants were categorized based on their disease reaction and data obtained were subjected to chi-square analysis to study the inheritance of disease resistance.

Chi-square analysis

Segregation for resistance was observed in different filial and back cross populations when 100 per cent plants of susceptible control were completely infected. Segregation ratios of resistant (R) and susceptible (S) disease reactions

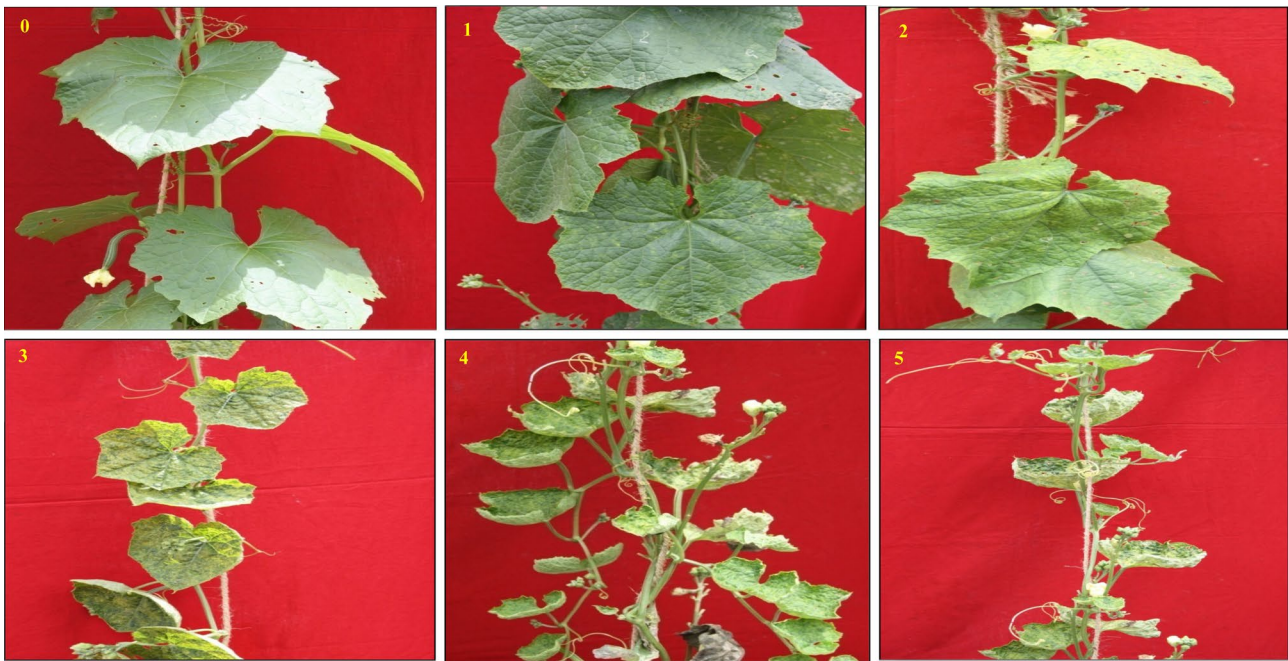


Fig.1 Score chart (0–5) for ToLCNDV screening in ridge gourd

of different generations were subjected to chi-square test to find out the goodness of fit to various classical mendelian ratios with the assumed phenotypic ratios of F_2 and back cross progenies as suggested by Panse and Sukhatme (1985). The expected values corresponding to the observed values were calculated on the ratio (hypothetical) presumed. The deviations from these were calculated by using the formula of chi-square test,

$$\chi^2 = \frac{\sum(\text{Observed number} - \text{Expected number})^2}{\text{Expected number}}$$

All statistical analysis was performed using WIN-DOSTAT version 8.6 (statistical software developed by Indostat Services, Hyderabad) licensed to LAN, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru.

Estimation of magnitude of gene effects and their interaction

The average score value of ToLCNDV for the parents (P_1 and P_2), F_1 's ($P_1 \times P_2$), F_2 's (F_1 's selfed) and their first generation back crosses ($BC_1 = F_1 \times P_1$ and $BC_2 = F_1 \times P_2$) from two crosses were calculated from the data pooled over three replications. This formed the base for the computation of various statistics. Before estimating the different parameters, scaling tests of Mather (1949) and Hayman and Mather (1955) were done to detect the presence of non-allelic interactions. The three parameters (m, d and h) were estimated

for non-interacting crosses using the formula proposed by Jinks and Jones (1958).

Results

Resistant parents, 'IIHR-Sel-1' and 'IIHR-137' were highly resistant throughout the growing period and did not show any disease symptoms. Individual plants of the F_1 , F_2 , BC_1 and BC_2 populations showed differential reactions, starting from no symptoms to severe curling and stunting of the plants as characteristic mark of yellow mosaic disease (Figs. 2, 3).

A total of 276 F_2 plants derived from the cross of Arka Prasan \times IIHR-Sel-1 were evaluated for disease response, out of which 76 plants were found to be resistant and 200 plants were susceptible. All plants from back-crosses with susceptible parent were susceptible to the disease. Upon subjecting F_2 population data to chi-square analysis; only the monogenic segregation ratio was satisfied and showed the segregation pattern of 3:1 (susceptible: resistant) with chi-square value of 0.95 with per cent probability of 33.00. Back cross population with the resistant parent (IIHR-Sel-1) was segregated into 40 resistant and 17 susceptible plants ($\chi^2 = 9.28$; $p = 0.002$). As the backcross population (BC_2) did not follow the mendelian segregation pattern (1:1) clearly, it was inconclusive, however based on F_2 population segregation, it is indicating towards the involvement of one

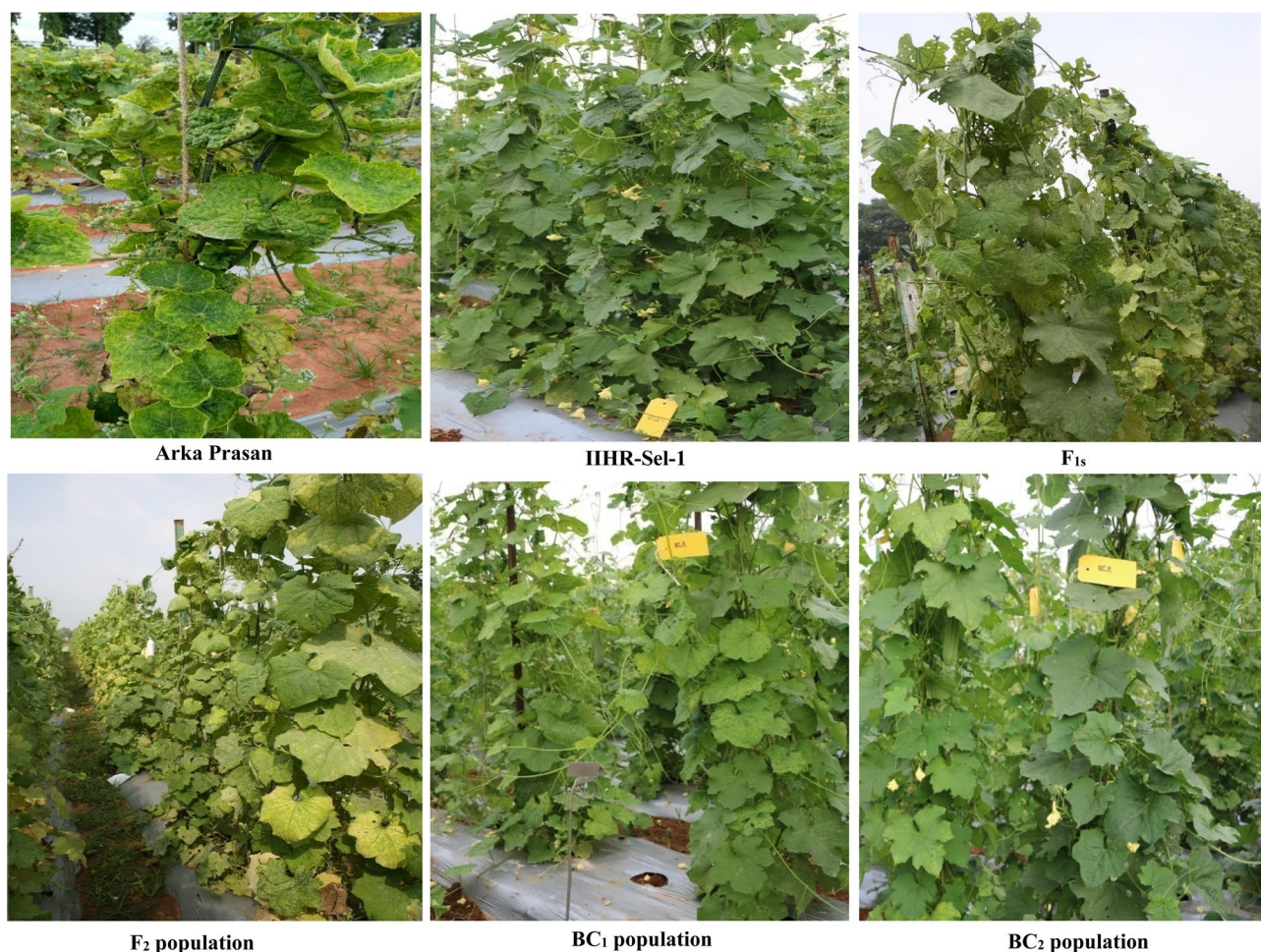


Fig.2 Screening of parents with the populations derived from the cross of Arka Prasan \times IIHR-Sel-1 under natural epiphytotic conditions

major recessive gene for yellow mosaic disease resistance in the genetic background of ridge gourd genotype, IIHR-Sel-1 (Table 1).

A total of 111 F_2 plants derived from the cross of Arka Prasan \times IIHR-137 were evaluated for disease response, out of which 29 plants were resistant and 82 plants were susceptible. Almost all the back-cross plants involving susceptible parent (Arka Prasan) were susceptible to the yellow mosaic disease. The F_2 population showed the segregation pattern of 3:1 (susceptible: resistant) ($\chi^2 = 0.07$; $p = 0.78$) (Table 1). The results pertaining to the inheritance pattern were confirmed by using back cross population ($F_1 \times$ IIHR-137) of 42 plants. Forty-two plants were segregated into 27 resistant and 15 susceptible plants which was best fit in 1:1 ratio for resistant and susceptible, respectively ($\chi^2 = 3.43$; $p = 0.06$), again confirming a monogenic recessive mendelian ratio against yellow mosaic disease resistance in the genetic background of 'IIHR-137' (Table 1).

The means for six generations calculated over all the individuals of given population in both the crosses were stated,

primarily to compare these populations means with one another. F_2 , BC_1 and BC_2 generations in both the crosses fell within parental range (Table 3). The variances in the non-segregating generation P_1 , P_2 and F_1 were relatively lower than those in segregating generations, F_2 , BC_1 and BC_2 (Table 2).

The results of the scaling tests and generation mean analysis for the two cross combinations are presented in Table 3. The scaling tests conducted on each of the two crosses were found to be non-significant which indicated the absence of epistasis. Therefore, both the crosses were of non-interacting types (Table 3) and are in conformity with the results of chi-square analysis.

Discussion

Yellow mosaic disease caused by the ToLCNDV has become major production constraint and results in considerable yield loss in ridge gourd and sponge gourd, especially in tropical and sub-tropical regions of India. Therefore, understanding the inheritance of disease resistance

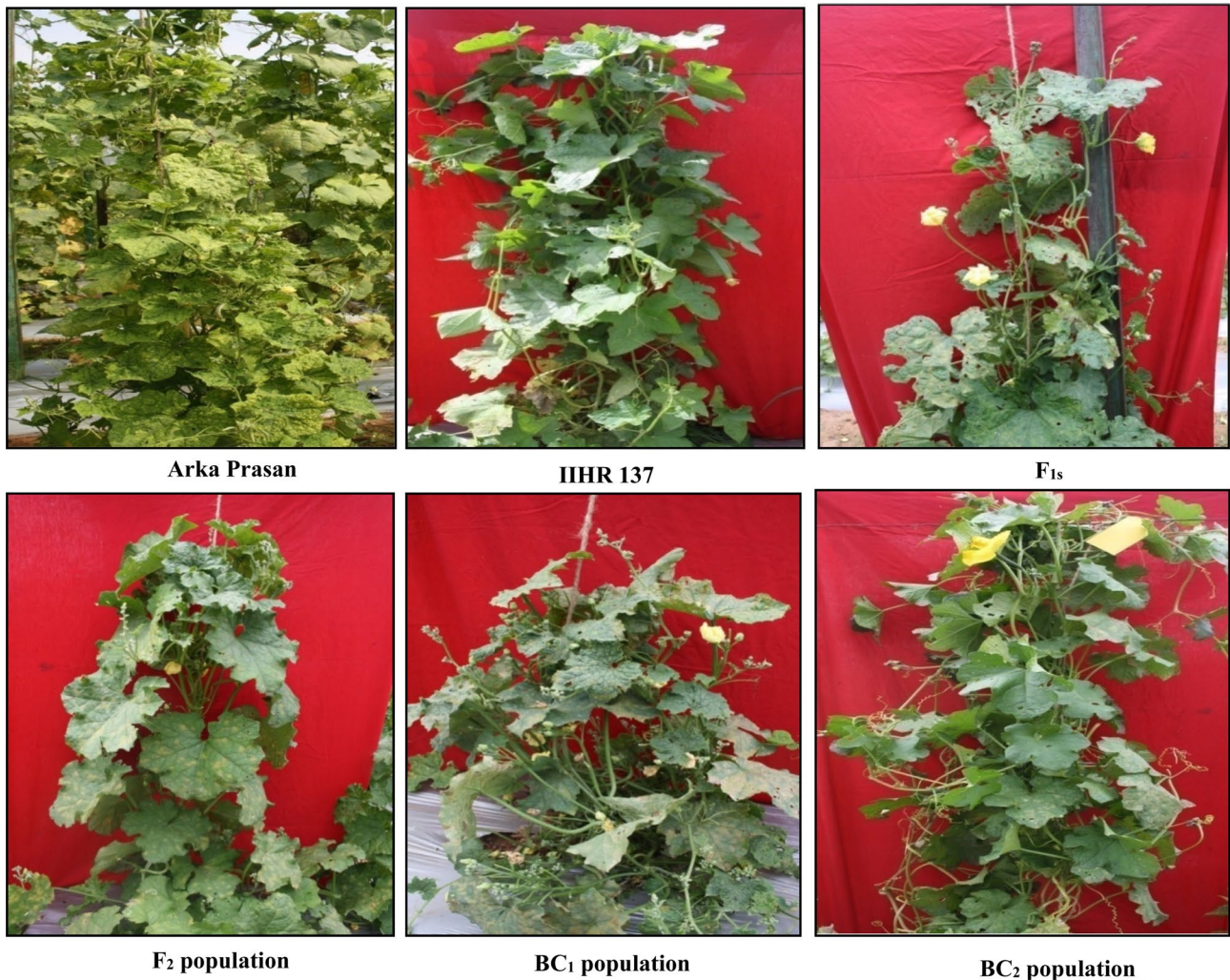


Fig.3 Screening of parents with the populations derived from the cross of Arka Prasan \times IIHR 137 under natural epiphytotic conditions

is useful to develop an effective breeding programme for crop improvement. In the present study, field screening was adopted to screen the segregating generations under high disease pressure conditions during spring–summer season. The spring–summer season was coinciding with high rate of whiteflies proliferation which is insect vector for disease transmission (Purohit et al. 2006). Susceptible check was transplanted randomly to avoid any chance of disease escape. The disease symptoms were characterized in previous study (Manpreet et al. 2020) and was followed for current study as well. Symptoms were observed after 18 days of transplanting in the susceptible checks (Figs. 2, 3). Similar observation has been recorded in ridge gourd germplasm screening through whitefly-mediated inoculation (Patil et al. 2017) and mechanical sap inoculation (Sohrab et al. 2013) and in sponge gourd germplasm screening under challenge virus inoculation (Islam et al.

2010). The inheritance pattern of yellow mosaic disease was studied in two crosses involving Arka Prasan as susceptible (female) parent and IIHR-Sel-1 and IIHR-137 as resistant donor (pollen) parents. As a confirmed source of resistance, IIHR-137 (Islam et al. 2010) was included in the study, additionally the interspecific hybrids of *L. acutangula* and *L. cylindrica* found to have strong heterosis effects (Wu et al. 2014). In the present experiment, after the artificial hybridization between ridge gourd and sponge gourd, interspecific hybrids (F_1) were partially sterile and fruit set was very low.

The F_1 plants of both the crosses were susceptible, indicating that resistance was inherited as a recessive trait. The segregation of F_2 populations into susceptible and resistant plants fit a 3:1 ratio, which indicated that susceptibility was dominant over resistance and ToLCNDV resistance was inherited through a homozygous recessive trait at a

Table 1 Estimates of chi square values and their probability for classical Mendelian ratios for ToLCNDV resistance in the crosses of Arka Prasan × IIHR-Sel-1 and Arka Prasan × IIHR-137

Cross	Generations	Description	Number of Plants			Genetic ratio	Chi square value	P value
			Susceptible	Resistant	Total			
I Arka Prasan × IIHR-Sel-1 (S × R)	P ₁	Arka Prasan	29	1	30	-	-	-
	P ₂	IIHR-Sel-1	3	27	30	-	-	-
	F ₁	Arka Prasan × IIHR Sel-1	30	0	30	-	-	-
	F ₂	Arka Prasan × IIHR Sel-1	200	76	276	3:1	0.95	0.33
	BC ₁	(Arka Prasan × IIHR Sel-1) × Arka Prasan	56	1	57	1:0	-	-
	BC ₂	(Arka Prasan × IIHR Sel-1) × IIHR-Sel-1	17	40	57	1:1	9.28	0.002
II Arka Prasan × IIHR-137 (S × R)	P ₁	Arka Prasan	29	1	30	-	-	-
	P ₂	IIHR-137	0	27	27	-	-	-
	F ₁	Arka Prasan × IIHR-137	17	4	21	-	-	-
	F ₂	Arka Prasan × IIHR-137	82	29	111	3:1	0.08	0.78
	BC ₁	(Arka Prasan × IIHR-137) × Arka Prasan	43	5	48	1:0	-	-
	BC ₂	(Arka Prasan × IIHR-137) × IIHR-137	15	27	42	1:1	3.43	0.06

Table 2 Generation means and variances for disease score for ToLCNDV resistance in the crosses of Arka Prasan × IIHR-Sel-1 and Arka Prasan × IIHR 137

Traits		P ₁	P ₂	F ₁	F ₂	BC ₁	BC ₂
Arka Prasan × IIHR-Sel-1	Mean ± SE	4.33 ± 0.18	0.5 ± 0.12	3.1 ± 0.26	2.62 ± 0.09	4.01 ± 0.14	1.14 ± 0.16
	Variance	0.92	0.47	2.09	2.47	1.09	1.55
Arka Prasan × IIHR-137	Mean ± SE	4.33 ± 0.18	0 ± 0.00	3 ± 0.33	2.5 ± 0.12	3.58 ± 0.2	1.55 ± 1.57
	Variance	0.92	0.00	2.3	1.62	1.91	0.19

single locus. In the first cross (Arka Prasan × IIHR-Sel-1), one of the backcross population (BC₂) has not followed the mendelian segregation (1:1) which might be attributed to a number of factors and needs reconfirmation. It depicts the study of first cross as inconclusive, however F₂ segregation has indicated the involvement of a major recessive gene for yellow mosaic disease resistance in ridge gourd. In the genetic background of *L. cylindrica* genotype 'IIHR-137' it was confirmed by the segregation pattern in both backcross populations that resistance to yellow mosaic disease is conferred by a single recessive gene (Table 1). Further,

the scaling tests conducted on each of the two crosses were found to be non-significant which indicated the absence of epistasis. Therefore, both the crosses were of non-interacting types (Table 3) and in conformity with the results of chi-square analysis.

Similar results of single recessive gene inheritance for ToLCNDV in melon accession 'IC-274014' (Romay et al. 2019) and leaf curl virus in interspecific cross in chilli peppers (PBC 535 × Bhut Jolokia) (Rai et al. 2014) and in cultivated resistant variety 'Punjab Lal' (Bal et al. 1995) have been reported. In tomato, one major recessive gene

Table 3 Individual scaling test and three parameter genetic model for ToLCNDV resistance in the crosses of Arka Prasan × IIHR-Sel-1 and Arka Prasan × IIHR-137

Traits	A	B	C	D	[m]	[d]	[h]
Arka Prasan × IIHR-Sel-1	0.602 ± 0.42 ^{ns}	-1.319 ± 0.43 ^{ns}	-0.541 ± 0.68 ^{ns}	0.088 ± 0.28 ^{ns}	1.917 ± 1.14 ^{**}	-0.388 ± 0.11 ^{**}	0.506 ± 1.54 ^{ns}
Arka Prasan × IIHR-137	-0.167 ± 0.55 ^{ns}	0.095 ± 0.51 ^{ns}	-0.351 ± 0.84 ^{ns}	-0.140 ± 0.37 ^{ns}	3.905 ± 1.44 ^{ns}	2.167 ± 0.09 ^{**}	1.322 ± 1.98 ^{ns}

[m] mean effect, [d] additive effect, [h] dominance effect, *significant at $p=0.05$, **significant at $p=0.01$, ^{ns} not significant

(*ty-5*) with one additional gene was reported in resistant source 'Fla 8753' (Hutton et al. 2012). In contrary, Islam et al. (2011) reported that yellow mosaic disease caused by ToLCNDV in sponge gourd is controlled by single dominant gene. A single dominant gene inheritance for leaf curl disease in chilli pepper (Thakur et al. 2019; Yadav et al. 2020), mungbean yellow mosaic India virus in black gram (Gupta et al. 2013), cucurbits yellow mosaic virus in *Cucumis melo* (Lopez-Sese and Gomez-Guillamon 2000) have been reported. The results suggested that resistance to yellow mosaic disease is governed by a major recessive gene in the genetic background of IIHR-Sel-1 and IIHR-137 genotype, however replicated experiments with expanded plant population is required to reconfirm the gene action while avoiding any chance of disease escape. Interspecific cross between *L. acutangula* and *L. cylindrica* need to be evaluated for different reproductive and fruit related traits for their possible use in breeding ToLCNDV resistant ridge gourd cultivars.

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Author's contribution Conceived and designed the experiments: BV, MK; Performed the experiments: MK Analyzed the data: MK, MK², RV; Contributed to the writing of the manuscript: MK, MK² BV; Development of F₁ populations: MK, BV; Screening of germplasm and F₂ Population: MK, MK² BV, BM; Contributed through intellectual input: ESR, MP, DCLR; All the authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical clearance No human subjects were used in the writing of the review manuscripts.

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