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RESEARCH ARTICLE

Studies on inheritance of yellow vein mosaic virus (YVMV) resistance in okra (*Abelmoschus esculentus* L.) cv. Pusa Bhindi-5 and effect of YVMV disease on fruit nutritional quality

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Abstract

The present investigation was carried out by involving six generations of okra, *i.e.,* P_1, P_2, F_1, F_2, B_1 (F_1 × Pusa Bhindi-5) and B_2 (F_1 × Pusa Sawani) developed from two contrasting parents, one susceptible cultivar Pusa Sawani (P_1) and another newly developed Bhendi yellow vein mosaic virus (YVMV) resistant variety Pusa Bhindi-5 (P_2). The experimental data showed the maximum PDI (percent disease incidence) in susceptible parent Pusa Sawani (95.56%) followed by B_2 (47.5%) and F_2 (29%) after 90 days of sowing, whereas Pusa Bhindi-5, B_1 and F_1 were recorded highly resistant to YVMV disease even after 90 days of sowing. Backcross population B_2 and F_2 fell into the moderately susceptible (MS) category, whereas parent Pusa Sawani was recorded as highly susceptible (HS). Based on F_2 and backcross data, the nature of inheritance of YVMV resistance in Pusa Bhindi-5 was found to be monogenic dominant. Analysis of t-test for the significance of the difference between the mean values of infected and healthy fruits was found to be highly significant with higher probability (*p-value* <0.05), indicating that the viral infection caused changes in the biochemical/nutrient contents such as protein, carbohydrate, dietary fiber, total ash and minerals. Most of the nutrients were negatively affected by the YVMV. Thus, this study ascertained the qualitative damage caused by this virus.

Keywords: Okra, inheritance, YVMV infestation, percent disease incidence, fruit nutritional quality

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench; 2n=2x=130] known as Bhindi or lady's finger is an important vegetable crop cultivated throughout the tropics and warmer parts of the temperate zone. India is the global leader in okra with a production share of more than 72% (6.095 million tonnes) from an area of 0.509 million hectares (NHB Database 2018). Okra is an economically significant vegetable with considerable earning through foreign exchange, accounting for around 13%of export fresh vegetables from India. However, in recent times, okra production in India is hampered by biotic stress, mainly bhendi yellow vein mosaic disease. This whitefly (*Bemisia tabaci*) transmitted virus severely affects yield and quality of the okra produced. Yield losses as high as 50-90% have been commonly recorded earlier by Singh and Singh (2000). Commonly, YVMV disease is indirectly managed by controlling whitefly vectors through synthetic pesticides, but okra, a vegetable with shorter harvesting intervals and fresh consumption, possesses residual hazards to consumers. Therefore, emphasis is now shifting in favor of host plant resistance for YVMV control. Use of resistant or tolerant varieties is the most economical and environmentally safe method of disease management. Therefore, the development of high-yielding and YVMV-resistant varieties has become the primary goal and integral part of okra improvement programs.

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Identifying a stable source of resistance and understanding the nature of resistance inheritance is a prerequisite to adopting the appropriate breeding methodology to evolve disease-resistant cultivars. The earliest study by Singh et al. (1962) in cultivar Pusa Sawani reported that the resistance to yvmv disease was governed by two recessive genes. Later on, Pusa Sawani became susceptible to yvmv disease. Sharma and Dhillon (1983) reported two complementary dominant genes in the cultivar Punjab Padmini. However, in some other varieties, resistance governed by a single dominant gene was also reported by Arora et al. (2008), Jambhale and Nerkar (1981) and Dutta (1991). Recently, Senjam et al. (2018) revealed the complex pattern of inheritance where a single dominant gene and some minor factors played key roles in resistance to YVMV disease. So, it is clear that the genetics of resistance varies from cultivar to cultivar, possibly affected by multiple factors, including genetic background, environment etc. Hence it is important to work out the inheritance pattern of resistance in the genotypes under the breeding pipeline.

Wild species of okra are regarded as stable and reliable sources of resistance to YVMV, but the pre and post-fertilization barriers hamper the transfer of resistance from wild species (Bedigar et al. 2024) and it is difficult to produce subsequent generations. In order to overcome such problems, cultivated line as a resistant source can be used to develop the resistant variety. Keeping this fact in mind the newly developed YVMV-resistant cultivar Pusa Bhindi-5 at IARI, New Delhi, has been utilized to work out the inheritance of YVMV resistance in okra, so that subsequently, this line can be utilized in a breeding program to develop resistant varieties/hybrids (Das et al., 2020). Many times, through phenotype-based screening in field or greenhouse screening, a plant that is a symptomless carrier of a virus goes undetected. Hence in the current study, the rolling circle amplification (RCA) technique (Blanco et al., 1989; Rockett et al., 2015) was utilized to confirm the presence or absence of virus to enhance the study's accuracy.

Materials and methods

The present investigation was carried out at the Research Farm of the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, situated at latitude 28° 40′ N, longitude 77°12′ E, and at an altitude of 228.6 m. The climate of Delhi is semi-arid with hot summers and cool winters. The soil of the experimental plot was sandy loam with good drainage and a nearly neutral reaction (pH 6.0–7.5). The experimental materials comprised of two contrasting parents, one susceptible cultivar Pusa Sawani (P_1) and another newly developed bhendi yellow vein mosaic disease resistant variety Pusa Bhindi-5 (P_2). The F_1 was developed using Pusa Sawani as the female and Pusa Bhindi-5 as the male parent. F_1 was selfed to obtain F_2 progenies and subsequently, F_1 hybrid plants were backcrossed with Pusa Bhindi-5 and Pusa Sawani to produce B_1 and B_2 populations, respectively. The final experiment was laid out with six generations *i.e.* P_1, P_2, F_1,F_2, B_1, and B_2 sown in Randomized Complete Block Design (RBD) with three replications during 2018 for analyzing gene action based on disease reaction in F_1 generation and segregation of trait in F_2 and backcross populations. To ensure viral load, Pusa Sawani was used as a susceptible check following the infector row method (Nene et al. 1972). The susceptible check was also grown along the borders of entire trial plots to provide an adequate virus source to the vector. The disease scoring was done at regular intervals, i.e., 30, 45, 60, 75 and 90 days after sowing (DAS). The response of the virus was assessed based on the percent disease incidence (PDI) in a given accession and disease severity (number of leaves having symptoms over total number of leaves in a single plant and averaged from five such plants).

$$\text{Disease severity} = \frac{\text{Number of leaves having symptom}}{\text{Total number of leaves}}$$

Based on the disease severity, symptom severity grades, designated with numerical values of 0–4, were assigned against each accession. A scale of response value (0–1) corresponding to such grades was denoted in Table 1 as described by Bag et al. (2014).

The coefficient of infection (CI) was calculated by multiplying the percent disease incidence (PDI) with the response value assigned for each severity grade using the following equation.

$$CI = PDI \times \text{Response value}$$

The goodness of fit of the observed segregation ratio for the segregation of resistance to YVMV is expected for a single gene, was tested using the classical chi-square (χ^2) test as suggested by Panse and Sukhatme (1985). The chi-square value was calculated using the following formula.

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

To study the effect of disease on fruit yield and quality in parental lines and backcross population, the fruits were collected from all generations, and in F_2 population fruits were bulked into seven categories ranging from healthy (F_20) to highly susceptible (F_2VI). Then these bulks were analyzed separately for various biochemical/ nutritional traits and the results were analyzed with t-test for comparing means of various bulks to know the effect of YVMV disease on quality traits.

Table 1. Scale for bhendi yvmv under natural epiphytotic condition

Symptom	Severity grade	Response value	Coefficient of Infection (CI)	Reaction
Symptom absent	0	0	0–4.0	HR (Highly resistant)
Very mild symptoms up to 25% leaves	1	0.25	>5–9.0	R (Resistant)
Appearance of symptoms in 26–50% leaves	2	0.50	>10–19.0	MR (Moderately Resistant)
Appearance of symptoms in 51–75% leaves	3	0.75	>20–39.0	MS (Moderately Susceptible)
Severe disease infection in symptom (>75% leaves)	4	1.00	>40–69.0	S (Susceptible)
			>70–100	HS (Highly Susceptible)

Rolling circle amplification for virus identification

DNA was extracted from leaf tissue of resistant plants and an infected leaf of susceptible plants from field after 90 days of sowing by the cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Rolling circle amplification (RCA) was performed to amplify full-length circular ssDNA genome of geminiviruses associated with bhendi yellow vein mosaic disease (Johne et al. 2009). After digestion, the amplified RCA product was analyzed on 1% agarose gel stained with ethidium bromide with a restriction enzyme having a single site in the viral genome.

RCA-restriction fragment length polymorphism

The RCA amplified concatamers were restriction digested using a set of restriction enzymes (*BamH*I, *EcoR*I, *Hind*III, *Sac*I, *Kpn*I). Two µL of RCA products corresponding to 300 to 400 ng DNA were digested with 10U of restriction enzymes (Thermo Scientific, Lithuania, EU), 2 µL of reaction buffer (10X), double distilled water to make the final volume 20 µL and incubated for 30 minutes at 37°C. Followed by separation on 1% agarose gel and stained with ethidium bromide. Potential enzymes for the release of full-length genomes or variable restriction patterns were selected.

Results

Scoring of YVMV disease and its pattern of inheritance

Analysis of variance (ANOVA) for generation means comprising six generations, i.e., P_1, P_2, F_1, F_2, B_1 and B_2 presented in the Table 2 revealed that P_2 (Pusa Bhindi-5), F_1 and B_2 (F_1 × Pusa Bhindi-5) populations were resistant to bhendi yellow vein mosaic disease (yvmv) up to 90 days after sowing.

However, Pusa Sawani recorded PDI of 12% followed by B_2 (8%) and F_2 (3%) at 30 days after sowing. The disease progress was recorded fastest in Pusa Sawani, which showed 27, 48, 74, and 95.6% disease incidence after 45 days, 60 days, 75 days, and 90 days after sowing, respectively. The data in Table 3 showed varied degree of disease incidence and Response Value (RV) of all six generations. Both PDI and RV were found to be high in Pusa Sawani, *i.e.,* 74% and 0.75 in 75 days after sowing. It implies a susceptible (S) response on the basis of a coefficient of infection (CI) value of 55.5, while P_2, F_1, and B_1 recorded a coefficient of infection (CI) value 0, indicating highly resistant (HR) reaction even after 90 Days of sowing (Table 3).

The segregating generation F_2 showed 3 resistant: 1 susceptible ratio. The observation indicates that a single dominant gene controlled the resistance to YVMV in Pusa Bhindi-5. In the backcross generation, B_2 (F_1 × Pusa Sawani) chi-square (χ^2) value (0.64) was less than the table value (3.64), revealing 1 resistant: 1 susceptible ratio, which is in accordance with the Mendelian monogenic dominant ratio. This further confirmed that the genetics of resistance of Pusa Bhindi-5 is due to a single dominant gene (Table 4).

Rolling circle amplification (RCA) for virus detection

The DNA analysis of okra leaf samples after 90 days of sowing for the presence of begomovirus through RCA technique showed that out of five restriction enzymes, namely *BamH*I, *EcoR*I, *Hind*III, *Sac*I, *Kpn*I, only *Hind*III could digest the circular

Table 2. Percent disease incidence (PDI) of six generations of cross Pusa Sawani × Pusa Bhindi-5 after 30, 45, 60, 75 and 90 days of sowing (DAS)

Generation		Percent Disease Incidence (PDI)				
	Population size	30 DAS	45 DAS	60 DAS	75 DAS	90DAS
P_1 (Pusa Sawani)	90	12	27	48	74	95.6
P_2 (DOV-66)	90	0	0	0	0	1
F_1	120	0	0	0	0	2
B_1 (F_1 × DOV-66)	120	0	0	0	0	2
B_2 (F_1 × Pusa Sawani)	120	8	16	33	38	47.5

DNA of begomoviruses and showed amplification in PCR. 3 Kb or ~2.7 Kb amplicon were obtained in Pusa Sawani and F_2 bulk, however, no amplication of 2.7 to 3 Kb was detected by other restriction enzyme. No band was found in the resistant parent, Pusa Bhindi-5 (Fig. 1).

Table 3. Disease reaction of six generations derived from the cross, Pusa Sawani × Pusa Bhindi-5 after 90 days of sowing

Generations	PDI (%)	RV	CI	Reaction category
	90 DAS	90 DAS	90 DAS	90 DAS
P_1 (Pusa Sawani)	95.6	1.00	95.6	HS
P_2 (DOV-66)	1	0.25	0.25	HR
F_1	2	0.25	0.50	HR
B_1 (F_1 × DOV-66)	2	0.25	0.50	HR
B_2 (F_1 × Pusa Sawani)	47.5	0.50	23.8	MS
F_2	29	0.50	14.5	MR

PDI = Percent disease incidence, PDS = Percent disease severity, CI = coefficient of infection

Effect of YVMV disease infestation on the nutritional quality of fruit

In an analysis of variance, mean square of protein (%), carbohydrates (%), fibre (%), total ash (%), potassium (mg/100 g), sodium (mg/100g), magnesium (mg/100 g), iron (mg/100 g), copper (mg/100 g) and zinc (mg/100 g) were found to be highly significant for all the six generations *i.e.* P_1, P_2, F_1, F_2, B_1 and B_2. This showed the presence of significant variation for these traits between the six generations. The mean values of different biochemical traits are presented in Table 5. Comparatively higher but non-significant protein content was recorded in F_1 (2.7%), followed by B_2 (2.14%) and F_2 (2.26) populations. F_1 recorded the maximum content of carbohydrates (5.56%) followed by B_1 (5.18%). There was no significant variation observed among the six generations with respect to dietary fiber content, i.e., P_1 (2.68%), P_2 (2.54%), F_1 (2.37%), and B_1 (2.36%). The maximum ash content was recorded in F1 (1.99%), which was significantly higher than in all other generations.

The highest potassium content (mg/100 g) was recorded in F_2 (223.72) followed by the P_2 (210) and B_2 (104.11) but the

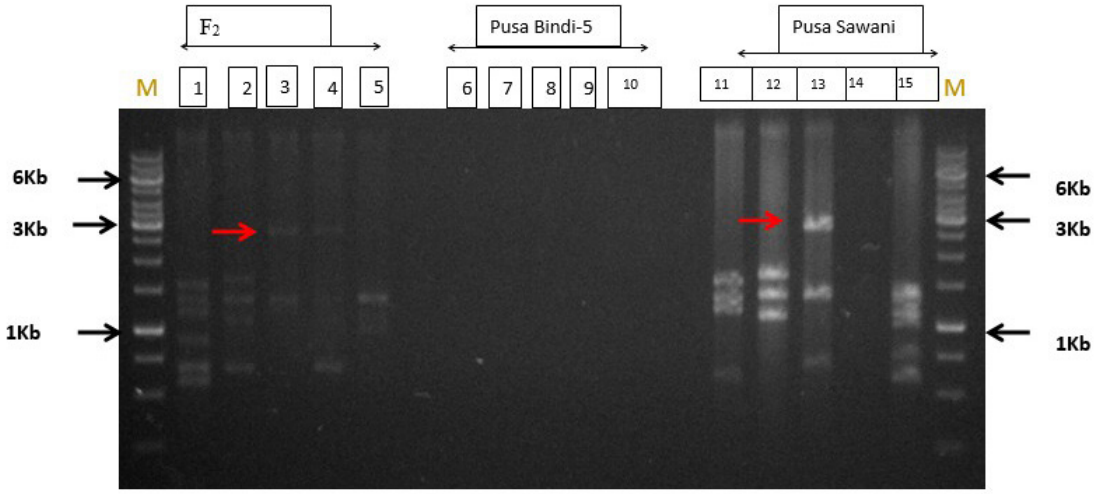

Fig. 1. Agarose gel analysis of rolling-circle amplification (RCA)-amplified DNA in okra genotypes: Pusa Sawani, 2: Pusa Bhindi-5, F_2 populations, lane 1, 6, 11 *BamH*I, lane 2, 7, 12 *EcoR*I, lane 3, 8, 13 *Hind* III, lane 4, 9, 14 *Sac*I, lane 5, 10, 15 *Kpn*I, lane 13 showed presence of begomovirus in Pusa Sawani

Table 4. Disease symptom and segregation pattern for yvmv resistance at 90 DAS in the cross of Pusa Sawani × Pusa Bhindi-5, F_1, F_2 and backcross generations of okra

S. No.	Generations	Number of Resistant plants	Number of Susceptible plants	Total number of plants	Expected ratio	χ^2 value (cal)	χ^2 value (tab)	P*
1	P_1 (Pusa Sawani)	4	86	90	-	-	-	-
2	P_2 (DOV-66)	90	0	90	-	-	-	-
3	F_1	120	0	120	-	-	-	-
4	B_1 (F_1 × DOV-66)	120	0	120	-	-	-	-
5	B_2 (F_1 × Pusa Sawani)	57	63	120	1:1	0.32	3.84	-
6	F_2	231	69	300	3:1	0.64	3.84	0.5–0.7

*P- Probability value

Table 5. Mean performance of generations of cross Pusa Sawani × Pusa Bhindi-5 for various biochemical traits

Genera-tions	Protein (%)	Carbo-hydrate (%)	Dietary fibre (%)	Total ash (%)	Potassium (mg /100g)	Sodium (mg /100g)	Magnesium (mg /100g)	Iron (mg /100g)	Copper (mg /100g)	Zinc (mg /100g)
P1	2.00 ± 0.02	4.06 ± 0.02	2.68 ± 0.1	1.31 ± 0.06	173.83 ± 8.81	6.61 ± 0.66	29.27 ± 0.37	2.09 ± 0.05	0.60 ± 0.01	0.35 ± 0.05
P2	2.10 ± 0.01	4.80 ± 0.10	2.54 ± 0.27	1.36 ± 0.04	210.16 ± 9.53	5.05± 0.08	31.82 ± 0.58	1.61 ± 0.01	0.48 ± 0.04	0.19 ± 0.01
F1	2.70 ± 0.02	5.56 ± 0.36	2.37 ± 0.18	1.99 ± 0.07	167.82 ± 4.62	6.86± 0.07	43.43 ± 0.30	2.74 ± 0.03	0.75 ± 0.05	0.30 ± 0.02
F2	2.26 ± 0.01	4.61 ± 0.03	2.64 ± 0.02	1.5 ± 0.01	223.72 ± 3.05	5.82± 0.14	36.51 ± 0.15	2.30 ± 0.07	0.58 ± 0.02	0.25 ± 0.01
B1	1.88 ± 0.16	5.18 ± 0.07	2.36 ± 0.18	1.41 ± 0.05	152.79 ± 8.32	4.99 ± 0.15	30.89 ± 0.28	2.29 ± 0.46	0.49 ± 0.08	0.21 ± 0.04
B2	2.14 ± 0.02	4.49 ± 0.04	2.70 ± 0.04	1.52 ± 0.02	204.11 ± 5.76	6.73 ± 0.06	34.77 ± 0.78	1.91 ± 0.03	0.57 ± 0.07	0.26 ± 0.03
SEm ±	0.04	0.08	0.10	0.02	4.28	0.14	0.29	0.11	0.03	0.29
C.D. (5%)	0.12	0.26	0.30	0.06	13.48	0.46	0.91	0.34	0.09	0.91
C.D. (1%)	0.18	0.36	0.43	0.09	19.18	0.65	1.30	0.48	0.12	1.30
C.V. (%)	3.12	2.93	6.49	2.17	3.93	4.17	1.46	8.67	8.16	1.46

difference was non-significant. Sodium content was also found to be at its maximum in the F1 (6.86) generation, which was slightly higher than the parent P_1 (6.61) and B_2 (6.73) generation. F_1 (43.43) recorded a maximum magnesium content significantly higher than the parental values. Iron content of P_2 (Pusa Bhindi-5) was significantly lower (1.61) than other generations such as P_1, F_1, B_1, B_2, which recorded 2.09, 2.74, 2.30, 2.29 mg/100 g, respectively. The maximum copper content (mg/100g) was recorded by F_1 (0.75). Zn content was recorded at the maximum in P_1, followed by F_1, B_2 and F_2.

Mean values (t-test) of biochemical traits of resistant and susceptible bulks of different generations of cross Pusa Sawani × Pusa Bhindi-5 were given in Table 6. The protein content in fruits of susceptible bulk (2.00) was significantly lower than the corresponding resistant bulk (2.45). A significant reduction of protein content was also observed in backcross population B_2 (F_1 × Pusa Sawani) susceptible bulk (1.97) versus the resistant bulk (2.31) and F_2 population bulks. The carbohydrate content was also significantly reduced with the incidence of disease i.e. resistant bulk (6.74) and the susceptible bulk (4.06) of Parent (Pusa Sawani) and segregating population (F_2), i.e. F_2 0 (5.37), F_2 I (5.27), F2II (5.07), F_2 III (4.51), F_2 IV (4.15), F_2 V (4.02) and F_2 VI (3.91).

The dietary fibre content significantly increased in the susceptible bulk (2.68) than that of the resistant bulk (2.28). The difference between the mean value of resistant bulk (2.06) and all other susceptible categories was found to be significant, except for the F_2 I category (2.19). A negative correlation was observed between total ash and the disease, which was recorded 1.58 and 1.46 for resistant and susceptible bulks, respectively. In F_2 population, a significant decrease in total ash content was observed with the progression of the disease. A significant increase in susceptible bulk was found as compared to that of the resistant bulk in parent and backcross population for sodium content in F_2 0 (5.03), F_2 I (5.05), and F_2 IV (6.10). The mean of susceptible bulk (173.83) was lesser than the resistant bulk (202.84) for potassium content. A negative relationship was observed between disease incidence and magnesium content on okra fruits in all populations. The rest of the bulks showed a significant decrease in magnesium content when compared with the resistant bulk in F_2. Iron content was found to be non-significantly higher in susceptible bulk (2.09) than the resistant bulk (1.95) for parents, whereas in backcross and F_2 populations iron content decreased with the disease incidence indicating a negative relationship. Increasing trend of zinc content with the disease incidence was observed in all the populations. Negative trends were recorded between the disease incidence and the copper content.

Discussion

By use of diverse germplasm in a hybridization program, the genetic yield potential of any variety can be improved. Availability of a desirable source of resistance is the prerequisite for developing resistant cultivars/hybrids. The resistance occurring within cultivated species is more desirable as this can

Table 6. Mean values (t- test) of biochemical traits of resistant and susceptible bulks of different generations of cross Pusa Sawani × Pusa Bhindi-5

Population	Dietary fibre (%)		Total ash (%)		Sodium (mg/100g)		Potassium (mg/100g)		Magnesium (mg/100g)	
	Mean	P value	Mean	P value	Mean	P value	Mean	P value	Mean	P value
PS (R)	2.28	0.00417*	1.57	0.003*	5.51	0.044761*	202.84	0.0067*	40.85	0.0029*
PS (S)	2.68		1.31		6.61		173.83		29.27	
B2 (R)	2.54	0.00578*	1.58	0.007	5.95	0.000011*	213.96	0.0173*	36.00	0.0448*
B2 (S)	2.85		1.46		7.51		194.26		33.54	
F2 0 (check)	2.06	0.09256	1.70	0.074	5.03	0.893046	256.22	0.243	39.54	0.4816
F2 I	2.19		1.58		5.05		245.34		39.28	
F2 0	2.06	0.01837*	1.70	0.007*	5.03	0.386087	256.22	0.0346*	39.54	0.0026*
F2 II	2.33		1.52		5.26		234.15		38.27	
F2 0	2.06	0.00014*	1.70	0.010*	5.03	0.000012*	256.22	0.0042*	39.54	0.0014*
F2 III	2.6		1.48		5.9		216.5		36.82	
F2 0	2.06	0.00128*	1.70	0.003*	5.03	0.083697	256.22	0.0036*	39.54	0.0003*
F2 IV	2.81		1.45		6.1		208.92		35.95	
F2 0	2.06	0.00006*	1.70	0.001*	5.03	0.00017*	256.22	0.0025*	39.54	0.0004*
F2 V	3.19		1.41		6.35		211.49		34.01	
F2 0	2.06	0.00015*	1.70	0.001*	5.03	0.00020*	256.22	0.0002*	39.54	0.0008*
F2 VI	3.31		1.37		7.09		193.40		31.70	
PS (R)	1.95	0.58096	0.21	0.01081*	0.61	0.829	6.74	0.00000005*	2.45	0.00167*
PS (S)	2.09		0.35		0.60		4.06		2.00	
B2 (R)	2.24	0.00013*	0.23	0.11145	0.58	0.93	5.27	0.00000019*	2.31	0.00007*
B2 (S)	1.57		0.28		0.57		3.72		1.97	
F2 0 (check)	2.78	0.42876	0.20	0.93314	0.60	0.055	5.37	0.0132356*	2.47	0.05724*
F2 I	2.65		0.21		0.58		5.27		2.38	
F2 0	2.78	0.03715*	0.20	0.00002*	0.60	0.004*	5.37	0.000340639*	2.47	0.01017*
F2 II	2.40		0.28		0.56		5.07		2.31	
F2 0	2.78	0.01561*	0.20	0.0879	0.60	0.023*	5.37	0.00000006*	2.47	0.00311*
F2 III	2.24		0.22		0.58		4.51		2.22	
F2 0	2.78	0.00487*	0.20	0.01096*	0.60	0.304	5.37	0.00000005*	2.47	0.00106*
F2 IV	2.08		0.26		0.59		4.15		2.18	
F2 0	2.78	0.00333*	0.20	0.00043*	0.60	0.033*	5.37	0.000000013*	2.47	0.00050*
F2 V	1.99		0.26		0.57		4.02		2.11	
F2 0	2.78	0.00230*	0.20	0.00009*	0.60	0.089	5.37	0.000000012*	2.47	0.00035*
F2 VI	1.93		0.28	0.01081*	0.59		3.91		2.03	

be more easily transferred (Dhankhar et al. 2005), for which knowledge of the gene action of resistance is also required. The generations mean analysis using six generations i.e. P_1, P_2, F_1, F_2, B_1 and B_2 developed from cross Pusa Sawani × Pusa Bhindi-5 was used to generate information on the nature of gene action for YVMV resistance. In addition, the effect of viral infection on nutritional quality trait was also worked out. In the present study, as per the results recorded the frequency distribution of F_2 generation of susceptible (S) × resistant (R) cross showed segregation in 3(R):1(S) pattern, which is in accordance with the monogenic dominant of Mendelian ratio. This monogenic dominant model was further confirmed by 1(R):1(S) ratio in the backcross population (B_2) as best fit with a high probability. However, it was observed that the inheritance pattern of YVMV resistance in okra differed from the source

of resistance (donar). Jambale and Nerkar (1981) reported single dominant gene resistance to YVMV disease in a cross between *A. esculentus* × *A. manihot* ssp. *manihot*. Senjam et al. (2017) also reported the prevalence of single dominant gene governing the YVMV disease resistance by crossing tolerant × susceptible cultivars of okra. Similar results were obtained by Dutta (1991) in *A. tetraphyllus* and cultivated okra. In contrast to these, two complementary dominant genes for resistance to YVMV were also reported (Thakur, 1976; Sharma and Dhillon 1983; Sharma and Sharma 1984 and Dhankar et al. 2005). Two recessive genes controlling YVMV resistance were reported in Pusa Sawani (Singh et al. 1962). Both, major and minor genes were reported by Arora et al. (2008) and Pullaiah et al. (1998) in inter-varietal crosses. Singh and Thakur (1979) reported the quantitative nature of YVMV resistance in wild *Abelmoschus*. While Ali et al. (2000) reported that tolerance in IPSA OKRA 1 is quantitative, with possibly two major factors and dependent on gene lineage with incompletely dominant gene action. They found that some progenies of B_1 (with resistant parent) exhibited susceptibility to YVMV. In contrast, epistatic interaction in resistance to viral infection was reported by Padmanabha et al. (2022). Therefore, it is clear that the genetics of resistance varies from germplasm to germplasm, hence, understanding the genetics of resistance in donor parent will help in the selection of a specific breeding strategy.

After 90 days of sowing, the okra leaf samples were analyzed for the presence of begomovirus through RCA technique. Result showed that out of five restriction enzymes, namely *BamH*I, *EcoR*I, *Hind*III, *Sac*I, *Kpn*I, only *Hind* III could digest the circular DNA of okra begomoviruses and showed amplification in PCR. 3 Kb or ~2.7 Kb amplicon, possibly could be any begomovirus, were obtained in Pusa Sawani and F_2 Bulk. However, no begomovirus was detected in sample with *Hind* III, *Pst* I restriction enzymes in Pusa Bhindi-5. The RCA was done to get it confirmed the presence of a symptom-less carrier in the resistant variety Pusa Bhindi-5. On the basis of RCA, it was concluded that the remittance to YVMV disease in variety Pusa Bhindi-5 had real as its plants were free from yellow vein mosaic virus symptoms and also showed absence of begomovirus in molecular analysis. Similar studies were also done by Blanco et al. (1989) and Rockett et al. (2015). Moreover, Hassan (2019) also identified the presence of new *Begomoviruses* through rolling circle amplification-based detection and recombination analysis of Squash leaf curl virus in Egypt.

The biochemical studies found significant differences between mean values of YVMV-infected and healthy fruits for nutrient contents, such as protein, dietary fibre, total ash, carbohydrate and minerals. In the present study most of the nutrients were negatively affected by the disease, whereas dietary fibre and sodium content showed enhancement with the progression of the disease. This is obvious as because of improper uptake, assimilation, and translocation of nutrients from root to shoot when plants are infected by the pathogen (Marschner 1995). The increase of dietary fibre content was observed in diseased fruits collected from infected plants of parents, backcross population B_2 (F_1 × Pusa Sawani), and in F_2 generation. The increased dietary fibre in diseased fruits might be due to the premature aging of fruits due to pathogen infection. Similarly, increase in sodium content in all populations, i.e., parent, backcross and F_2 populations, was observed. An increase in sodium content was also observed in Witches' broom (caused by *Candidatus Phytoplasma aurantifolia*) infected plants of Acid limes (*Citrus aurantifolia*) as reported by Al-Ghaithi et al. (2016). The reduction in nutrient content of diseased plants might be due to less synthesis of these nutrients in infected fruits due to reduced chlorophyll pigment in leaves. The reduction in nutrient content was significant for most of the minerals (Ragaee et al. 2006); however, no clear-cut trend was found in zinc and copper content. However, in general, Zn and Cu-deficient plants are highly susceptible to plant diseases (Grewal et al. 1996 in wheat and Helfenstein et al., 2015 in soybean) as these minerals protect plants against pest and disease infestations (Marschner, 1995). Khaskheli et al. (2017) reported that the yellow vein mosaic virus significantly reduced plant height, number of leaves, flowers, fruits, and overall pickings and yield of okra. Most resistant genotypes were reported to be very poor in yield and fruit quality (Nizar et al. 2004). Similarly, YVMV incidence has considerably affected the nutritional content of okra fruits. Due to the huge quantitative yield loss caused by YVMV, its effect on fruit quality and nutrition is often overlooked. Hence, in the current study an effort was made to ascertain the qualitative loss caused by this virus. Furthermore, the nutritional profile of the cultivar Pusa Bhindi-5 was obtained, and it showed an acceptable level. In the future, more work need to be done to generate information related to the variable effect of virus infection on the accumulation of minerals/ nutrition and associated defense mechanisms.

Authors' contribution

Conceptualization of Research (RKY, VND); Designing of experiments (RKY, VND); Contribution of the experimental materials (RKY, VND); Execution of the field/lab experiments and data collection (VND, RKY, AT, VKB, BBS, AD); Analysis of the data and interpretation (VND, AT, SL, VKB); Preparation of the final manuscript (VND, RKY, AT, VKB, BBS, SL, AD).

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