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PHENOTYPIC SCREENING UNDER NATURAL INFECTION CONDITIONS IDENTIFIES OKRA GENOTYPES WITH RESISTANCE TO YELLOW VEIN MOSAIC VIRUS

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ABSTRACT

Yellow Vein Mosaic Virus (YVMV) is a devastating viral disease transmitted through white fly (*Bemisia tabaci*) in okra. The disease adversely affects the quality of fruit and yield. A total of 45 okra genotypes, including wild species and cultivated varieties, were screened for resistance to YVMV under natural infection conditions. The resistance classification ranged from Highly Resistant (HR) to Highly Susceptible (HS), based on per cent disease incidence (PDI) and coefficient of infection (CI). Wild species such as *A. moschatus*-UAHS1, *A. manihot*-UAHS6 and *A. ficulneus*-UAHS3 exhibited high resistance with yields ranging from 315.00 g to 330.00 g per plant and low PDI (4.5%-6%). Cultivated genotypes, such as Arka Anamika and Hybrid-135, were classified as moderately resistant (MR), with yields ranging from 190.40 g to 220.10 g per plant and moderate disease incidence (15.5%-45%). Genotypes such as Pusa Sawani and PKV-2 were highly susceptible (HS), exhibiting yields of less than 100 g per plant and PDI values approaching 100%. The variability in disease response underscores the importance of incorporating resistant genotypes into future crop improvement efforts for sustainable okra production. This study highlights the presence of stable YVMV resistance in certain wild species, which can be utilized in breeding programs for resistant okra varieties.

Keywords : Okra, Yellow Vein Mosaic Virus, Natural infection screening, Disease resistance, Wild *Abelmoschus* species.

Introduction

Okra (*Abelmoschus esculentus* L. Moench), also known as bhindi, is a crucial vegetable crop in tropical and subtropical regions, particularly in India, where it holds significance in both domestic consumption and export markets (Suma *et al.*, 2023). The cultivated species i.e., *A. esculentus* have somatic chromosome number 130. Okra appears to have originated in South Africa or Asia (Thompson and Kelley, 1957). Young okra fruit contain good amount of vitamins A, B, C as well as protein, carbohydrates, fats, minerals, iron and iodine. 100 g of fresh okra fruits provides 20%, 15%, and 50% of the daily requirement of calcium, iron and ascorbic acid, respectively (Gemedede *et al.*, 2015). The old mature fruits are also used in processed products (Dantas *et al.*, 2021). However, one of the primary

biotic constraints affecting okra production is Yellow Vein Mosaic Virus (YVMV), which is transmitted by the whitefly (*Bemisia tabaci*). YVMV causes severe damage to the crop, characterized by symptoms such as chlorosis, yellowing of leaf veins, stunting and malformed fruits, leading to significant yield loss (Mubeen *et al.*, 2021).

The first report of YVMV in India dates back to 1924 and since then, the virus has become widespread, especially during the rainy season when environmental conditions are favourable for the whitefly vector (Mishra *et al.*, 2017). Yield losses due to YVMV can range from 50% to 94%, depending on the timing of infection. Early infections, particularly within the first 20 days after germination, can result in near-total crop failure, emphasizing the importance of developing

resistant varieties to mitigate these losses (Mishra *et al.*, 2017). Efforts to control YVMV in okra through chemical treatments, such as insecticides, have been largely ineffective due to environmental pollution, pest resistance and health risks associated with excessive pesticide use. The non-selective use of insecticides also leads to resurgence, new biotype mutation and also damages the environmental health. Therefore, identifying and development of resistant variety is an effective way for mitigation of these diseases. Genetic resistance, being more sustainable, remains the most effective strategy for managing YVMV (Singh and Maitreya, 2021).

Previous breeding programs have focused on identifying sources of resistance to YVMV in cultivated okra varieties. However, resistance in these varieties has often been short-lived due to the continuous evolution of viral strains and the breakdown of existing resistance mechanisms (Vidya *et al.*, 2023). As a result, there has been a growing need to search for new, stable sources of resistance, particularly in the wild relatives of okra, which are known to harbor more robust resistance traits. Research has shown that wild species such as *A. moschatus*, *A. manihot*, and *A. ficulneus* contain significant levels of resistance to YVMV (Sandeep *et al.*, 2022). In the past, *A. manihot ssp. manihot* and *A. manihot ssp. tetraphyllus* have been used for the development of YVMV disease resistant okra cultivars such as Punjab-7, Punjab Padmini, Parbhani Kranti, Arka Abhay and Arka Anamika. Presently, all these varieties have become susceptible, thus necessitating a constant search and transfer of newer sources/accessions of YVMV disease resistance (Mishra *et al.*, 2017). These wild species have demonstrated high resistance in various studies, making them ideal candidates for introgression into cultivated okra varieties through breeding programs.

This study aimed to evaluate 45 okra genotypes, including wild species and cultivated varieties, for their

response to YVMV under natural field conditions. The genotypes were assessed based on the per cent disease incidence (PDI) and coefficient of infection (CI), combining disease severity and the extent of infection. The results of this screening are critical for identifying stable sources of resistance that can be incorporated into breeding programs aimed at developing high-yielding, disease-resistant okra varieties.

Methods and Materials

Experimental Design and Site Description

The experiment was conducted during the 2021 *kharif* cropping season at the Zonal Agricultural and Horticultural Research station, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga. The field conditions were conducive to the natural spread of YVMV, with whitefly populations present in significant numbers. A total of 45 genotypes were included in the study, representing both wild species and cultivated varieties of okra. The wild species used in this study included *A. moschatus*, *A. manihot* and *A. ficulneus*, while the cultivated varieties included popular genotypes such as Pusa Sawani, Arka Anamika, and Hybrid-135. The experimental design was a randomized block design (RBD) with three replications to ensure reliable and statistically significant results. Each entry was sown at 60 x 30 cm spacing accommodating 30 plants in each plot. The standard recommended packages of practices were followed for raising a healthy crop.

Disease Screening and Resistance Classification

To assess the resistance of each genotype to YVMV, per cent disease incidence (PDI) was recorded at 60 days after sowing. The PDI was calculated using the formula:

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Table 1 : Scale used to classify the genotypes (Santhiya *et al.*, 2022).

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

In addition to PDI, the coefficient of infection (CI) was calculated by multiplying the PDI by a severity rating scale. The severity scale ranged from 0

(immune) to 4 (highly susceptible). Plants showing no symptoms were rated as 0, and those showing severe symptoms (over 75% leaf damage) were rated as 4.

The CI values allowed for a comprehensive assessment of disease incidence and severity across the genotypes.

Yield and Agronomic Observations

At the end of the cropping season, the yield per plant was recorded from each picking for each genotype. Yield measurements were taken to assess the impact of YVMV resistance on overall productivity. High-yielding genotypes were identified and their disease resistance was compared to those with lower yields. This data helps determine not only the level of resistance to YVMV but also the potential for using these genotypes in breeding programs to develop high-yielding, disease-resistant okra varieties.

Results and Discussion

Disease Incidence and Resistance Classification

The resistance of 45 okra genotypes, including both wild species and cultivated varieties, to YVMV was assessed under natural infection conditions. Based on per cent disease incidence (PDI) and coefficient of infection (CI), the genotypes were classified into five categories: Highly Resistant (HR), Moderately Resistant (MR), Moderately Susceptible (MS), Susceptible (S), and Highly Susceptible (HS).

Highly Resistant (HR) Genotypes

Among the 45 genotypes, three wild species *viz.*, *A. moschatus* UAHS1, *A. manihot* UAHS6 and *A. ficulneus* UAHS3 stood out as Highly Resistant (HR) to YVMV. These wild species exhibited remarkably low PDI values (4.5%–6%) and low CI values (10–14), reflecting robust resistance mechanisms against YVMV. These genotypes also maintained high yields, ranging from 315.00 g to 330.00 g per plant, further supporting that high resistance to YVMV can be associated with good productivity. *A. moschatus*-UAHS1 showed a yield of 315.00 g per plant with a PDI of 5.10% and a CI of 12.20, making it one of the top-performing genotypes for YVMV resistance. *A. manihot*-UAHS6 exhibited a yield of 330.00 g per plant, a PDI of 4.50%, and a CI of 10.00, which reinforces its potential as a highly resistant genotype. *A. ficulneus*-UAHS3 also demonstrated high resistance with a yield of 320.00 g per plant, a PDI of 6.00%, and a CI of 14.40. These findings align with earlier studies identifying wild relatives as valuable sources of YVMV resistance in okra (Kaur *et al.*, 2020). These species provide a genetic reservoir for resistance traits, making them critical for breeding programs aiming to develop YVMV-resistant cultivars.

Table 2: Classification of okra genotypes into resistance groups based on Percent Disease Index (PDI), Coefficient of Infection (CI) and yield under natural YVMV epiphytotic conditions.

Sl. No.	Genotype	Resistance Class	Yield (g/plant)	PDI (%)	CI
1	<i>A. moschatus</i> -UAHS1	HR	315.00	5.10	12.20
2	<i>A. manihot</i> -UAHS6	HR	330.00	4.50	10.00
3	<i>A. ficulneus</i> -UAHS3	HR	320.00	6.00	14.40
4	<i>A. moschatus</i> -UAHS4	MR	273.63	7.20	16.12
5	<i>A. ficulneus</i> -UAHS5	MR	255.35	12.80	28.94
6	<i>A. manihot</i> -UAHS2	MR	240.25	14.30	31.65
7	UAHS1	MR	220.10	15.50	34.10
8	UAHS2	MR	210.00	16.50	35.50
9	UAHS3	MR	200.00	18.00	38.00
10	UAHS4	MR	190.10	20.00	40.00
11	<i>A. moschatus</i> -UAHS7	MR	180.00	21.20	42.40
12	<i>A. manihot</i> -UAHS8	MR	170.00	22.50	44.20
13	<i>A. ficulneus</i> -UAHS9	MR	160.00	24.00	48.00
14	<i>A. moschatus</i> -UAHS10	MR	150.00	26.00	50.00
15	<i>A. ficulneus</i> -UAHS11	MR	140.00	27.00	52.00
16	<i>A. manihot</i> -UAHS12	MR	130.00	28.00	54.00
17	UAHS5	MS	125.50	30.00	56.00
18	UAHS6	MS	120.00	32.00	58.00
19	UAHS7	MS	115.50	34.00	60.00
20	UAHS8	MS	110.00	36.00	62.00
21	JHN-4	S	105.60	60.00	120.00
22	Dhanraj	S	95.50	75.00	150.00
23	Pusa Badami	S	88.00	85.00	170.00
24	PKV-1	S	90.50	76.00	140.00
25	Pusa Sawani (Check)	HS	110.30	100.00	400.00

26	Pusa Bhindi-1	S	95.50	74.00	145.00
27	Arka Anamika	MR	220.10	15.50	34.10
28	Hybrid-135	MS	210.20	45.00	65.00
29	Prerna 321	MS	200.30	48.00	68.00
30	Arka Abhay	MR	190.40	50.00	70.00
31	S-8	MS	175.50	53.00	75.00
32	TNAU-15	MS	165.20	55.00	80.00
33	RDO-3	MS	160.50	58.00	85.00
34	TNAU-12	MS	155.60	60.00	90.00
35	Hybrid-143	S	150.00	63.00	95.00
36	Pusa Makhmali	S	145.00	65.00	100.00
37	JOL-1	HS	100.00	90.00	180.00
38	Arka Satya	HS	95.50	92.00	185.00
39	JOL-2	HS	90.00	95.00	190.00
40	Pusa Sawani-2	HS	85.00	97.00	195.00
41	Pusa Bhindi-2	HS	80.00	99.00	200.00
42	PKV-2	HS	75.00	100.00	210.00

Moderately Resistant (MR) Genotypes

A total of 15 genotypes were classified as Moderately Resistant (MR), with disease incidence ranging from 7.20% to 45% and CI values between 16.12 and 70. These genotypes, while exhibiting moderate resistance, demonstrated acceptable yields ranging from 130.00 g to 220.10 g per plant. UAHS1 displayed a yield of 220.10 g per plant with a PDI of 15.50% and a CI of 34.10, signifying its moderate resistance to YVMV. Hybrid-135 showed a yield of 210.20 g per plant, with a PDI of 45% and a CI of

65.00, placing it in the moderately resistant category. Arka Anamika, one of the commonly grown varieties, exhibited a yield of 220.10 g per plant, a PDI of 15.50%, and a CI of 34.10, indicating its potential for moderate resistance in breeding programs (Vinay *et al.*, 2024). These genotypes likely harbour partial resistance mechanisms, which limit the spread and severity of the virus within plants, providing an opportunity for high productivity under field conditions despite the virus presence.



Fig. 1: Symptoms of Yellow Vein Mosaic Virus (YVMV) in okra under natural field conditions showing prominent leaf chlorosis and vein yellowing.

Moderately Susceptible (MS) Genotypes

Ten genotypes were categorized as Moderately Susceptible (MS), with PDI values ranging from

30.00% to 60.00% and CI values between 56.00 and 100.00. These genotypes exhibited higher disease incidence and severity but showed a moderate level of

resistance when compared to the S and HS genotypes, with yields ranging from 95.50 g to 170.00 g per plant. S-8 showed a yield of 175.50 g per plant with a PDI of 53.00% and a CI of 75.00, indicating moderate susceptibility. TNAU-15, another MS genotype, had a yield of 165.20 g per plant with a PDI of 55.00% and a CI of 80.00. The MS genotypes may serve as intermediates for breeding programs, offering a balance between disease tolerance and desirable agronomic traits such as fruit quality or drought tolerance (Deshmukh *et al.*, 2011). These genotypes are not entirely immune to YVMV but demonstrate sufficient resistance to sustain moderate productivity levels.

Susceptible (S) Genotypes

A group of genotypes, including JHN-4, Dhanraj, Pusa Badami and PKV-1, were classified as Susceptible (S), with PDI values ranging from 60.00% to 85.00% and CI values from 120.00 to 170.00. These genotypes exhibited high disease incidence and severe symptoms of YVMV, resulting in substantial yield reductions. Yields for these genotypes ranged from 75.00 g to 105.60 g per plant. JHN-4 yielded 105.60 g per plant, with a PDI of 60.00% and a CI of 120.00, indicating susceptibility. Dhanraj exhibited a yield of 95.50 g per plant, with a PDI of 75.00% and a CI of 150.00. These genotypes represent the most vulnerable cultivars to YVMV and are not suitable candidates for YVMV-resistant breeding programs. However, they can serve as controls in disease management studies, allowing researchers to test the efficacy of various treatments (Mkhabela *et al.*, 2022).

Highly Susceptible (HS) Genotypes

Check variety Pusa Sawani and others, such as JOL-1, Arka Satya, and Pusa Bhindi-2, were classified as Highly Susceptible (HS) with PDI values from 90.00% to 100.00% and CI values between 180.00 and 400.00. These genotypes showed the most severe symptoms and the lowest yields, highlighting their vulnerability to YVMV. For example, Pusa Sawani yielded only 110.30 g per plant, with a PDI of 100.00% and a CI of 400.00. JOL-1, Arka Satya, and Pusa Bhindi-2 also showed similar susceptibility, with severe disease expression and reduced yields. The high disease incidence and yield loss in these genotypes highlight the urgent need for breeding programs to focus on improving resistance to YVMV in commercially cultivated varieties (Alphy *et al.*, 2020).

Implications for Breeding Programs

The results from this study have significant implications for the development of YVMV-resistant okra varieties. The identification of highly resistant

wild species, such as *A. moschatus*-UAHS1, *A. manihot*-UAHS6, and *A. ficulneus*-UAHS3, provides a valuable genetic resource for breeding programs. These wild species can be used as donors for resistance genes, which can be introgressed into cultivated varieties using conventional breeding or advanced molecular methods like marker-assisted selection (MAS) (Dhankhar 2016). Moderately resistant genotypes, such as Arka Anamika and Hybrid-135, also offer valuable options for breeding programs targeting YVMV resistance. These genotypes provide a source of resistance while maintaining acceptable yield levels, making them suitable for hybridization programs to combine high yield potential with disease resistance.

Moreover, the moderate resistance observed in MS genotypes offers opportunities for developing varieties suitable for regions with moderate disease pressure. These genotypes could serve as sources of intermediate resistance, balancing disease tolerance with other desirable traits. The results underline the importance of maintaining genetic diversity in okra breeding programs. By incorporating both wild species and cultivated varieties with different levels of resistance, breeders can create a more diverse genetic pool, increasing the likelihood of developing durable resistance to YVMV (Mishra *et al.*, 2021).

Genetic Insights and Future Research Directions

Future research should focus on elucidating the molecular and genetic mechanisms underlying YVMV resistance in these wild species. Techniques like genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping can help identify the specific genes responsible for resistance. The identification of these genes will enable marker-assisted breeding programs, significantly speeding up the development of resistant varieties. Genetic transformation technologies, such as the use of viral resistance genes (e.g., coat protein genes) or RNA interference (RNAi) and mutagenesis could offer alternative strategies for managing YVMV in okra (Abhishek *et al.*, 2023; Hazra *et al.*, 2023). Exploring transgenic approaches for enhancing YVMV resistance could further diversify the tools available for managing this virus. The long-term stability of YVMV resistance in the identified genotypes should also be monitored. Over time, the virus may evolve to overcome existing resistance mechanisms, so continuous evaluation of resistance stability is crucial. Breeders should work toward developing new sources of resistance as the virus continues to evolve.

Conclusion

This study highlights the potential of wild species such as *A. moschatus*-UAHS1, *A. manihot*-UAHS6, and *A. ficulneus*-UAHS3 as valuable sources of stable resistance to YVMV in okra. The moderate resistance observed in cultivated varieties like Arka Anamika and Hybrid-135 also offers potential for breeding high-yielding, disease-resistant varieties. The integration of wild species into breeding programs is essential for developing sustainable solutions to YVMV, ensuring that okra production remains viable in regions affected by this devastating virus.

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Competing Interests

The authors declare no competing interests.

Contributions

Conceptualization, B.M.D. and S.N.; methodology, S.N, B.M.D. and V.R.; software, S.N; formal analysis, S. N; investigation.; data curation, S. N.; writing-original draft preparations. S. N., B.M.D.; supervision, B.M.D.; All authors have read and agreed to the published version of the manuscript.

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