*Review Article*

Yellow Vein Mosaic Disease (YVMD) resistance in okra (*Abelmoschus esculentus* L. Moench) through conventional breeding; opportunities and challenges

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ABSTRACT

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| Yellow Vein Mosaic Disease (YVMD), caused by begomovirus, represents the most detrimental threat to okra cultivation worldwide, significantly compromising yield, productivity and quality. As chemical interventions offer inadequate and often impractical control, the development of resistant or tolerant cultivars stands as the most practical long-term strategy. Substantial research efforts have identified potential resistance sources and elucidated the genetic basis of YVMD resistance. In India, public research institutions and private companies have employed various conventional breeding approaches including pure line selection, pedigree breeding, interspecific hybridization with backcrossing, and mutation breeding, to develop YVMD-resistant varieties. Effective utilization of okra genetic resources in resistance breeding programmes requires comprehensive knowledge of resistance sources in both cultivated and wild gene pools. This review synthesizes current knowledge on YVMD resistance sources across cultivated and wild okra germplasm, examines conventional breeding strategies for developing resistant cultivars, identifies major constraints encountered during breeding efforts, and proposes approaches to overcome these limitations. By consolidating the existing knowledge, this article provides a strategic framework to guide future okra breeding programmes focused on developing YVMD-resistant varieties with superior agronomic performance. |

*Keywords: Okra, YVMD, conventional breeding, source of resistance, wild relatives*

1. INTRODUCTION

 Okra [*Abelmoschus esculentus* (L.) Moench; 2n = 2x = 130], an important and popular warm-season vegetable belongs to the Malvaceae family, is globally recognized by various local names, including; lady's finger, gumbo, and bhindi in different regions of the world. This crop provides an affordable array of essential nutrients, such as carbohydrates, minerals, proteins, vitamins, dietary fiber, and beneficial phytonutrients. Furthermore its seeds yield edible oil rich in unsaturated fatty acids (Anwar *et al.*, 2020). Okra exhibits broad adaptability, thriving in tropical, subtropical, and warm temperate climates (Kochhar, 1986). India stands as the primary global producer of okra, with an annual production of 7.16 million tonnes and a productivity of 13.0 tonnes/ha across a cultivation area of 0.55 million ha (FAOSTAT, 2023). Cytogenetic studies suggest that the cultivated okra is likely an amphidiploid derived from *Abelmoschus tuberculatus* (2n=58) and an unidentified species with 2n=72 (Datta & Naug 1968). While primarily valued for its immature pods, okra also offers diverse applications. The leaves, flowers and buds of okra are also incorporated into culinary practices, in the West African countries. The dried seeds can be used as source of protein, oil, vegetable curd, and even a coffee additive or substitute. Additionally, okra foliage and stem possess industrial application, in that they may be utilized for paper pulp processing or as source of fuel (Sharma 1993).

 Biotic and abiotic stresses are major constraints limiting okra productivity. Even though okra is considered as a hardy crop, its production and yield worldwide are substantially constrained by Yellow Vein Mosaic Disease (YVMD). It is a detrimental disease caused by Yellow vein mosaic virus (YVMV), a species belonging to genus Begomovirus, within Geminiviridae family (Fauquet & Stanley 2005), affecting quality and yield in okra and it is transmitted through the vector white fly (*Bemisia tabaci*). YVMD is characterized by a distinctive reticulate pattern of yellow veins enclosing green islands of tissue on the leaves. In case of severe infections, leaves may become entirely yellow or creamy. The severity of YVMD infection in okra decreases with later infection timing (Venkataravanappa *et al.,* 2013). Studies have reported yield losses ranging from 50–94% depending on the plant's growth stage at the time of infection (Sastry and Singh 1974, Pun and Doraiswamy 1999).

 Given the unsustainable nature, economic feasibility and environmental consequences of chemical control measures, developing resistant okra varieties is the most viable long-term solution to YVMD. Conventional breeding methodologies, encompassing introduction, selection and hybridization can be can be utilized to achieve this objective. Furthermore, interspecific hybridization followed by backcrossing and selection in segregating generations has proven effective in developing disease-resistant interspecific okra hybrids. Alternative breeding strategies include backcross breeding followed by pedigree selection, as well as mutation breeding techniques (Pitchaimuthu 2020).

 Arumugam and Muthukrishnan (1978) opined that *Abelmoschus esculentus* lacks endogenous resistance to YVMD. Consequently, they recommended redirecting search for resistance towards related wild species. There are several wild varieties of okra known to be immune to YVMD. Utilizing these YVMD-resistant wild germplasm to introgress resistance into cultivated okra, offer an effective and well-established approach to address this challenge.

2. Genetic Resources for YVMD Resistance

**2.1 Resistance Sources in Cultivated Germplasm**

 The fundamental step of breeding for disease resistance is the identification of suitable resistance sources, ideally within the cultivated species itself or in closely related wild relatives. While resistance genes from wild species can be valuable, sources within the cultivated gene pool are often prioritized due to the relative ease of their introgression into otherwise agronomically superior but susceptible varieties (Dhankhar *et al*., 2005).

 *Abelmoschus* *esculentus* variety 'IC 1542' from West Bengal was found to be symptomless, carrier, which is field resistant to YVMD and it is one of the parent involved in development of 'Pusa Sawani', first YVMD-tolerant cultivar in India (Singh *et al*., 1962).

Subsequent field trials have further expanded the list of identified resistant genotypes. Screening of sixty okra genotypes for YVMD resistance under field conditions, revealed seven accessions (KRCO-15, KRCO-28, KRCO-10, KRCO-3, MHO-30, MHO-10 and MHO-24) having resistance to YVMD (Prakash *et al.,* 2017). Similarly, Nizar *et al*. (2004) screened 62 high yielding okra accessions, for field resistance of YVMD under natural epiphytotic condition, finding no immune or highly resistant lines, but identifying EC 305619, IC 69286, and IC 218887 as possessing resistance. In another study involving 19 okra varieties, Hybrid No.-10 and Soumya F1 (OH-4002) showed moderate resistance, whereas Hybrid No.-8 exhibited resistance against YVMV (Saurabh *et al*., 2016). Batra and Singh (2000) documented complete absence of YVM disease symptomatology in some okra cultivars, namely P-7, LORM-1, Ok No.-6 and VRO-3. Consistent with these findings, field screening of 12 okra germplasm lines by by Rashid *et al.* (2002) identified OK-285 and OK-292 as resistant to YVMV infection. Sanwal *et al* (2014 b) in a field screening identified several cultivated lines *viz*., VROB 178, VRO 109, 307 10-1, No. 315 and VRO 104 that exhibited complete freedom from YVMD symptoms, representing valuable germplasm resources for future okra improvement programs. Out of 565 genotypes screened, by Jamir *et al. (*2020) only BCO-1 exhibited resistance to YVMD, emphasizing the relative scarcity of highly resistant sources in some germplasm collections. More recently, Puneeth *et al*. (2022) screened 64 okra genotypes for resistance against YVMD and found the most resistant cultivated genotypes were DOV-89, DOV-92 and Pusa Bhindi-5 (DOV-66), highlighting their potential for exploitation in future breeding programs aimed at enhancing YVMD resistance.

**2.2 Wild *Abelmoschus* species as resistance source**

 Wild relatives of okra represent potential germplasm resources for developing YVMD resistant hybrids. The possibility of using these wild relatives of okra (*Abelmoschus* ssp.) possessing inherent resistance to yellow vein mosaic disease in Indian breeding programs aimed at developing resistant varieties has been described (Nerkar, 1991). *Abelmoschus manihot* ssp manihot has been identified as an excellent source of Yellow Vein Mosaic Virus (YVMV) resistance (Sharma & Sharma 1984) and has been extensively utilized in resistance breeding programs. These genetic resources offer significant potential for developing YVMV-resistant okra hybrids. True resistance to Yellow vein mosaic has been documented in several *Abelmoschus* species, including certain forms of *A. manihot*, *A. crinitus*, *A. pungens*, *A. caillei*, *A. angulosus, A. panduraeformis,* *A. tetraphyllus* and *A. vitifolius*. *A. manihot* subspecies *manihot,* *A. manihot* subspecies *manihot* variety 'Ghana' and *A. tuberculatus* were found to be asymptomatic carrier for YVMV (Dhankar and Mishra 2004, Singh *et al*., 2007). Out of which the highest number of resistant germplasm lines was found in the wild taxa of *A. tetraphyllus*, followed by *A. callei*, both of which hold potential for developing resistant varieties.

 In a study evaluating YVMD resistance across four seasons, Prabu *et al*. (2007) screened various wild and cultivated okra lines. Their findings indicated that *Abelmoschus angulosus,* the wild species was completely asymptomatic. A high level of resistance was observed in *A. manihot* ssp. *tetraphyllus, A. tetraphyllus* (lines 1, 2, 3, 4), *A. caillei*-2, and *A. moschatus* (lines 1, 2, 3, 4, 5). Additionally, lines classified as resistant included *A. manihot* ssp. *manihot, A. manthot* (L.) Medikus*,* and *A. tetraphyllus*-5. Sanwal *et al.* (2014a) reported resistance to YVMD in specific accessions of several wild *Abelmoschus* species. These resistant accessions included *A. moschatus* (NIC5952), *A. tuberculatus* (IC90340 and IIVR Tube-1) *A. enbeepeegeerense* (IC582757), and *A. manihot* (Jpn/N-2176).

 Screening of 24 wild genotypes under natural epiphytotic conditions for Yellow Vein Mosaic Disease (YVMD) resistance led to the identification of three highly resistant accessions: *Abelmoschus caillei* (Sikkim accession), *A. moschatus* (IC 141055) and *A. tetraphyllus* (IC 90476-1) (Santhiya *et al*., 2022). Seth *et al*. (2016) reported that, *A. caillei* and *A. manihot* are highly promising candidates for YVMD resistance breeding programs as they exhibited the highest levels of resistance to the disease along with other desired attributes.

 Singh *et al*. (2023) evaluated sixteen accessions representing seven distinct wild okra species, alongside two cultivated okra cultivars (Pusa Sawani and Punjab Padmini), for their resistance to YVMD. They identified accessions; IC-203833 and IC-470751 of *Abelmoschus angulosus* and accessions; SBT-12592 and SBT-12557 of *Hibiscus acutetus* as new sources, demonstrating a highly resistant reaction to the disease.

**3. BREEDING FOR YVMD RESISTANCE THROUGH CONVENTIONAL MEANS**

 Various breeding approaches are utilized in okra improvement, including plant introduction, pureline selection, and hybridization. Interspecific hybridization combined with backcrossing and selection within segregating populations represents the optimal strategy for developing interspecific okra hybrids. Additional effective breeding methodologies include backcrossing followed by pedigree selection, mutation breeding, polyploidy breeding and integrated approaches that combine conventional techniques with marker-assisted backcross breeding (Bisht and Bhat, 2006, Pitchaimuthu, 2020).

 Prior to 1950, India lacked improved okra cultivars. Dr. Harbhajan Singh was the pioneer in okra breeding, who initiated germplasm collection and varietal improvement efforts in the 1950’s in India. His significant contribution was the development of Pusa Sawani, India's first Yellow Vein Mosaic Disease (YVMD) resistant variety, derived from an inter-varietal cross between IC-1542 (asymptomatic carrier of YVMD from West Bengal) and Pusa Makhmali. Pusa Sawani demonstrated field resistance to YVMD while exhibiting superior agronomic traits (Singh *et al*., 1962). It remained the predominant YVMD-resistant variety for several decades until eventually becoming susceptible to the disease. The National Bureau of Plant Genetic Resources (NBPGR) identified a highly YVMD-resistant introduction from Ghana belonging to *Abelmoschus manihot* ssp. *manihot* This valuable germplasm resource was subsequently incorporated into various breeding programs throughout India during the 1980s, facilitating the development of numerous resistant breeding lines and leading to the release of several commercially important resistant varities *viz*., Punjab Padmini (Sharma, 1982), Punjab-7 (PAU) (Thakur & Arora, 1988), and Parbhani Kranti (VNMKV) (Jambhale & Nerkar, 1986), developed through interspecific hybridization combines with backcrossing and selection. Whereas, varieties such as Varsha Uphar, Hisar Unnat (CCSHAU), Kashi Pragati Kashi Vibhuti, Kashi Sathdhari, Kashi laila, Kashi Mohini and Kashi Kranti (IIVR, Varanasi) were developed by different institutes using pedigree method of breeding all exhibiting field tolerance or resistance to YVMD (Sanwal *et al*., 2016) Resistance to YVMD from another wild relative of okra *Abelmoschus tetraphyllus* was successfully introgressed into cultivated okra, despite encountering incompatibility barriers. The cultivars Arka Anamika and Arka Abhay are notable outcomes of this interspecific breeding strategy (Dutta 1991).

 Mutation breeding has proven successful in developing disease-resistant okra. Anjitha and Manjima are high yielding YVMD resistant okra varieties developed by KAU, Thrissur. The experimental work involving the gamma irradiation (300 Gy) of F1 seeds from an *A. esculentus* × *A. manihot* cross indicated that this dose could be effective for inducing mutations in okra (Manju and Gopimony, 2009). An induced mutant from Pusa Sawani developed using 1% EMS, namely Punjab-8 (EMS 8) resistant to YVMD has been released from, PAU, Ludhiana (Sharma and Arora 1990).

 In India, private sector seed companies also make prominent contributions to breeding YVMD resistant/tolerant okra hybrids, largely through inter-varietal crosses. This approach has led to the introduction of several high-yielding resistant hybrids, such as; EXP HY NO.2, MAHY 28, MAHY 55 (MAHYCO), Sarvottam, Selvam (Nunhems), OH 102, OH 517 (Syngenta), Avantika, Nandini (Bioseed), JKOH 502, JK Harit, JKOH 5608 (JK Agri Genetics) Jaani, Ratna, Navya (Advanta seeds), and NS 7801 (Namdhari seeds).

**4. CHALLENGES FACED IN CONVENTIONAL BREEDING FOR YVMD RESISTANCE**

 While resistance breeding is a primary strategy for controlling viral diseases in crops, the durability of resistance incorporated into cultivated gene pools often proves limited. Such resistance can deteriorate within a 5 to 8-year timeframe, eventually rendering previously resistant cultivars susceptible once more (Mishra *et al*., 2021). This breakdown of resistance is primarily attributed to the evolution of novel viral variants, predominantly through genetic recombination of virus strains (Sanwal *et al*., 2014b). Further complicating viral disease management is the increasing prevalence of the polyphagous 'B' biotype of *Bemisia tabaci*, whose expanded host range facilitates viral transmission to, and subsequent infection of crop species previously not considered primary hosts or those that were unaffected (Chowda-Reddy *et al.,* 2012). Consequently, wild okra varieties are recognized as important and consistent reservoirs of YVMD resistance. However, the introgression of these valuable resistance traits into cultivated okra is significantly challenged by reproductive incompatibilities. These incompatibilities act as barriers to successful gene transfer and the development of viable subsequent generations (Badiger *et al*., 2024). Occurance of pre-zygotic and post-zygotic reproductive barriers impedes gene flow from wild species to cultivated species in okra, which operates through all stages of reproductive pathway results in limited fertilization, evidenced by low seed set and embryo formation, often followed by seed abortion (Joseph *et al.,* 2013). The successful introgression of traits among *Abelmoschus* species is frequently complicated by these barriers stemming from variations in chromosome numbers and prevalent polyploidy. These factors lead to incongruity between and within species, thereby necessitating a preliminary assessment of crossability relationships before initiating hybridization programs (Rajamony *et al.*, 2006). Incongruity describes a suite of reproductive barriers that disrupt the normal progression of pollen-pistil interactions and prevent fertilization. These can manifest as issues with pollen adhesion and germination on the stigma, obstruction of pollen tube penetration through the transmitting tract of the style, or the pollen tube’s failure to correctly navigate to and reach the ovary. (Pickersgill 1993, Shivanna 1996). Patil *et al*. (2013) observed delayed pollen tube growth alongside structural abnormalities such as; swelling, twisting, high branching, bi-furcated tip and variation in callose deposition in the interspecific cross including *A. manihot* subsp. *tetraphyllus* var. *pungens* × *A. esculentus*, making the cross highly incompatible. In contrast, the cross *A. manihot* subsp. *tetraphyllus* var. tetraphyllus × *A. esculentus* exhibited partial compatibility, while *A. esculentus* × *A. caillei* demonstrated full compatibility.

 Another significant challenge in interspecific hybridization is the occurrence of post-zygotic barrier, primarily manifesting as hybrid sterility. This phenomenon is typically attributed to the disparities in chromosome number or limited homology between the parental genomes in the resulting hybrids, which culminate in F1 progeny that are incapable of producing viable seeds following self-pollination or backcrossing (Patil *et al.,* 2013).

**4.1 Overcoming hybridization barriers in okra breeding**

 Effective utilization of wild species germplasm in okra improvement programme necessitates understanding and mitigating various hybridization barriers that that impede successful interspecific crosses.. Pre-fertilization barriers can be successfully overcome through various specialized pollination techniques, including stump pollination, bud pollination, growth hormone application, use of irradiated mentor pollen and *in vitro* fertilization. Whereas post-fertilization barriers can be addressed through *in vitro* methods such as embryo rescue (Bhat & Sarla, 2004). For instance, an embryo rescue protocol for overcoming post fertilization barriers in interspecific crosses involving *A. esculentus* and *A. tetraphyllus* was successfully developed by Rattan and Kumar (2020), demonstrating the potential of such techniques in widening the genetic base of cultivated okra.

 Fertility restoration in F1 hybrids derived from crosses between cultivated okra (*Abelmoschus esculentus*) and wild relatives (*A. tuberculatus* and *A. tetraphyllus*) has been successfully achieved through colchicine treatment. Application of 0.1% colchicine to emerging seedlings at the two-leaf stage effectively induced amphidiploidy, thereby restoring fertility (Joseph *et al*., 2013; Suma *et al*., 2023). This approach, involving colchicine-induced amphidiploidization of the F1 hybrid followed by selfing and backcrossing (using the amphidiploid as the seed parent), has also proven effective in facilitating the introgression of desirable traits from *Abelmoschus manihot* var. tetraphyllus and *A. moschatus* into *A. esculentus*, as demonstrated by Badiger *et al*. (2024).

**5. CONCLUSION**

 Conventional breeding programmes aimed at improving okra have prioritized tackling Yellow Vein Mosaic Disease (YVMD), a significant biotic stress. Through field evaluation and screening, potential resistant sources have been identified within both cultivated germplasm and wild relatives. Deploying host plant tolerance to YVMD is considered the most economically viable and environmentally sound approach to managing this disease. Consequently, several YVMD-tolerant okra varieties have been developed in India through the application of conventional breeding methodologies. Crop wild relatives of okra serve as valuable reservoirs of resistance genes; however, their effective utilization in breeding is often constrained by presence of various pre- and post-zygotic barriers. Advancing the successful introgression of disease resistance genes from these wild species into the cultivated gene pool requires comprehensive knowledge of species variability, crossability dynamics, and effective conservation strategies. Hence, systematic efforts are imperative to collect and pool diverse okra germplasm, encompassing commercial varieties, landraces, and related *Abelmoschus* species, with the ultimate goal of developing okra varieties exhibiting durable resistance or tolerance to YVMD.

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**ABBREVIATIONS**

*YVMD: Yellow Vein Mosaic Disease;* ha:hectare*; YVMV: Yellow vein mosaic virus; A.: Abelmoschus;* NBPGR: National Bureau of Plant Genetic Resources; PAU: Punjab Agricultural University; VNMKV: Vasantrao Naik Marathwada Krishi Vidyapeeth; CCSHAU: Chaudhary Charan Singh Haryana Agricultural University; IIVR: Indian Institute of Vegetable Research; KAU: Kerala Agricultural University; Gy: Gray; EMS: Ethyl Methanesulfonate