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Utilization of wild species for biotic stress breeding in okra: a review

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Introduction

Okra (*Abelmoschus* spp.) is traditionally grown as a common vegetable crop with significant area under cultivation in Africa and Asia having huge socio-economic potential. Its robust nature of cultivation, ample dietary fibres and distinct seed protein balanced in both lysine and tryptophan amino acids enhance its nutritional importance for consumption. Although it is robust in nature, considerable yield losses have been reported from growing regions due to the incidence of a number of biotic and abiotic stresses. The major limitation is Yellow vein mosaic disease (YVMD) transmitted by white fly (*Bemisia tabaci*) in okra which results in substantial losses of yield up to 95% in India and also deterioration of fruit quality of the vegetable depending on the stage of crop growth at which infection occurs.[1] Besides YVMD, Okra Enation leaf curl virus, cercospora leaf spot/ blight and powdery mildew also damaging the crop significantly. Apart from diseases, several pests also cause serious damages on okra, such as jassids, mites and spotted bollworm considered as the most destructive pest of okra (Table 1).

Recombination breeding for improved varieties/ hybrids

The inheritance of resistance to yellow-vein mosaic disease (YVMD) has been reported both in intervarietal and interspecific crosses. The domesticated varieties mostly belong to *A. esculentus* due to its wide adaptation and earliness even under erratic growing conditions besides of its amphidiploid nature. Therefore, recombination breeding was observed to be a suitable means to overcome the major biotic stresses. In this line various efforts have been made since 1960's. Potential spp. are being mentioned in Table 2 with their cultivation status which suggests wide array of scope making them suitable to be used for pre-breeding material. However, the cytogenetics and contrasting features of two most commonly used spp. (*A. esculentus* and *A. caillei*) for recombination breeding are listed in Table 3.

Yellow vein mosaic disease (YVMD)

The YVM belongs to the genus Begomovirus, family Geminiviridae. At least 27 begomoviruses reported to infect okra. They have high recombination rate and the emergence of 'B' biotype whiteflies is promoting for epidemics of begomoviruses in okra. An okra variety Pusa Sawani identified as resistant to YVMD, by incorporating resistance from the strain IC 1542 and it has been widely cultivated since then. It exhibited field resistance for some years but later on became susceptible. In various investigations, different cultivars of *A. esculentus* has been screened but there is no resistance among cultivated varieties. Therefore, the search of resistance invariably be shifted to related wild species. *A. caillei* is gradually replacing common okra in the tropical-humid region (Figure 1A&1B) because of its better adaptation under humid zone and tolerance to biotic stresses.[2] Indeed under very limited and erratic rainfall in the growing regions, earliness of *A. esculentus* (being amphidiploid) as compared to *A. caillei* (being amphipolyploid) was preferred during early domestication. In Asia, *A. caillei* has been utilized as a resistant source to breed Yellow vein mosaic virus resistant common okra variety.[3] The inter-specific cross between *A. caillei* and *A. esculentus* is successful with the possibility of gene transfer, although the partial hybrid breakdown barrier must be overcome.[4] The study on geographical distribution and extent of natural outcrossing in Benin and Togo suggests that genetic integrity of these two species is not threatened.[5]

Table 1 Damages caused by disease & pests and their symptoms

Diseases	Symptoms
Yellow vein mosaic disease	Clearing of small veins, usually starts at various points near the leaf margins in about 15-20 days after infection of plants
Okra Enation leaf curl disease	Small, pin-headed enations on leaves, leaf curling, followed by warty and rough texture of the leaves. The under surface of the leaves is characterized by mild, bold and prominent enations.
Cercospora leaf spot (Cercospora abelmoschi)	Brown and irregular leaf spots or sooty black and angular leaf spots, severe defoliation during humid seasons
Powdery mildew (Erysiphe cichoracearum)	Mainly older leaves, petioles and stems are affected. A large part of the leaf surface is covered by the talc-like powder composed of fungal spores. Spores are easily blown by winds and helps disease to spread.
Root rot	Yellowing and wilting of infected plants, browning of root
Pests	
Red spider mites	Colonies of mites can be found feeding on ventral surface of leaves, resulting in yellow spots on dorsal surface.
Jassids	Leaves turn pale and curl upward. In case of severe infestation, the leaves have a burnt look and fall down.
Spotted bollworm	Fruits become distorted and unfit for human consumption.
Root knot nematode (Meloidogyne spp.)	Plants wilt and appearance of root galls/knots of different sizes and infected roots also become enlarged and distorted.

Table 2 *Abelmoschus* spp. for pre-breeding utilization as a source of resistance or tolerance

Sr. No.	Species	Status	YVMV	OELCV	PM	CLS	Mites	Jassids	Fruit borer
1	*A. esculentus*	Cultivated for common consumption							
2	*A. moschatus*	Cultivated for aromatic seeds							
3	*A. ficulneus*	Wild							
4	*A. tuberculatus*	Wild							✓
5	*A. crinitus*	Wild	✓	✓				✓	
6	*A. manihot*	Wild	✓	✓					
7	*A. tetraphyllus*	Wild	✓						
8	*A. pungens*	Wild							
9	*A. angulosus*	Wild	✓	✓					
10	*A. caillei*	Wild						✓	

Table 3 Most widely used spp. for recombination breeding

Species	Cytogenetics	Contrasting traits
A. esculentus (common okra) 95% cultivated area	Amphidiploid (2*n*=130-140): *A. tuberculatus or A. ficulneus* (2*n*-58-60) x unknown?	Poor adaptation in humid zone, more susceptible to biotic stresses, less vigorous, short life cycle (suitable for short rainy season areas), usually day neutral, cultivated in both rainy (rain fed) and dry (irrigated) seasons
A. caillei (West African okra) 5% cultivated area	Amphipolyploid (2*n* = 196-200): *A. esculentus* (2*n*=130-140) x *A. manihot* (2*n* = 60-68)	Better adaptation in humid zone, tolerant/ resistant to biotic stresses, more vigorous, longer life cycle, mostly photoperiod sensitive, cultivated mainly in dry season

Figure 1 Disease reaction and symptoms of Okra: (A) Resistance to YVMV (B) Heavy infestaion of YVMV (C) Incidence of Cercospora leaf spot.

The IPSA Okra 1 variety, tolerant to Yellow Vein Mosaic Virus (YVMV) was crossed to 3 susceptible genotypes viz. Parbhani Kranti, SL-44 and SL-46 to determine the nature of inheritance of tolerance of IPSA Okra 1.[6] Grafting test was also done to know the nature of tolerance. It was revealed from the results of grafting test that the tolerance in IPSA Okra 1 is genetic, not due to escape. The F1 hybrids were tolerant to YVMV. From the segregation pattern for disease reaction in F2 and BC1 generations of the 3 crosses, it could be hypothesized that the tolerance to YVMV in IPSA Okra 1 is quantitative, with possibly two major factors, and dependent on gene dosage with incompletely dominant gene action. The Regional station of National Bureau of Plant Genetic Resources, Thrissur, Kerala conducted a field experiment from 1999–2001 to identify high yielding okra germplasm coupled with acceptable fruit quality and field resistance to yellow vein mosaic virus (YVMV). During the preliminary germplasm evaluation, 62 high yielding accessions were identified and were subjected to further evaluation in order to confirm the stability of desired traits for which they were selected. The indigenous accession IC 265147 collected from Thrissur district of Kerala performed better than all others for fruit yield per plant. Accessions IC 43735, 43736, 43720, 128088, 39137A, 111500 and 265147 recorded high fruit yield per plant coupled with better fruit quality and may be considered for incorporation into breeding programme. However, none of the entry reported as immune or highly resistant during screening programme under natural epiphytotic condition. Among entries 43 were 'moderately resistant', three accessions viz., IC 218887, IC 69286 and EC 305619 were 'resistant' and the rest were susceptible. The results also revealed that none of the yellow vein mosaic virus disease resistant accessions were exceptionally high yielding or *vice-versa*.[7] The *A. caillei* and *A. tetraphyllus* have been utilized for improvement of conventional varieties *i.e.* Punjab Padmini, P-7, Parbhani Kranti, Arka Anamika and Arka Abhay since time long which needs to be improved with emerging biotypes of begomovirus.[8]

Okra enation leaf curl virus (OELCV)

It was first reported in India at ICAR-IIHR, Bengaluru in year 1984. However, the OELCV disease is more serious in North India and parts

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of South India. This virus is transmitted by white fly (*Bemisia tabaci*). Initial symptoms appear on leaves in form of pin-headed enations followed by rough texture of the leaves. There is twisting of main stem, lateral branches and leaf petiole and the leaves become thick and leathery. Leaf curling and enations are more prominent in middle-aged leaves. In case of heavy infestation, the twisting and bending of the stem are so severe that the entire plant seems spreading on the soil surface. The infected plants either do not produce fruits or produce few deformed and small fruits unfit for marketing and consumption. The yield loss varies from 30% to 100% depending upon the age of the plant at the time of infection.[8] Screening of available germplasm observed twenty four lines from *A. esculentus* and some wild taxa for resistance to OELCV under field condition. Amongst these lines VRO-5, VRO-6, KS-410, VRO-4, 111-8-9 (Red), 131-8-1 (Red) and IIVR-10 were free from infection. Infection was absent in the wild taxa viz. *A. pungens, A. manihot, A.crinitus, A. ficulneus and A. angulosus*. Nine lines of *A. tetraphyllus* and 8 lines of *A. tuberculatus* expressed less than 10% infection while 29 lines of *A. tetraphyllus* and 2 lines of *A. tuberculatus* were observed free from disease infection.[9]

Cercospora leaf spot

In India, two species of *Cercospora* produce leaf spots in okra *i.e. C. malayensis* and *C. abelmoschii.* Irregular spots with brown colour patches are typical for leaf spot caused by *C. malayensis* whereas characteristic angular black coloured spots obtained from *C. abelmoschii* (Figure 1c). Till date no wild or cultivated species has been reported as resistance source for cercospora leaf spot. However, a project CRP on agrobiodiversity is being coordinated by ICAR-NBPGR at various research centres of India pertaining to all available accessions of okra for evaluation, characterization, seed multiplication and reactions against important diseases like YVMV, OELCV, Cercospora leaf spot etc.[10]

Powdery mildew

A white coloured powdery growth appears on the upper leaf surfaces of okra. This growth slows photosynthesis and resulting in reduction of economic yield. The leaves detached and fall to the ground in heavy infestation. The pathogen spreads *via* airborne conidia and survives on the infected plant debris. None of the species showed resistant reactions among *A. esculentus, A. caillei, A. tuberculatus and A. ficulneus*. Screening of 20 elite varieties/germplasm under field condition of Maharastra showed moderate resistance reaction for eight germplasm and twelve material showed moderately susceptible reaction against powdery mildew.[11]

Root rot

It has been widely spread to Asian countries like India, Pakistan and China. This is caused by *Fusarium solani*. Recently, China province reported the yellowing symptoms on leaves of infected plants and wilting alongwith browning of root portion.[12] The *A. caillei* and *A. tetraphyllus* were found resistant to this disease. Also, *A. tuberculatus, A. moschatus, A. crinitus* and *A. pungens* may be used as source of resistance to root rot disease. Two accessions each of *A. manihot* and *A. angulosus* were moderately resistant and none of the lines of *A. ficulneus* was found resistant. Four lines of *A. esculentus i.e.* IC-90293, V-4680, V-4365 and IC-42495 B were also found to be resistant to this disease.[9]

Insect pests

Jassid [*A. biguttula biguttula* (Shir.)]

It cause serious damage in okra by heavy desapping of leaves leading to phytotoxemia, known as hopperburn. None of the lines of some of the species like *A. moschatus, A. ficulneus* and *A. crinitus* species expressed immune reaction to jassid infestation and therefore yellowing, cupping or necrosis symptoms were not observed. However, most of the germplasm of *A. tetraphyllus* and *A. esculentus* showed cupping symptoms. Resistance of okra to leafhopper feeding is reported in some lines *viz.* VRO-5, VRO6 and the cultivar Red Long while AOL-03-02, AOL-04-03, AOL-04-05, Pusa Sawani and Gujarat Okra -2 were moderately resistant.[13]

Fruit borer (*Earias vitella*)

The accessions of *A. tuberculatus* recorded less than 10% fruit damage which may be due to the presence of tubercles on the surface of fruit wall. Though small tubercles does not inhibit the insects to lay eggs on these plants but the toughness of the fruit wall filter neonate larvae to feed and survive causing premature death of the insect.

Red spider mite (*Tetranychus urticae*)

Field screening on the basis of mite population in the lower, middle and upper leaves indicated resistance of varying level particularly in the wild accessions. *A. angulosus* were found free from red spider mite in the field conditions. Three accessions of *A. caileii* (EC-305656, EC-305664 and EC-305696) did not harbour any mite population exhibiting complete immunity and 36 accessions were resistant with 1-5 mites/cm^2 only. Most of the accessions of *A. tetraphyllus*, *A. moschatus, A. crinitus* and *A. ficulneus* were free from to be red spider mite infestation. Sheeba et al.[14] reported that Kasturi Bhendi (R), Dharmapuri local (MR), Parbhani Kranti (MR), Pusa Sawani (MR), COBhH1 (MR) and Mahyco 10 may have potential against Red spider mite improvement programme. The resistant variety Kasturi Bhendi can be effectively utilized to develop okra varieties with improved resistance to two spotted spider mite.

Conclusion

Wild or semi-wild cultivars play specific role for many crop improvement programs for development of biotic stress resistance. Field evaluation and detailed investigation in okra indicated differential reaction of wild cultivars to a specific biotic stress which may be utilized for development of resistant varieties/hybrids. Although, the botanical distance or polyploidy nature of *A. esculentus* and other wild relatives act as a barrier in successful hybridization programme for transfer of desirable resistant genes. In this context, advanced molecular biological tools and techniques viz. RNA-Sequencing, TILLING approaches and Reverse genetics open the scope for better exploration of wild cultivars in okra to overcome the biotic stresses. Wild relatives of okra from India, other parts of Asia and Africa are yet to be explored and important for their inherent resistance to one or more biotic stresses. Further, intermediate forms resulting from natural hybridization is also the rule of nature which is easy to utilize and these should be collected for future breeding programme.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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