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Screening of wild okra (*Abelmoschus moschatus*) germplasm for okra yellow vein mosaic disease resistance in India

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ABSTRACT

Okra yellow vein mosaic disease (OYVMD) is a major viral disease of okra crop caused by *Okra yellow vein mosaic virus* (OYVMV). The disease is responsible for direct yield loss in terms of both quantity and quality of capsules (fruits). Initially, DNA-A of coat protein, intergenic region and partial rep protein sequences were determined using OY2395F/OY680R specific primer and the amplified 1.2 Kb band product showed presence of OYVMV. Two years field screening of wild okra accessions (*Abelmoschus moschatus* ssp. *moschatus*) carried out during *kharif* 2017 and 2019 against OYVMD in agro-ecological conditions of New Delhi. Among seventy six (76) wild okra accessions 10 accessions, viz. EC360586, EC360794, EC360830, EC360900, EC359730, EC359836, EC359870, EC360351, EC361171 and EC361111 exhibited resistant (R) response in *kharif* 2017. While in 2019, out of these 10 promising accessions, four accessions, viz. EC360794, EC360586, EC360830 and EC361171 further showed R response. Rest of the tested lines showed either moderately resistant (MR) or susceptible (S) response. Average percent disease incidence (PDI) value was 19.51% and range value was 4.46 to 64.06% for the first year of field screening. Whereas the recorded average PDI were 21.77% with the range value of 4.36 to 67.33% in the second year. For both the years, out of 76 accessions, four accessions, viz. EC360794, EC360586, EC360830 and EC361171 were found promising and exhibited R response consistently. These promising lines could be utilized in breeding programmes for development of varieties resistant to OYVMV.

Keywords: Field screening, OYVMD, OYVMV, Resistance source, Wild okra

Okra [*Abelmoschus esculentus* (L.) Moench] is also known as Lady's finger, is an important vegetable crop cultivated throughout tropical and sub-tropical low altitude regions of Asia and Africa. In India, it is grown in an area of 0.53 million ha with annual production of 6.36 million tonnes and a productivity of 11.9 tonnes/ha (Anonymous 2015). It is a rich source of essential micro and macronutrients (Fajinmi and Fajinmi 2010). Unfortunately, okra is more vulnerable to many viral diseases, viz. okra yellow vein mosaic disease (OYVMD) and okra enation leaf curl disease (OELCuD). Among the viral diseases, OYVMV is considered as major threat to okra cultivation (Kulkarni 1924, Uppal *et al.* 1940, Capoor and Verma 1950). OYVMD is caused by OYVMV belonging to genus begomovirus (DNA-A) and family gemini viridae. It is associated with DNA β satellite molecule (Jose and Usha 2003).

OYVMV is transmitted by whitefly (*Bemisia tabaci*) in a persistent circulative mode (Ghanem 2003). Initial symptom showed interwoven yellow vein pattern on leaves. Loss in fruit yield ranged from 50 to 94% based on the stage of crop infection (Sastry and Singh 1975). Other factors for yield loss comprises poor level of disease resistance to OYVMD (Bora *et al.* 1992), Occurrence of novel biotypes of whitefly vectors and development of resistance against many insecticides has been reported (Rashida *et al.* 2005). To curtail yield loss, use of synthetic pesticide spray against whitefly vector is commonly followed by farmers but it is costly and has adverse impact on non-target species and environment. Thus, attention is given to develop resistant variety (Eigenbrode and Trumble 1994, Nataraja *et al.* 2013). So far, the resistant varieties released at national/state level failed to show durable and stable resistance. Evaluation of large number of okra germplasm in natural epiphytotic condition could not show resistant genotypes, whereas it was reported that wild species of okra possesses resistance to OYVMD. Therefore, current study was undertaken to find out OYVMD resistance sources by screening wild okra, i.e. musk okra (*A. moschatus* ssp. *moschatus*) germplasm to utilize in resistance breeding programme.

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MATERIALS AND METHODS

Germplasm collection and screening: The present study

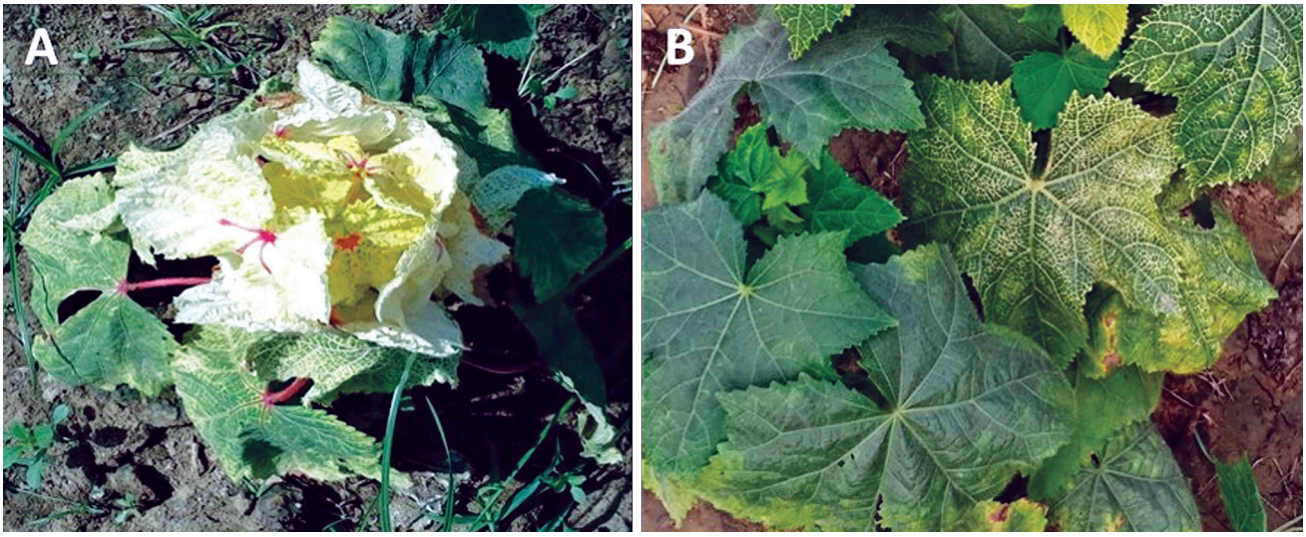

Fig 1 Okra plant showing okra yellow vein mosaic disease (OYVMD) symptom (A) Pusa Sawani as susceptible check, (B) Wild okra (*Abelmoschus moschatus* ssp. *moschatus*).

was conducted on 76 wild okra, i.e. musk okra accessions along with four resistant and susceptible checks, viz. Arka Anamika (resistant check), VRO-6 (resistant check), Pusa Sawani (susceptible check) and Parbhani Kranti (susceptible check) (Table 1).

Screening of germplasm resources of wild okra: All the accessions of wild okra along with four checks were sown in ICAR-NBPGR New Area Farm, Pusa in Augmented Block Design (ABD) during *kharif* 2017 and 2019 with one row for each accession maintaining plant spacing 30 cm × 30 cm and row to row spacing 75 cm × 75 cm. Ten plants were maintained in one row per accession. Occurrence and incidence of OYVMD was monitored in natural condition. Symptomatic leaf samples were collected for identification of the associated virus.

Disease incidence and data analysis: Experimental field was monitored thrice at an interval of 25 days during vegetative stage of the crop and recorded the percent disease incidence (PDI) on 10 plants of each genotype. Disease scoring scale (0-9) was adopted as given by Mayee and Datar (1986) with slight modifications. The PDI was computed using the following formula:

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

The PDI data of OYVMD were subjected to Augmented Block Design (ABD) statistical analysis using R Programming (version Ri386 3.6.2) to compute standard error, critical difference and coefficient of variance. Germplasm cluster was constructed using DARwin 6.0.21 software (http://darwin.cirad.fr).

Molecular identification of the viral pathogen: Total nucleic acid extracted from diseased leaf tissues collected from field using cetyltrimethyl ammonium bromide method with slight modifications (Doyle and Doyle 1990). OYVMV (DNA-A, coat protein, intergenic region and partial Rep protein) partial DNA region of DNA-A component was amplified using begomovirus specific primer pair (OY2395F/OY680R), OY2395F 5'-GCTCCCTGAATGTTCGGATGGA-3', OY680R 5'-GTTCTCR TCCATCCATATCTTAC-3' and *Okra yellow vein mosaic virus* (OYVMV) was detected using these primers (Venkataravanappa *et al.* 2012).

The PCR reactions were performed in a DNA Engine (Peltier thermal cycler) machine. The total volume of PCR reaction prepared was 25 µl (100 pmol DNA template, 1.5U Taq DNA Polymerase, 25 mM $MgCl_2$, 2.5 mM dNTPs, 25 pmol of each primer and nuclease free water). Total number of amplification cycles used was 35 with initial denaturation at 94°C for 4 min and final extension at 72°C for 10 min. The cycling conditions were denaturation at 94°C for 45s, annealing at 55°C for 50s, and extension at 72°C for 90s. Amplified PCR products were electrophoresed (1 h at 80 volts) on 1% agarose gel and seen on a Gel documentation system. Desired size of 1.2 Kb amplified products corresponding to the OYVMV of okra leaf was obtained. The PCR product of 1.2 Kb has been sequenced and sequence similarity was analysed using BLAST.

RESULTS AND DISCUSSION

Disease occurrence in germplasm: Field observation on PDI of OYVMD in wild okra accessions (*A. moschatus* ssp. *moschatus*) at New Delhi location during *kharif* 2017 and 2019 (Table 1). A total of 76 wild okra accessions showed varied OYVMD symptoms (Fig 1). Majority (>70%) of these accessions exhibited typical leaf vein yellowing and stunted plant growth during field screening.

Disease reaction of okra germplasm and categorization: Wild okra accessions showed a varied disease reaction response to OYVMD. In the first year of field screening the minimum, average and maximum PDI values were 4.46, 19.50 and 64.06%, respectively, while in the second year recorded 4.36, 21.77 and 67.33%, respectively, which clearly indicated increasing trend of disease progression

Table 1 Incidence of okra yellow vein mosaic disease in germplasm accessions of wild okra

Accession	Reaction against OYVMD			
	Kharif 2017		*Kharif* 2019	
	PDI	Reaction	PDI	Reaction
EC359730	4.46^{a}	R	13.33^{bcd}	MR
EC361111	4.46^{a}	R	12.70^{b}	MR
EC359870	4.76^{a}	R	14.73^{bcdefgh}	MR
EC360830	5.03^{a}	R	4.90^{a}	R
EC360794	5.13^{a}	R	5.90^{a}	R
EC360586	5.16^{a}	R	4.36^{a}	R
EC361171	5.40^{a}	R	5.90^{a}	R
EC359836	5.60^{a}	R	16.80^{hijklmn}	MR
EC360900	5.63^{a}	R	23.40^{r}	S
EC360351	5.90^{a}	R	16.00^{efghijk}	MR
EC360787	12.96^{b}	MR	13.06^{bc}	MR
EC361137	13.03^{bc}	MR	13.83^{bcdef}	MR
EC360736	13.20^{bcd}	MR	14.83^{bcdefgh}	MR
EC361006	13.20^{bcd}	MR	14.16^{bcdefg}	MR
EC360672	13.46^{bced}	MR	16.43^{ghijklm}	MR
EC360826	13.66^{bcdef}	MR	15.33^{cdefghi}	MR
EC360927	13.70^{bcdef}	MR	13.06^{bc}	MR
EC360735	13.73^{bcdef}	MR	17.80^{ijklmno}	MR
EC361003	13.76^{bcdef}	MR	15.46^{cdefghij}	MR
EC360377	13.80^{bcdef}	MR	15.26^{cdefghi}	MR
EC361129	13.80^{bcdef}	MR	13.10^{bc}	MR
EC361200	14.03^{bcdefg}	MR	13.70^{bcde}	MR
EC361014	14.10^{bcdefg}	MR	13.63^{bcde}	MR
EC360964	14.13^{bcdefg}	MR	13.26^{bcd}	MR
EC361019	14.16^{bcdefgh}	MR	15.56^{cdefghij}	MR
EC360484	14.20^{bcdefgh}	MR	15.93^{efghijk}	MR
EC361018	14.23^{bcdefgh}	MR	12.46^{b}	MR
EC361264	14.23^{bcdefgh}	MR	17.16^{hijklmn}	MR
EC360820	14.33^{bcdefghi}	MR	15.56^{cdefghij}	MR
EC359653	14.46^{bcdefghij}	MR	13.76^{bcdef}	MR
EC360900-A	14.80^{bcdefghijk}	MR	14.96^{bcdefgh}	MR
EC360911	14.90^{bcdefghijkl}	MR	12.53^{b}	MR
EC361020	14.90^{bcdefghijkl}	MR	13.70^{bcde}	MR
EC361022	14.90^{bcdefghijkl}	MR	14.23^{bcdefg}	MR
IC141055	14.93^{bcdefghijkl}	MR	19.06^{nop}	MR
EC361231	14.96^{bcdefghijkl}	MR	17.00^{hijklmn}	MR
EC361284	15.00^{bcdefghijkl}	MR	15.26^{cdefghi}	MR
EC361044	15.10^{bcdefghijklm}	MR	13.33^{bcd}	MR
EC361261	15.13^{bcdefghijklm}	MR	14.33^{bcdefg}	MR
EC359878	15.16^{cdefghijklm}	MR	16.43^{ghijklm}	MR
EC360853	15.23^{defghijklm}	MR	17.86^{jklmno}	MR
EC360554	15.26^{defghijklm}	MR	14.06^{bcdefg}	MR
EC359709	15.30^{defghijklm}	MR	16.60^{ghijklm}	MR
EC360665	15.40^{defghijklm}	MR	16.66^{hijklmn}	MR
EC360945	15.40^{defghijklm}	MR	16.43^{ghijklm}	MR
EC360819	15.43^{efghijklm}	MR	16.20^{fghijkl}	MR
EC361007	15.43^{efghijklm}	MR	13.63^{bcde}	MR
EC361138	15.43^{efghijklm}	MR	20.03^{op}	S
EC361178	15.43^{efghijklm}	MR	17.33^{ijklmn}	MR
EC329408	15.53^{efghijklmn}	MR	14.00^{bcdefg}	MR
EC360629	15.60^{efghijklmn}	MR	15.33^{cdefghi}	MR
EC360332	15.66^{fghijklmn}	MR	18.70^{lmno}	MR
EC361131	15.73^{fghijklmno}	MR	22.76^{qr}	S
EC360953	16.03^{ghijklmno}	MR	18.30^{klmno}	MR
EC359715	16.10^{ghijklmno}	MR	15.83^{defghij}	MR
EC361067	16.10^{ghijklmno}	MR	12.73^{b}	MR
EC316073	16.30^{hijklmno}	MR	14.13^{bcdefg}	MR
EC359787	16.43^{ijklmno}	MR	23.90^{r}	S
EC359906	16.63^{jklmno}	MR	17.66^{ijklmn}	MR
EC360675	16.73^{klmno}	MR	14.96^{bcdefgh}	MR
EC361132	16.86^{klmno}	MR	53.53^{t}	HS
EC360410	16.93^{klmno}	MR	20.36^{opq}	S
EC361082	17.10^{lmno}	MR	21.56^{pqr}	S
EC360245	17.23^{mno}	MR	18.03^{klmno}	MR
EC360095	17.26^{mno}	MR	18.96^{mnop}	MR
EC360828	17.63^{no}	MR	16.20^{fghijkl}	MR
EC360331	17.90^{o}	MR	42.93^{s}	S
EC360915	61.66^{p}	HS	63.30^{u}	HS
EC361170	62.13^{pq}	HS	63.26^{u}	HS
EC361148	63.16^{pq}	HS	55.36^{t}	HS
EC359828	63.20^{pq}	HS	62.46^{u}	HS
EC360193	63.36^{pq}	HS	62.70^{u}	HS
EC360855	63.73^{pq}	HS	62.56^{u}	HS
IC140985	63.76^{pq}	HS	62.10^{u}	HS
EC329394	63.80^{pq}	HS	63.36^{u}	HS
EC316077	64.06^{q}	HS	67.33^{u}	HS
Parbhani Kranti (Check)	71.86^{r}	HS	73.06^{v}	HS
Arka Anamika (Check)	71.90^{r}	HS	72.20^{v}	HS
VRO-6 (Check)	72.43^{r}	HS	72.93^{v}	HS
Pusa Sawani (Check)	72.46^{r}	HS	72.30^{v}	HS
SEd	1.10		1.24	
CD (P=0.05)	2.17		2.45	

The values within a column with different letters are significantly different at 5% level of probability. R: Resistant, MR: Moderately Resistant, S: Susceptible and HS: Highly Susceptible.

in the second year. Out of 76 lines, nine lines exhibited highly susceptible (HS) disease reaction, 57 lines were moderately resistant (MR) and 10 lines remained resistant (R) in *kharif* 2017 (Table 1). On the other hand in *kharif* 2019, six lines showed susceptible (S) reaction, 56 lines showed MR reaction, 10 lines showed HS reaction and 4 lines remained resistant (Table 1).

Ten lines, viz. EC360586, EC360794, EC360830, EC360900, EC359730, EC359836, EC359870, EC360351, EC361171 and EC361111 clearly exhibited R reaction in first year. But in the second year of field screening of these 10 lines, only four lines, viz. EC360794, EC360586, EC360830 and EC361171 showed R reaction (Table 1) and remaining six lines, viz. EC360900, EC359730, EC359836, EC359870, EC360351 and EC361111 exhibited either MR or S reaction, hence these four lines were recognized as most promising.

UPGMA hierarchial analysis revealed that all 76 germplasm as well as four checks categorized into 7 clusters comprising R (2 cluster), MR (3 cluster) and HS (2 cluster), based on the level of OYVMD incidence in *kharif* 2017, whereas in *kharif* 2019 genotypes were categorized into a 9 clusters representing R (2 cluster), MR (3 cluster), S (3 cluster) and HS (1 cluster).

Detection and confirmation of OYVMV: PCR amplification of OYVMV (DNA-A of coat protein, intergenic region and partial rep protein) using OY2395F/OY680R specific primer pair amplified 1.2 Kb for all the six randomly selected wild okra symptomatic accessions (EC360855, EC316077, EC329394, EC359828, EC360193 and EC360915). The PCR product of 1.2 Kb has been sequenced and found 98.16–98.31% sequence similarity with *Okra yellow vein mosaic virus* (OYVMV).

Begomoviruses are emerging threat to majority of economically important crops in tropical and sub-tropical region (Polston and Anderson 1997). Being warm tropical climate in India and intensive cultivation, the okra crop is highly susceptible to OYVMD transmitted by whitefly, which supports survival of whitefly population round the year except severe winter months especially in northern region. Presently, no cultivable variety of okra found resistant to OYVMD. Therefore present study was focused to screen exotic wild okra genotypes for resistance to OYVMD. Begomoviruses exhibited a wider range of adaptability due to increased mutation (Varma and Malathi 2003) and recombination (Padidam *et al.* 1999). Among all begomoviruses, OYVMV of okra is spreading quickly all over the country. Okra is susceptible to the virus attack at all stages of the crop growth resulting into quality and yield loss. In our study, majority (>90%) of genotypes showed symptoms of OYVMD during vegetative stage, i.e. late infection. OYVMD of okra is considered as most serious begomovirus associated disease in India (Capoor and Varma 1950). Based on visual OYVMD like symptoms and whiteflies presence on okra plants, OYVMV infection was suspected. PCR amplification using OY2395F/OY680R primer pair (Venkataravanappa *et al.* 2012) confirmed the presence of OYVMV. Host plant resistance against OYVMD is only practical and eco-friendly solution to avoid yield loss in okra.

Field screening of wild okra accessions was carried out during *kharif* 2017 and 2019. During *kharif* 2017, based on disease reaction genotypes were grouped into R, MR and HS categories, whereas during *kharif* 2019, it was grouped into four categories exhibiting R, MR, S and HS disease reactions. During first season, average PDI was 19.50% ranging within 4.46 to 64.06%. However, during the second season (*kharif* 2019), average PDI was 21.78% within the range of 4.36–67.33%. Four checks, viz. Arka Anamika, VRO-6, Pusa Sawani and Parbhani Kranti showed average PDI of 72.05, 72.68, 72.38 and 72.46% respectively. In both seasons of field screening indicate that resistance has been broken down in Arka Anamika and VRO-6. Among all four checks, highest PDI was noticed in VRO-6. Present finding revealed four genotypes, viz. EC360794, EC360586, EC361171 and EC360830 as resistant to OYVMD. The future research needs to identify the actual resistance mechanism in wild okra genotypes for utilizing them as efficient disease resistance donor with a target to develop suitable cultivars which will help to minimize the yield and quality loss due to OYVMD in India.

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