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Screening of wild okra (*Abelmoschus esculentus*) against yellow vein mosaic and enation leaf curl diseases

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ABSTRACT

Okra yellow vein mosaic (OYVMD) and enation leaf curl diseases (OELCuD) are the most important biotic diseases which cause yield and quality loss in okra (*Abelmoschus esculentus* (L.) Moench). Therefore, development of viral resistance varieties and lines become important breeding objective worldwide. Wild relatives are the natural source for disease and pest resistance. Hence an experiment was conducted at research farm of ICAR-Indian Agricultural Research Institute, New Delhi during rainy (*kharif*) season 2020 and for screening of 24 wild genotypes to identify the resistance source for OYVMD and OELCuD diseases of okra. PDI (Percent disease incidence) for OYVMD varied from 3.12–93.72, while PDI for OELCuD ranged from 0.00–83.29. The area under disease progress curve for both the diseases indicated that the rate of disease incidence was in increasing trend with the growth of the crop. Based on the natural epiphytotic screening *A. moschatus* (IC 141055), *A. tetraphyllus* (IC 90476-1) and *A. caillei* (Sikkim) were 3 top most genotypes from different species which showed very less incidence of OYVMD and no incidence of OELCuD. These genotypes were grouped in highly resistant category for both the diseases; other genotypes grouped in this category were *A. moschatus* (EC 360900), *A. tetraphyllus* (IC 90515 and IC 470735). These genotypes can be used further for interspecific breeding programme and resistant variety development in okra.

Keywords: Okra, OYVMD and OELCuD disease, Screening wild relatives

Okra (*Abelmoschus esculentus* (L.) Moench) also called bhendi or lady's finger is nutritionally significant for its fibre content, vitamins, minerals, like calcium, potassium and iodine (Hughes 2009). In different parts of the world okra is consumed in various forms, like salad, stews and soups, while in India it is consumed in cooked, roasted and fried forms (Salameh 2014). India is the largest producer with more than 70% of world's total okra production share (NHB 2020) and exporter which contribute to 60% of export among fresh vegetables (Singh *et al*. 2014). Having its own importance, the cultivation of okra has many challenges of which okra yellow vein mosaic virus (OYVMV) and okra enation leaf curl virus (OELCuV) are two different viral diseases which belong to the same genus (*Begomovirus*) and family (Geminiviridae) are transmitted by vector whitefly (*Bemicia tabaci*). These viral diseases cause significant loss to okra production worldwide (Venkataravanappa *et al.* 2013) leading to 50–90% yield loss depending upon stage of infection (Chakraborty *et al.* 1997). Symptoms of YVMV includes chlorosis and yellowing of veins and veinlets, leaves, plants and fruits (Venkataravanappa *et al*. 2012,) while in in case of enation leaf curl virus, curling of new leaves, shortening and thickening of leaves, twisting of petiole, stem bending, thick and deformed lower leaf surface are the major symptoms (Singh and Dutta 1986, Singh 1996, Garcia-Cano *et al.* 2008, Sayed *et al.* 2014, Sanwal *et al.* 2014, Venkataravanappa *et al*. 2022). 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. Therefore, present study was carried out to identifying the new sources of stable resistance for OYVMD and OELCuD.

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MATERIALS AND METHODS

Screening of genotypes: Twenty-four genotypes (Supplementary Table 1) from 5 different species of *Abelmoschus,* viz. *A. esculentus* L. (Moench)*, A. moschatus* (Medik)*, A. tetraphyllus* (Roxb. ex Hornem)*, A. caillei* (A. Chev.) and *A. ficulneus* (Wight & Arn.) were used for screening under natural epiphytotic condition during rainy (*kharif*) season of 2020 and 2021 at research farm of Indian Agricultural Research Institute, New Delhi. Two standard susceptible checks (infector lines) Pusa Sawani and DOV 22 for Okra yellow vein mosaic virus (OYVMD) and Okra

enation leaf curl virus (OELCuD) diseases respectively were used following the infector row method (Nene *et al*. 1972). These infector lines were planted after every three treatments to ensure enough sources for disease spread. Spacing adapted for planting was 60 cm × 30 cm. All the recommended agronomic practices were followed to maintain healthy crop, except the plant protection measures to control whitefly. Disease assessment was carried out at every 15-day interval after 30 days of crop sowing until 90 days. The incidence of disease in infector lines Pusa Sawani (OYVMD) and DOV 22 (OELCuD) was considered as benchmark. Per cent disease incidence (PDI) and coefficient of infection were calculated as per methods given by Singh and Singh (2000), Bag *et al*. (2014) and Nazeer *et al.* (2014). Table 1 shows the scale used to classify the genotypes based on their reaction to the viral diseases, i.e. OYVMD and OELCuD (Bag *et al*. 2014, Venkataravanappa *et al.* 2022).

Crop loss under an epidemic disease was estimated using area under disease progress curve (AUDPC). AUDPC which estimates disease progress over a period of crop growth was calculated using equation given by Shaner and Finney (1977). Disease severity observed at 15 days interval from 30–90 days of crop was used in AUDPC calculation.

$$A_k = \sum_{i=1}^{Ni-1} \frac{\left(yi + i\left(yi+1\right)\right)}{2}\left(\left(ti+1\right)-ti\right)$$

RESULTS AND DISCUSSION

Evaluation of genotypes for OYVMD resistance: On an account of field evaluation of 24 genotypes under natural field epiphytotic condition, per cent disease incidence (PDI) ranged from 3.12 (*A. moschatus* IC 141055) to 93.72 (Pusa Sawani) at 90 days after sowing. Genotypes which showed lowest PDI were *A. moschatus* accession (IC 141055) (3.12) and *A. tetraphyllus* (IC 90476-1) (8.21) which were found resistance to OYVMD. The lowest disease severity was recorded for *A. moschatus* IC 141055 (0.08) and highest for Pusa A4 (0.79) which showed maximum yellowing in veins next only to the check Pusa Sawani (0.80). The plants with highest disease severity showed maximum symptom for disease, similar report have been made by Venkataravanappa *et al*. (2022). The progress of disease severity at different time intervals for the most resistant genotype *A. moschatus* (IC 141055) was compared with the check Pusa Sawani in Fig 1. This disease severity was used to estimate severity grade, response value (R) and coefficient of infection (CI) as given in Table 2. Based on reaction to disease, all 24 genotypes were grouped into 6 groups, i.e. Highly Susceptible (HS), Susceptible (S), Moderately Susceptible (MS), Moderately Resistant (MR), Resistant (R), Highly Resistant (HR). The genotypes which were grouped under highly resistance (HR) category were *A. moschatus* (EC 360900 and IC 141055), *A. caillei* (SKM), *A. tetraphyllus* (IC 90515, IC 90476-1 and IC 470735). Pasupathi *et al.* (2019) also reported that, one wild accession of *A. moschatus* was immune to YVMV. Area under disease progress curve (AUDPC) value was calculated based on disease severity which indicated the progress of disease between two-time intervals (Table 2). The cumulative AUDPC for Pusa Sawani and Pusa A4 were 31.09 and 29.36 respectively which indicated high rate of disease progress in these two genotypes. However, AUDPC value for *A. moschatus* (IC 141055) and *A. tetraphyllus* (IC 90476-1) were 1.20 and 1.43 respectively indicating very slow progress of disease incidence in these two species. The images of these two lines are given in Fig 5. Based on above screening top three genotypes which were identified to be highly resistant were included as *A. moschatus* accession IC 141055 and *A. tetraphyllus (*IC-90476-1) and *A. caillei* (SKM). Similar to this study *A. caillei* and *A. manihot* were reported to show resistance to YVMV and can be used in future breeding program (Seth *et al.* 2016). Earlier workers also reported resistance to YVMV in cultivated and wild species of okra, especially in *A. tetraphyllus* (Prabu and Warade 2009, Badiger and Yadav 2019, Puneeth *et al.* 2022). PDI at different intervals for most resistant genotypes was compared with Pusa Sawani (infector line) (Fig 2) showed that there was significant increase in disease with the growth of crop in susceptible check, however in resistant lines the rate of increase was very meagre. This was in accordance with findings of Venkataravanappa *et al*. (2022) and Jamir *et al*. (2019).

Evaluation of genotypes for OELCuD resistance: Simultaneously all the 24 genotypes were screened based on the incidence of OELCuD. The PDI for OELCuD at 90 days after sowing varies from 0.00–83.29 (Table 3). The highest PDI was recorded for *A. ficulneus* (Sel 1) which indicated its susceptibility to ELCV. The disease severity

Table 1 Scale used to classify the genotypes

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)

Table 2 OYVMD incidence in okra wild species after 90 days of sowing

Genotype	PDI 90 day	DS 90 day	Severity grade	R VAL	CI	Reaction	AUDPC
Pusa A4	93.72	0.79	4	1.00	93.72	HS	29.36
Pusa Sawani (OYVMD susceptible check)	95.21	0.80	4	1.00	95.21	HS	31.09
A. moschatus (EC 360900)	15.60	0.21	1	0.25	3.90	HR	4.73
A. moschatus (IC 141055)	3.12	0.08	0	0.00	0.00	HR	1.20
A. moschatus (IC 140986)	20.00	0.24	1	0.25	5.00	R	6.68
A. moschatus (IC 141065)	23.19	0.18	1	0.25	5.80	R	9.08
A. moschatus (IC 393008)	31.25	0.36	2	0.50	15.63	MR	9.68
A. moschatus (IC 316073)	44.97	0.42	2	0.50	22.49	MS	17.70
A. moschatus (IC 141056)	55.29	0.53	3	0.75	41.47	S	17.59
A. moschatus (IC 140970)	30.33	0.28	2	0.50	15.17	MR	11.33
A. caillei (Sel 1)	29.07	0.22	1	0.25	7.27	R	10.50
A. caillei (Sikkim)	9.22	0.11	1	0.25	2.31	HR	3.15
A. tetraphyllus (IC 90515)	10.12	0.12	1	0.25	2.53	HR	4.73
A. tetraphyllus (IC 90511)	25.14	0.26	2	0.50	12.57	MR	11.25
A. tetraphyllus (IC 90476-1)	8.21	0.11	0	0.00	0.00	HR	1.43
A. tetraphyllus (IC 90409)	25.31	0.28	2	0.50	12.66	MR	7.76
A. tetraphyllus (IC 90508)	27.64	0.25	1	0.25	6.91	R	8.93
A. tetraphyllus (IC 141045)	32.45	0.35	2	0.50	16.23	MR	16.50
A. tetraphyllus (IC 385287)	19.77	0.21	1	0.25	4.94	R	7.28
A. tetraphyllus (IC 470735)	14.71	0.13	1	0.25	3.68	HR	3.38
A tetraphyllus (Sel 1)	18.22	0.19	1	0.25	4.56	R	8.10
A ficulneus (IC 90364)	42.27	0.45	2	0.50	21.14	MS	17.55
A ficulneus (Sel 1)	36.45	0.33	2	0.50	18.23	MR	9.68
DOV 22	75.23	0.71	3	0.75	56.42	S	28.16
CD (P= 0.05)	4.55	0.07					
SEm (±)	1.54	0.02					
CV (5%)	6.67	10.92					

at 90 day was also highest for *A. ficulneus* (Sel 1) (0.79) next only to the check DOV 22 (0.88). Many genotypes recorded no incidence of OELCuD as their PDI and disease severity were nil (0.00) which showed the ability of these genotypes to resist the OELCuD occurrence which may be due to wild characters of genotypes as well as less severity of OELCuD as compared to OYVMVD. All these genotypes were grouped in HR (highly resistant) category. Only two genotypes showed high incidence of OELCuD, viz. *A. ficulneus* (Sel 1) along with check DOV 22 which were grouped as highly susceptible genotypes. Phenotypic screening of okra for YVMV and ELCV was in accordance with the work of Venkataravanappa *et al*. (2022). It was also reported that wild okra species have disease resistant

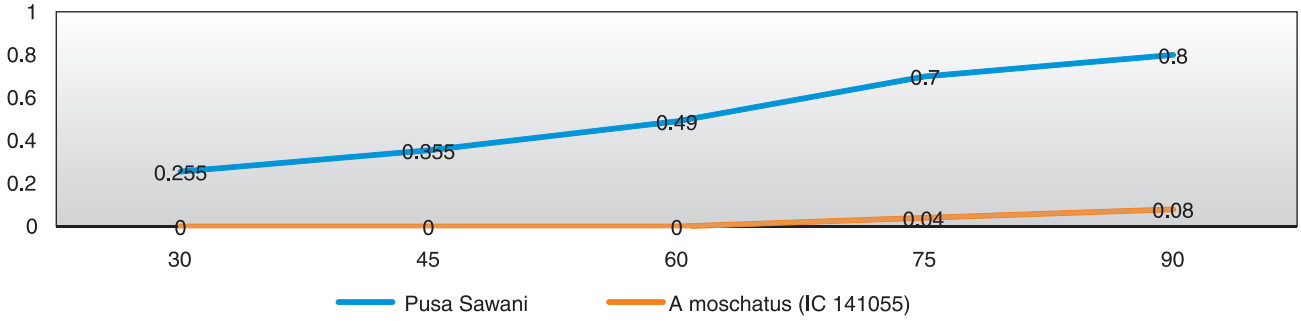

Fig 1 Progress of Disease severity for OYVMD in most resistant genotypes compared to standard check (Pusa Sawani). X-axis: Time interval (Days); Y-axis: Disease severity

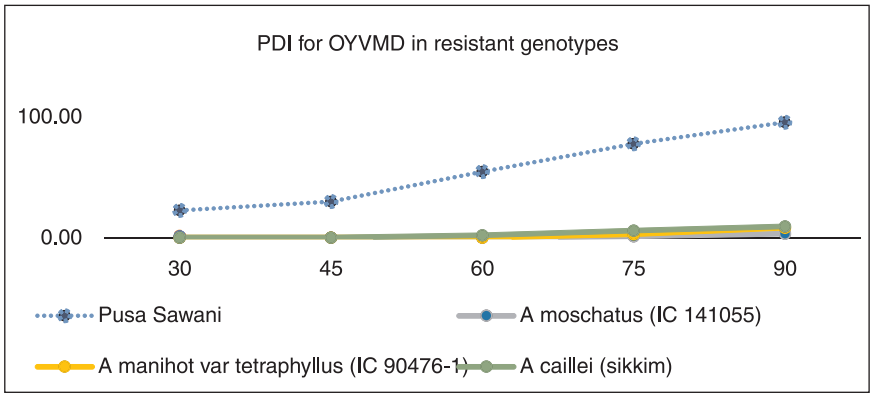

Fig 2 Per cent disease incidence of resistant genotypes based on OYVMD disease. X-axis: Time interval (days); Y-axis: Percent disease Incidence (PDI)

genes for pest and disease resistance (Singh *et al.* 2007). Kumari *et al*. (2021) also screened 76 accessions of *A. moschatus* against okra enation leaf curl virus under Delhi condition and reported different level of resistance within this species. In accordance to our findings, Venkataravanappa *et al*. (2022) also reported 125 wild accessions highly resistant to OELCuD out of 178 cultivated/wild okra genotypes studies. Similarly wild okra species are reported to act as resistant source for YVMV and ELCV viruses (Sanwal *et al.* 2014, Badiger and Yadav 2019).

Cumulative AUDPC which was calculated based on disease severity at 15 days interval also showed high intensity of disease progress in check DOV 22 followed by *A. ficulneus* (Sel 1) which ensured the species susceptibility. PDI at different time interval for 3 resistant genotypes and most susceptible genotype (A. *ficulneus* (Sel 1)) along with check DOV 22 has been depicted in Fig 3.

The phenotypic screenings for both the diseases

Table 3 OELCuD incidence in okra wild species after 90 days of sowing

Genotype	PDI 90 day	DS 90 day	Severity grade	R VAL	CI	Reaction	AUDPC
Pusa A4 (yvmv susceptible check)	23.65	0.28	2	0.5	11.83	MR	8.48
Pusa Sawani (yvmv susceptible check)	22.45	0.27	2	0.5	11.23	MR	7.50
A moschatus (EC360900)					0.00	HR	0.00
A moschatus (IC 141055)					0.00	HR	0.00
A moschatus (IC 140986)					0.00	HR	0.00
A moschatus (IC 141065)					0.00	HR	0.00
A moschatus (IC 393008)					0.00	HR	0.00
A moschatus (IC 316073)	10.11	0.13	1	0.25	2.53	HR	2.58
A moschatus (IC 141056)	17.76	0.18	1	0.25	4.44	R	4.43
A moschatus (IC 140970)	12.47	0.15	1	0.25	3.12	HR	3.17
A caillei (Sel 1)	21.38	0.23	1	0.25	5.35	R	9.60
A caillei (Sikkim)						HR	0.00
A. tetraphyllus (IC 90515)						HR	0.00
A. tetraphyllus (IC 90511)	18.43	0.19	1	0.25	4.61	R	4.88
A. tetraphyllus (IC 90476-1)	0.00	0.00	0	0	0.00	HR	0.00
A. tetraphyllus (IC 90409)	3.25	0.09	1	0.25	0.81	HR	1.35
A. tetraphyllus (IC 90508)	2.12	0.10	1	0.25	0.53	HR	0.98
A. tetraphyllus (IC 141045)	2.64	0.09	1	0.25	0.66	HR	0.98
A. tetraphyllus (IC 385287)	3.54	0.12	1	0.25	0.89	HR	1.88
A. tetraphyllus (IC 470735)						HR	
A. tetraphyllus (Sel 1)						HR	
A ficulneus (IC 90364)	70.69	0.72	3	0.75	53.02	S	16.84
A. ficulneus (Sel 1)	83.29	0.79	4	1.00	83.29	HS	24.83
DOV 22 (OELCuD susceptible Check)	80.17	0.88	4	1.00	80.17	HS	31.69
CD (P=0.05)	3.53	0.01					
SEm (±)	1.20	0.02					
CV (5%)	10.95	13.66					

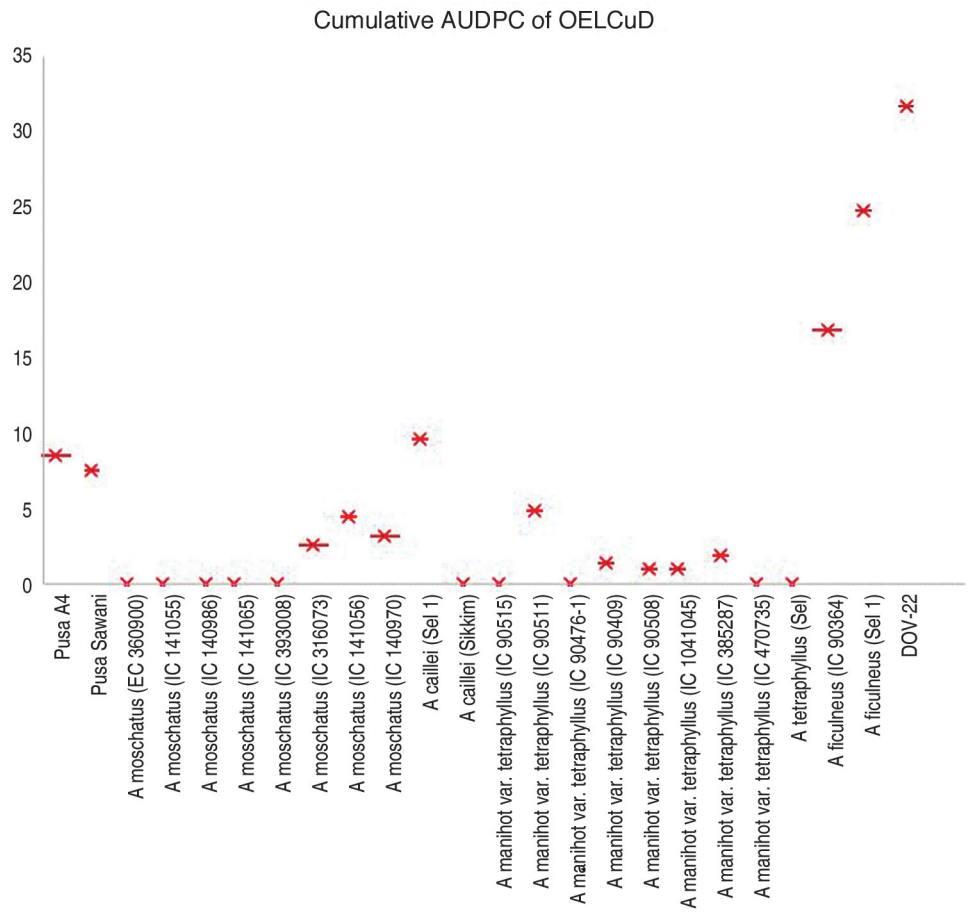

Fig 3 Cumulative value of AUDPC for all genotype based on OELCuD incidence. X-axis: Genotypes; Y-axis: AUDPC value

indicated that genotypes had resistance to OELCuD were more in number as compared to the number of genotypes showed resistance to OYVMD. This might be due to less incidence of enation leaf curl virus disease in Delhi condition as compared to Southern and Coastal region of the India. These resistant genotypes need further screening for enation leaf curl virus disease in hot spot area like, Surat, Gujarat and Guntur, Andhra Pradesh. Similar results have been reported by Pasupathi *et al.* (2019). The identified resistant wild accessions can further be exploited to develop resistant line or variety which can be used in okra cultivation. The genotypes with dual resistance (OYVMD and OELCuD) will be more useful in future, when both the diseases may become a serious threat to okra cultivation. These wild genotypes can be utilized as one of the parents in interspecific hybridization programme to develop a resistant variety or in the development of pre-breeding material.

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