Screening of germplasm of Abelmoschus against biotic stresses

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ABSTRACT

Okra [Abelmoschus esculentus (L.) Moench] is an important vegetable of which India is the leading producer, but its yield potential is hampered due to Bhendi Yellow Vein Mosaic Virus (BYVMV) disease and insect pests like whitefly, leafhopper, and shoot and fruit borer. The present study was done to identify the stable sources of resistance. Total 44 accessions of 6 wild Abelmoschus species and 2 check cultivars of A. esculentus (Pusa Sawani and Pusa A-4) were screened under natural field conditions at Division of Vegetable Science, ICAR-IARI, New Delhi during kharif season 2015. Both the check varieties were found susceptible to BYVMV, Jassids, whiteflies, and shoot and fruit borer. Out of 44 accessions of 6 wild Abelmoschus species, 3 accessions, viz. IC306722, IC90476-1 and IC141055 were found highly resistant to BYVMV, only two accessions, viz. IC141055 and IC140986 were resistant to Jassids and only one accession 141055 was resistant to whiteflies. While all the accessions of A. tuberculatus and more than 50% of the accessions of A. manihot var. tetraphyllus and A. moschatus were found resistant to fruit borer. However, one of the wild accession IC141055 showed multiple resistance, i.e. Jassids, whiteflies and fruit borer along with resistance to BYVMV disease indicated its possible importance as source of multiple biotic stress resistance. The mentioned promising accessions can be further screened at different hotspots for confirmation and can be exploited in future breeding programmes for okra improvement.

Key words: Abelmoschus esculentus, BYVMV, Jassids, Shoot and fruit borer, Whiteflies

Okra [Abelmoschus esculentus (L.) Moench] is one of the important warm-season vegetable grown extensively in tropics, sub-tropics and during warmer seasons of the temperate areas across the world. It is a good source of iron, calcium, manganese, and magnesium, vitamins A, B, C, and K, as well as iodine (Moaward et al. 1984). Okra mucilage has its potential use in food, non-food and medicinal purposes. Okra is an important vegetable that contributes to India by earning foreign exchange through its fresh export to different parts of the world accounting 13% of export of fresh vegetables (Singh et al. 2014). Although India is the largest producer of okra with 6.0 million tonnes production from 0.5 million hectare area in the world (Anonymous 2018), its yield potential is low due to an array of biotic stresses adversely affecting the yield and quality of the produce. The important biotic bottlenecks are Bhendi Yellow Vein Mosaic Virus (BYVMV), Enation Leaf Curl Virus and insects like whitefly (Bemisia tabaci), leafhopper (Amrasca biguttula biguttula) and shoot and fruit borer (Earias vitella). BYVMV is a major menace responsible for 50-90% yield losses depending upon the age of the plant at the time of

¹Breeder (Okra) (maheshhort536@gmail.com), Seed Works International Pvt Ltd, Hyderabad, Telangana; ²Principal Scientist (rkyadavneh@gmail.com), Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. infection (Chakraborty *et al.* 1997). Shoot and fruit borer is responsible for 8.4–73.2% fruit damage depending on the season (Kumar and Urs 1988). Chemical control of these pests has several ecological problems like toxicity hazards, environmental pollution, pest resurgence, etc. Therefore, use of genetic resistance is an effective strategy to manage these biotic stresses. Wild relatives of okra are important genetic sources of resistance to biotic stresses specifically BYVMV (Dhankar *et al.* 2005). Frequent breakdowns of viral disease resistance have been observed in popular okra varieties like Parbhani Kranti, P-7, Arka Anamika, Arka Abhay (Sanwal *et al.* 2014). Keeping these facts in view, present study aimed at shedding light on alternative sources of resistance to major biotic stresses such as BYVMV, whiteflies, leafhopper, and shoot and fruit borer.

MATERIALS AND METHODS

The present investigation was carried out at the Research Farm of Division of Vegetable Science, during *kharif* of the year 2015 ICAR-Indian Agricultural Research Institute, Pusa, New Delhi India, located at 28°35'N, 70°18'E, altitude 226 m amsl. Experimental material consisted of 44 accessions of 6 wild okra species and 2 check cultivars – Pusa Sawani and Pusa A-4 of cultivated species (*A. esculentus*). The species were *Abelmoschus caillei* (3), *A. manihot* var. *tetraphyllus* (17), *A. moschatus* (16), *A. tuberculatus* (3), *A. ficulneus* (2) and *A. angulosus* var. *grandiflorus* (3). The experiment

was laid out in Randomized Complete Block Design (RCBD) with two replications. All the scientific agronomic packages of practices were followed to raise a healthy crop. No insecticides were applied to control the pests. The accessions were screened under natural epiphytotic condition for BYVMV following the infector row method (Nene et al. 1972). To ensure even distribution of viral disease pressure throughout the experimental field a susceptible check cv. Pusa Sawani was used as 'infector line' after every three treatments (test accessions) and also as 3 rows around each block to ensure adequate source of virus inoculums. The disease incidence was recorded at 15 days interval during the crop growing season (kharif 2015) starting 15 days after sowing (DAS). The response of the virus was assessed based on per cent disease incidence [PDI = (Number of diseased plants/ Total number of plants) × 100] in a given accession and disease severity designated with numerical values of 0-4 were assigned against each accession and a scale of response value (0–1) corresponding to such grades were denoted (Table 1) as described by Bag et al. (2014). The coefficient of infection (CI) was calculated by multiplying the PDI and Response value (Bag et al. 2014). The disease reaction was finally deduced based on the CI value and six levels of response-categories were developed (Table 1).

Abelmoschus germplasm was screened under field condition against leafhopper and whiteflies as per Mudgalkar et al. (2014). Population of leafhoppers (nymphs) and whiteflies (adults) were recorded on five randomly selected tagged plants from three leaves (top, middle and bottom) in each replication during morning hours (6.30 AM to 8 AM) from 15 DAS to 105 DAS at 15 days interval. Later the population was averaged and expressed in mean number of insects per leaf. All the accessions were categorized into resistant (<5), moderately resistant (5–10), moderately susceptible (>10–20), susceptible (>20–30) and highly susceptible (>30) as suggested by Iqbal et al. (2008) in

Table 1 Scale for wild *Abelmoschus* accessions against BYVMV under natural epiphytotic condition

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

okra. For shoot and fruit borer infestations, the number of infested fruits along with total number of healthy fruits in each accession were counted at each harvest and added up to get the per cent infestation. All the accessions were categorized into resistant (<20 %), moderately resistant (20–40 %), moderately susceptible (>40–60 %), susceptible (>60–80 %) and highly susceptible (>80 %) based on the per cent fruit infestations (Mishra *et al.* 1988). The quantitative data was analysed for statistical significance as suggested by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Screening for BYVMV under field condition revealed that PDI and CI values ranged from 7.73 (IC141055) to 71.33 (Pusa Sawani) and 1.93 (IC141055) to 71.33 (Pusa Sawani) respectively (Table 2). The disease pressure was high in the experimental plot as evident from the susceptible check Pusa Sawani that showed highly susceptible reaction (CI = 71.33). Species wise disease reaction category (Table 3) revealed that two varieties of cultivated okra (Pusa Sawani and Pusa A-4) were found to be highly susceptible. In A. caillei accessions only (IC306722) was highly resistant. In the accessions of A. manihot var. tetraphyllus only IC90476-1 was highly resistant to BYVMV disease. While, among 16 accessions of A. moschatus only IC141055 was found highly resistant and 4 more accessions were resistant. Among the A. tuberculatus accessions, one was moderately resistant (IC90343). Both the accessions of A. ficulneus were moderately susceptible to BYVMV. Among the 3 accessions of A. angulosus var. grandiflorus, only IC470752 was moderately resistant. Availability of resistance to BYVMV in A. manihot spp. tetraphyllus was earlier reported by Prabhu et al. (2009), Rajmony et al. (1995), Bag et al. (2014) and Gangopadhyay et al. (2016). Prabhu et al. (2009) opined that higher amount of phenols and their oxidation products like quinines formed by increased peroxidase and polyphenol oxidase may be responsible for reduced virus multiplication which finally could have led to resistance reaction in wild okra and their interspecific hybrids.

None out of A. tuberculatus, A. ficulneus and A. angulosus var. grandiflorus was resistant to BYVMV. Rajmony et al. (1995) extracted some BYVMV resistant accessions from A. ficulneus and A. angulosus, respectively, which indicated prevalence of accessional variation within the species. Many BYVMV resistant varieties have been developed using wild species A. manihot since 1970 (Thakur 1976) including Parbhani Kranti (Jambhale and Nerkar 1985). Later breakdown of resistance was observed due to pan India use of sole species as source of resistance. Therefore, BYVMV resistant accessions identified in the present investigation can be further screened under hotspots to reaffirm their resistance potential across the regions and thereafter deployed into breeding programmes. Combining resistance from diverse species into cultivated background helps in development of broad based and durable source of resistance.

Mean number of leafhoppers per leaf ranged from 4.43 (IC141055) to 24.85 (Pusa Sawani) (Table 2). Low mean

Table 2 Incidence of BYVMV and infestation of Jassids, whiteflies and fruit borer

PDI Pusa Sawani 71.33 Pusa A4 57.81 A. caillei 27.65 IC306722 14.71 A. caillei (SKM-1) 21.08 IC90511 26.18 IC141025 20.58 IC141040 34.74 IC470735 24.80	1.00 1.00 0.50 0.25 0.50 0.50 0.50	CI 71.33 57.81 13.82 3.68 10.54 13.09	Reaction category HS S MR HR MR	Mean no./leaf 24.85 23.81 21.16 15.20	Reaction category S S S	Mean no./ leaf 23.52 21.44	Reaction category	Fruit borer infestation (%) 82.44	Reaction category HS
Pusa A4 57.81 A. caillei 27.65 IC306722 14.71 A. caillei (SKM-1) 21.08 IC90511 26.18 IC141025 20.58 IC141040 34.74	1.00 0.50 0.25 0.50 0.50	57.81 13.82 3.68 10.54 13.09	HS S MR HR	24.85 23.81 21.16	S S	23.52	S		
Pusa A4 57.81 A. caillei 27.65 IC306722 14.71 A. caillei (SKM-1) 21.08 IC90511 26.18 IC141025 20.58 IC141040 34.74	1.00 0.50 0.25 0.50 0.50	57.81 13.82 3.68 10.54 13.09	S MR HR	23.81 21.16	S			82.44	LIC
A. caillei27.65IC30672214.71A. caillei (SKM-1)21.08IC9051126.18IC14102520.58IC14104034.74	0.50 0.25 0.50 0.50 0.50	13.82 3.68 10.54 13.09	MR HR	21.16		21.44		o z	
IC306722 14.71 A. caillei (SKM-1) 21.08 IC90511 26.18 IC141025 20.58 IC141040 34.74	0.25 0.50 0.50 0.50	3.68 10.54 13.09	HR		S		S	64.30	S
A. caillei (SKM-1)21.08IC9051126.18IC14102520.58IC14104034.74	0.50 0.50 0.50	10.54 13.09		15.20		7.48	MR	67.93	S
IC90511 26.18 IC141025 20.58 IC141040 34.74	0.50 0.50	13.09	MR		MS	10.14	MS	72.62	MR
IC141025 20.58 IC141040 34.74	0.50			11.40	MS	10.80	MS	61.9	MR
IC141040 34.74			MR	12.01	MS	10.55	MS	23.04	MR
	0.75	10.29	MR	9.11	MR	11.09	MS	45.97	MS
IC470735 24.80		26.05	MS	22.79	S	9.28	MR	40.03	MS
	0.50	12.40	MR	12.61	MS	11.03	MS	48.73	MS
IC470743 54.39	0.75	40.79	S	13.97	MS	9.09	MR	22.16	MR
IC141045 32.68	0.75	24.51	MS	13.07	MS	9.09	MR	27.25	MR
IC9548 55.37	0.75	41.53	S	13.67	MS	10.68	MS	49.79	MS
IC90409 22.63	0.25	5.66	R	11.96	MS	10.43	MS	7.22	R
IC90499 41.96	0.50	20.98	MS	15.22	MS	10.36	MS	19.79	R
IC90508 23.05	0.50	11.53	MR	23.79	S	10.51	MS	13.98	R
IC90476-1 8.61	0.25	2.15	HR	8.86	MR	8.23	MR	21.56	MR
IC90515 16.98	0.25	4.24	R	13.03	MS	9.26	MR	19.50	R
IC90505 28.77	0.75	21.58	MS	13.57	MS	9.87	MR	40.16	MS
A. tetraphyllus 28.47	0.50	14.24	MR	15.65	MS	11.07	MS	15.36	R
IC47092 11.00	0.50	5.50	R	14.76	MS	10.33	MS	14.54	R
IC111500 18.44	0.75	13.83	MR	15.06	MS	8.88	MR	12.26	R
IC385287 20.89	0.50	10.44	MR	13.18	MS	9.69	MR	21.14	MR
IC141065 22.60	0.50	11.30	MR	9.53	MR	6.15	MR	14.74	R
IC140985 23.06	0.50	11.53	MR	9.82	MR	5.90	MR	22.89	MR
IC140970 26.36	0.50	13.18	MR	11.13	MS	6.22	MR	16.85	R
IC316073 42.08	0.50	21.04	MS	10.39	MS	6.56	MR	20.18	MR
IC393008 28.85	0.50	14.42	MR	12.23	MS	7.00	MR	39.37	MR
IC469584 27.44	0.75	20.58	MS	12.09	MS	5.65	MR	15.75	R
IC339520 54.86	1.00	54.86	S	10.47	MS	6.49	MR	48.99	MS
	0.50	20.73	MS	10.99	MS	5.48	MR	23.62	MR
IC140986 17.29	0.25	4.32	R	4.84	R	4.67	MR	9.60	R
IC140113 19.41	0.25	4.85	R	12.84	MS	5.51	MR	25.71	MR
IC212557 33.63	0.25	8.41	R	10.55	MS	5.64	MR	7.25	R
IC141056 49.90	0.75	37.43	MS	11.03	MS	7.44	MR	9.98	R
EC316077 18.97	0.25	4.74	R	11.74	MS	6.54	MR	10.84	R
IC141055 7.73	0.25	1.93	HR	4.43	R	4.55	R	12.59	R
NIC4676 35.64	0.75	26.73	MS	10.70	MS	8.30	MR	31.87	MR
Egypt-R 40.04	0.50	20.02	MS	9.97	MR	6.29	MR	41.59	MS
IC436706 50.72	0.50	25.36	MS	13.93	MS	11.17	MS	14.45	R
IC90396 40.44	0.50	20.22	MS	13.90	MS	11.61	MS	5.43	R
IC90343 24.56	0.50	12.28	MR	12.78	MS	9.47	MR	14.02	R
IC90364 44.18	0.50	22.09	MS	12.45	MS	10.60	MS	31.66	MR
IC550661 55.27	0.50	27.64	MS	10.14	MS	10.61	MS	23.34	MR
IC213314 46.47	0.50	23.23	MS	10.71	MS	10.84	MS	21.10	MR
IC470752 32.26	0.50	16.13	MR	11.38	MS	12.17	MS	39.01	MR
IC203834 41.44	0.50	20.72	MS	11.23	MS	10.99	MS	16.91	R

Table 3 Species wise reaction categories against BYVMV, leafhopper (Jassids), whiteflies, and shoot and fruit borer infestation

Abelmoschus species	Insect pest	Highly resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible
4. esculentus	BYVMV					Pusa A-4	Pusa Sawani
	Jassids					Pusa Sawani, Pusa A-4	
	Whiteflies					Pusa Sawani, Pusa A-4	
	fruit borer					Pusa A-4	Pusa Sawani
	BYVMV	IC306722		A. caillei& A. caillei (Sikkim)			
	Jassids				IC306722, A. caillei (Sikkim)	A. caillei	
	Whiteflies				A. caillei	IC306722, A. caillei (Sikkim)	
	fruit borer		A. caillei			IC306722, A. caillei (Sikkim)	
	BYVMV	IC90476-1	IC90515, IC47092, IC90409	IC90511, C141025, IC141040, IC470735, IC90508, IC111500, I C 3 8 5 2 8 7, A. tetraphyllus	IC141045, IC90499, IC90505	IC470743, IC9548	
	Jassids			IC141025, IC90476-1	IC90511, IC470735, IC470743, IC141045, IC9548, IC90409, IC90499, IC90515, IC90505,A. tetraphyllus, IC47092, IC111500, IC385287		
	Whiteflies			IC141045, IC90476- 1, IC90515, IC90505,	IC90511, IC141025, IC470735, IC9548, IC90409, IC90499, IC90508, A. tetraphyllus, IC47092		
	fruit borer		IC90409, IC90499, IC90508, IC90515, IC111500, A. tetraphyllus,		IC141025, IC141040, IC470735, IC9548, IC90505		
A. moschatus	BYVMV	IC141055	IC47092 IC140986, IC140113, IC212557, EC316077		IC316073, IC469584, IC141056, NIC4676, Egypt-R, IC470737	IC339520	
	Jassids		IC140986, IC141055	IC141065, IC140985, Egypt-R	IC140970, IC316073, IC393008, IC469584, IC339520, IC470737, IC140113, IC212557 IC141056, EC316077, NIC4676		

Table 3 (Concluded)

Abelmoschus species	Insect pest	Highly resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible
	Whiteflies		IC141055	IC141065, IC140985, IC140970, IC316073, IC393008, IC469584 IC339520, IC470737, IC140986, IC140113, IC212557, IC141056, EC316077, NIC4676, Egypt-R			
	Fruit borer		IC141065, IC140970, IC469584, IC140986, IC212557, IC141056, EC316077, IC141055	IC140985, IC316073, IC393008, IC470737, IC140113, NIC4676	IC339520, Egypt-R		
	BYVMV			IC90343	IC436706, IC90396		
	Jassids				IC436706, IC90396, IC90343		
	Whiteflies			IC90343	IC436706, IC90396		
	fruit borer		IC436706, IC90396, IC90343				
A. ficulneus	BYVMV				IC90364, IC550661		
	Jassids				IC9034, IC550661		
	Whiteflies				IC90364, IC550661		
	fruit borer			IC90364, IC550661			
A. angulosus	BYVMV			IC470752	IC213314, IC203834		
var. grandiflorus	Jassids				IC213314, IC470752, IC203834		
	Whiteflies				IC213314, IC470752, IC203834		
	fruit borer		IC203834	IC213314, IC470752			

number of hoppers per leaf was also observed in IC140986 (4.84), IC90476-1 (8.86), IC141065 (9.53), IC140985 (9.82) and Egypt-R (9.97). After categorising into 5 different categories, IC141055 (4.43) and IC140986 (4.84) formed resistant category (Table 3). Both the cultivated varieties were found susceptible (Pusa Sawani, and Pusa A4). None of the accessions from A. caillei and A. manihot var. tetraphyllus were resistant. Out of 16 accessions of A. moschatus screened for jassids, only IC141055 and IC140986 were resistant. Present findings were in accordance with Prabhu et al. (2009) and Dharavath et al. (2016). Hooda and Dhankhar (1992) and Singh et al. (2007) who reported that the species A. moschatus due to its laminal hairiness was found to be highly resistant to hopper. Hopper (Jassid) resistant cultivars were reported to possess higher trichome density, longer trichome length and higher concentration of sugars, silica, potassium, tannins and phenols in the leaves.

Over all mean number of whiteflies per leaf ranged from 4.67 (IC140986) to 23.52 (Pusa Sawani). Data in Table 3

showed that cultivated varieties Pusa Sawani and Pusa A4 were found susceptible to whiteflies. Only IC141055 of *A. moschatus* was resistant. None of the accessions of *A. tetraphyllus*, *A. caillei*, *A. tuberculatus*, *A. ficulneus* and *A. angulosus* var. *grandiflorus* were found resistant. Resistance of *A. moschatus* might be attributed to presence of dense hairs on stem, upper and lower surface of leaves. Availability of whitefly resistance among wild relatives was also reported earlier by Dharavath *et al.* (2016), Prabhu *et al.* (2009) and Narayanan *et al.* (2016). Borad *et al.* (1993) and Pun *et al.* (2005) reported a highly significant positive correlation between adult whitefly population density and the incidence of BYVMV disease in okra. White fly resistant accession IC141055 was also found resistant to BYVMV indicating its importance in strategic BYVMV resistance breeding.

Data presented in Table 2 revealed that fruit borer infestation ranged from 5.43 (IC90396) to 82.44 (Pusa Sawani). Based on fruit borer infestation 46 accessions were divided into 5 categories (Table 3). All three *A. caillei*

accessions were found susceptible to fruit borer. Out of 17 accessions of A. manihot var. tetraphyllus only 7 were resistant to borer. Among 16 accessions of A. moschatus 8 were resistant. All the 3 accessions of A. tuberculatus were found resistant to fruit borer. Two A. ficulneus accessions included in the study were moderately resistant. In A. angulosus var. grandiflorus accessions, one accession was resistant. None of the wild species accessions were susceptible to fruit borer. These findings corroborated with Gangopadhyay et al. (2016). High level of resistance to shoot and fruit borer in A. tuberculatus might be due to tubercles on the surface of the fruit wall. Singh et al. (2007) also opined the same regarding A. tuberculatus resistance to fruit borers. While the presence of dense trichomes on the stem and fruit surfaces of A. manihot var. tetraphyllus and A. moschatus provided them resistance to fruit borer. Negative correlation between trichome density and borer infestation in okra was observed by Sharma and Singh (2010). Lengthy fruits were found more suitable for damage by Earias as they harbored more larvae per fruit in okra (Muthukumaran and Ganesan, 2017). In the present investigation highest fruit length was observed in Pusa Sawani (14.09 cm) and among the wild accessions it varied from 1.5 to 7.5 cm. Accordingly, highest fruit borer infestation was observed in Pusa Sawani and none of wild Abelmoschus accessions were recorded under the susceptibility category.

The results obtained indicate that one wild accession IC141055 (*A. moschatus*) was found resistant to Jassids, whiteflies and fruit borer along with resistance to BYVMV which in turn indicated its possible importance as multiple biotic stress resistance source. So far no attempts have been made to exploit the biotic stress tolerance from *A. moschatus* which might be due to scarce information available about its crossability with cultivated *Abelmoschus esculentus*. Thus, *A. moschatus* can be further studied at different hotspots for confirmation.

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