Plant Genetic Resources: Characterization and Utilization

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Interspecific hybridization as a way of resistance transfer against viruses in okra: Hindrances and way forward

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Research Article

Cite this article: Patel BN, Hegde GK, Manu TG (2021). Interspecific hybridization as a way of resistance transfer against viruses in okra: Hindrances and way forward. *Plant Genetic Resources: Characterization and Utilization* **19**, 357–362. https://doi.org/10.1017/S1479262121000423

Received: 22 October 2020
Revised: 14 August 2021
Accepted: 20 August 2021
First published online: 16 September 2021

Key words:
Abelmoschus esculentus; *Abelmoschus tetraphyllus*; ELCV; okra; wide hybridization; wild relatives; YVMV

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Abstract

Okra (*Abelmoschus esculentus* L. Moench) is considered as a treasure house of nutrients and it is one of the major vegetables widely spread all over tropical, subtropical and warm temperate regions of the world. Yellow vein mosaic virus (YVMV) and enation leaf curl virus are the most destructive diseases of okra as they affect both crop growth and yield. Due to the frequent breakdown of resistance and lack of a stable source of resistance in the cultivated species, interspecific hybridization is considered as a reliable approach for durable resistance. Cultivated species from The United States Department of Agriculture and wild accessions from The National Bureau of Plant Genetic Resources were screened at YVMV hotspot (Guntur, Andhra Pradesh) to identify the potential donors for disease resistance. Accessions IC141032 and IC141012 were found to be free from both viruses and categorized as resistant lines. Interspecific hybridization between *A. tetraphyllus* and *A. esculentus* revealed a high crossability index of around 80% when *A. esculentus* was utilized as a female parent. The bottleneck of hybrid sterility was partially overcome by the colchicine treatment of interspecific F1 hybrids. Good seed set was observed when raw colchiploids were backcrossed to the recurrent parent.

Introduction

Owing to its nutritional benefits, medicinal value, ease in cultivation and adaptability, okra (*Abelmoschus esculentus* L. Moench) has emerged as an important vegetable crop in recent years (Reddy *et al*., 2013). It is extensively cultivated in warm, moderate, tropical and subtropical areas across the globe. The cultivated species of okra is an amphidiploid of *A. tuberculatus* ($2n = 58$) and an unknown species with $2n = 72$. The genome of the *Abelmoschus* genus is quite complex and there is a wide variation in the chromosome number. Datta and Naug (1968) reported that the chromosome numbers – $2n = 72$, 108, 120, 132, and 144 are in a regular series of polyploids ($n = 12$). The highest chromosome number reported for *A. manihot* var. *caillei* is approximately 200, and the lowest chromosome number reported for *A. angulosus* is $2n = 56$.

India is globally the largest okra producer; however, the biotic and abiotic stresses pose a major hindrance to the enhanced productivity of the crop. The yield and quality of fruits are significantly threatened by enation leaf curl virus (ELCV) and yellow vein mosaic virus (YVMV) (Ayam *et al*., 2018). The colossal loss (ranging from 17 to 96%) is attributed to the growth stage of crops during which the infection takes place (Jamir *et al*., 2020). Resistance breeding is a dependable and feasible approach (Senjam *et al*., 2018). However, breeding for disease resistance is quite a daunting task because of the complex genome of the crop (Mishra *et al*., 2017) less understood genetics of inheritance of diseases, and lack of potential sources of resistance in cultivated species (Ayam *et al*., 2018). The availability of stable and potential sources of resistance in cultivated species is meager (Sastry and Zitter, 2014). However, wild species such as *A. tetraphyllus*, *A. pungens*, *A. panduraeformis*, *A. tuberculatus*, *A. vitifolius*, *A. crinitus*, *A. angulosus* and *A. manihot* have been reported to be the potential sources of YVMV and ELCV resistance (Singh *et al*., 2007). A number of characters have been transferred from these crop wild relatives to cultivated types through wide hybridization, at the intergeneric or interspecific levels (Nomura and Makara, 1993). Samarajeeva *et al*. (1998) reported the variation in the magnitude of sexual compatibilities of wild relatives with cultivated okra. Several pre-fertilization and post-fertilization barriers exist and the crossability index varies with the species. Crosses involving *A. tetraphyllus*, which has medium crossability with the cultivated species, have been attempted but hybrid sterility is the bottleneck (Singh *et al*., 2007). It is sometimes possible to attain first-generation hybrids in crosses between *A. tetraphyllus* and *A. esculentus*; however, the process is blocked at

the second generation (Hamon, 1988). Sureshbabu and Dutta (1990) used colchicine treatment and produced completely fertile amphidiploid *A. tetraphyllus* × *A. esculentus*.

However, durability of resistance transferred from the wild species is not exemplary. The resistant varieties developed by interspecific hybridization have succumbed to diseases, probably due to new virus strains or due to the ineffective contribution of the resistant genes transferred from the wild species because of the lack of adapted gene complexes, which exist in the wild species (Prabu and Warade, 2013). This study attempts to identify the latent source of resistance for YVMV and its subsequent transfer into cultivated background through wide hybridization and ways to overcome the hybrid sterility which is the major hindrance in the gene transfer process.

Materials and methods

Screening for YVMV and ELCV

Screening

The wild and cultivated species of *Abelmoschus* were screened for their reaction to YVMV and ELCV. Screening was taken up at Nutakki village, Mangalgiri, Guntur district of Andhra Pradesh, which is considered as the hotspot for both YVMV and ELCV. The humid nature of the location is favourable for the multiplication of the white flies, which serve as a vector for these viruses. Screening of 120 accessions from NBPGR and USDA was done in the summer and rainy seasons of 2019 to see the reactions of accessions in different climatic conditions.

The experimental material was evaluated in two replications with a spacing of 45 × 30 cm. The susceptible accession (PI 370028) was sown 15 days prior to the test entry along the border of the screening plot and it was sown after every 10 rows of test accessions to ensure uniform distribution of the inoculum. Precautions were taken to avoid the insecticide sprays that affect the vector population in the field. The crop was maintained by following all the agronomic practices mentioned in the standard package of practices (DoH and TNAU, 2019).

Scoring

Okra plants were scored for the incidence of both ELCV and YVMV separately, but plants with combined symptoms were counted for both the diseases. Plants were scored for virus incidence at 30, 60 and 90 days after sowing. The number of infected plants was recorded during the scoring. Per cent disease index was calculated after the scoring, the reaction was categorized based on 1–4 scale, and the severity was scored on the 0–7 ratings given by Das *et al.* (2013).

YVMV severity was assessed using the following scoring pattern (Das *et al.*, 2013).

Sl. No.	Score	Description
1	0	No disease
2	1	Up to 15% of leaf area affected
3	3	30–45% of leaf area affected
4	5	45–60% of leaf area affected
5	7	Greater than 60% of leaf area affected

Incidence of the disease was calculated by:

PDI (%) = (Number of infected plants/Total number of plants observed) × 100

YVMV disease was scored in a scale of PDI values (Das *et al.*, 2013).

Sl. No.	Reaction	PDI
1	Resistant (R)	≤10%
2	Moderately resistant (MR)	11–15%
3	Moderately susceptible	16–45%
4	Highly susceptible	>45%

PDI was calculated for ELCV using the formula as mentioned below and the reaction was categorized based on 1–6 scale used for tomato leaf curl virus by Kuldeep (2014).

PDI (%) = (Number of infected plants/Total number of plants observed) × 100

Grade	Reaction	Per cent disease incidence
1	Immune	No plants infected
2	Resistant	0–5% plants infected
3	Moderately resistant	5–10% plants infected
4	Moderately susceptible	10–25% plants infected
5	Susceptible	25–50% plants infected
6	Highly susceptible	>50% plants infected

The severity of the ELCV was graded by a scale suggested by Alegbejo (1997) and Jamir *et al.* (2020).

Scale	Description of symptom
0	No symptom
1	No visible disease symptom
3	Top leaves curled and slight stunting of plant
5	All leaves curled, twisting of petiole and slight stunting of plant
7	Severe curling of leaves, twisting of petiole, stunting of plant and proliferation of auxiliary branches

After calculating the PDI for both diseases, accessions that showed resistance to both viruses were considered as resistant accessions because only virus symptoms were considered for scoring.

Parental material selection

The inbred line NBO-9, which was found to be superior in all horticultural traits except YVMV and ELCV susceptibility was selected as the recipient parent. The obtained resistant wild accessions at the end of the screening were used as the donor parent for interspecific hybridization and for the transfer of virus resistance.

Interspecific hybridization

Hybridization between the recipient parent NBO-9 (superior horticulturally, but susceptible to ELCV and YVMV) and YVMV and

https://doi.org/10.1017/S1479262121000423 Published online by Cambridge University Press

Fig. 1. *Abelmoschus tetraphyllus* accession IC141032.

ELCV-resistant donor parent (*Abelmoschus tetraphyllus*) was carried out during the post rainy season of 2019 at Research Station, Bangalore. Ten *A. esculentus* flowers were used each for both direct and reciprocal crosses to study the difference in the fruit set. Hand emasculation and pollination were followed for hybridization. In order to avoid the contamination of pollen, the flower buds of *A. esculentus* (supposed to open next day) were hand emasculated and covered with butter paper. Hand pollination was carried out the morning after. Butter paper was used to cover cross-pollinated flowers for avoiding out-crossing. Harvesting of dry and fully mature crossed fruits, extraction of F1 hybrid seeds, and counting of the number of seeds set on *A. esculentus* was carried out at 35 days after pollination. Seed germinability was studied in F1 seeds.

Germination and dormancy of interspecific F1 hybrid seed

One hundred seeds collected from crosses were sun dried and the seed germination was studied using the paper towel method. The following formula was used to compute the germination percentage.

Germination percentage = (Number of seeds germinated/Number of seeds kept for germination) × 100.

Colchicine treatment in interspecific F1s to overcome sterility

Fully mature and properly dried F1 hybrid seeds, obtained from the crosses between *A. esculentus* and *A. tetraphyllus*, were collected and sown in the germination trays to raise seedlings of F1 hybrids for colchicine treatment during the summer season of 2020. Cotton swab method was used to treat the seedlings of interspecific crosses at the pseudocotyledonary (two-leaf) stage with 0.1% colchicine on apical meristem four times in a day, from the fourth day to the seventh day after germination, at a 3 h interval (from 9.00 a.m. to 6.00 p.m.) (Reddy, 2015). The seedling mortality was observed.

Results and discussion

The frequent breakdown in the resistance for YVMV and ELCV has raised a serious concern in ensuring the enhanced productivity of okra. The scarce availability of resistance sources in the cultivated species has led to the use of wild relatives for resistance transfer (Reddy, 2015). Hardiness and profuse branching are other desirable traits that can be transferred along with resistance. However, fertilization barriers, which obstruct interspecific hybridization, are a setback for resistance transfer (Dutta, 1984; Jambhale and Nerkar, 1981; Sureshbabu, 1987; Hamon, 1988; Rajamony *et al.*, 2006; Jatkar *et al.*, 2007). Fertile F1 progenies are the prerequisite for resistance transfer from the wild species. It would be difficult to carry out backcross and produce the subsequent generations, if sterility is encountered at the F1 level (Joshi and Hardas, 1976; Singh and Bhatnagar, 1976; Siemonsma, 1982a, 1982b; Hamon and Yapo, 1986; Hamon, 1988). However, the crossability index was found to be satisfactory as most of the cultivated and well-adapted genotypes of okra were crossable with the wild species. The present study results highlight the possibility of overcoming the major hurdle of hybrid sterility and thereby the possibility of successful transfer of resistance from *A. tetraphyllus*.

YVMV and ELCV screening

YVMV and ELCV incidence and severity were observed at 30-day intervals up to 90 days. Susceptible accessions, i.e., NBO-3 (for YVMV) and NBO-8 (for ELCV), showed 100% disease incidence with high severity and suggest the adequate inoculum level in the field. Resistant check NBO-33 was completely free from both viruses. Testing of 120 accessions of United States Department of Agriculture and National Bureau of Plant Genetic Resources belonging to *A. esculentus* and *A. tetraphyllus* species was done for disease reaction. Out of these, only two accessions IC141032 and IC141012 belonging to *A. tetraphyllus* were found to be completely free from both diseases (Fig. 1). Apart from these two accessions, seven other accessions were identified to be free from YVMV, but were moderately susceptible to ELCV (11–18%) with moderate severity (Table 1). Resistance nature of *A. tetraphyllus* was also described by Shetty *et al.* (2013). Singh *et al.* (2007) reported that about 57% accession of *A. tetraphyllus* was found to be free from YVMV and 29 accessions of *A. tetraphyllus* were completely free from ELCV. Manjua *et al.* (2018) reported that wild accession IC344598 and two cultivated accessions, *viz.*, PSRJ-12952 and RJR-124, did not show any signs of YVMV infection throughout the crop period and exhibited immune reaction. They opined that the incidence of less YVMV was probably due to the less population of whiteflies in these accessions. These results suggest that *A. tetraphyllus* accessions are a source of

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Table 1. Reaction of okra wild accessions for YVMV and ELCV

		ELCV			YVMV		
Sl. No.	Accession number	PDI	Severity	Reaction	PDI	Severity	Reaction
1	IC141032	0.00	0	Resistant	0.00	0	Resistant
2	IC141012	0.00	0	Resistant	0.00	0	Resistant
3	IC90343	17.86	5	Moderately susceptible	0.00	0	Resistant
4	IC90386	17.14	3	Moderately susceptible	0.00	0	Resistant
5	IC90388	11.11	3	Moderately susceptible	0.00	0	Resistant
6	IC90404	16.67	3	Moderately susceptible	0.00	0	Resistant
7	IC90461	18.42	5	Moderately susceptible	0.00	0	Resistant
8	IC90503	12.12	3	Moderately susceptible	0.00	0	Resistant
9	IC90529	13.64	3	Moderately susceptible	9.09	3	Resistant
10	Susceptible check (NBO-8)	100.00	7	Susceptible	30.00	5	Moderately Susceptible
11	Susceptible check (NBO-3)	43.33	7	Susceptible	100.00	7	Susceptible
12	Resistant check (NBO-33)	0.00	0	Resistant	0.00	0	Resistant

Fig. 2. Pollen load in *A. tetraphyllus* accession.

Table 2. Germination percentage in different crosses

Sl. No.	Cross	Germination (%)
1.	NBO-9×IC141032	90
2.	NBO-9×IC141012	75

YVMV and ELCV resistance and our results are in accordance with these reports.

Interspecific hybridization

Hybridization between in-house developed line NBO-9 and wild accessions IC141032 and IC141012 of *A. tetraphyllus* was successful with a crossability index of 80% (eight pods were set out of 10 pollinated flowers). The utilization of *A. esculentus* as a female parent resulted in this higher per cent (Fig. 2). This result is in line with the study conducted by Teshima (1933) and Sujatha (1983), who found that the cross was compatible only when

Fig. 3. Shrivelled seeds in untreated F1.

https://doi.org/10.1017/S1479262121000423 Published online by Cambridge University Press

Fig. 4. Partial seed set in colchicine-treated F1.

A. manihot was used as a male parent and *A. esculentus* was used as a female parent. The successful F1s were obtained in both direct cross and reciprocal cross between *A. tetraphyllus* var. *tetraphyllus* and *A. esculentus* cultivars by Jambhale (1980) and Sheela (1986). This high crossability index is because *A. tetraphyllus* and *A. esculentus* belong to ploidy level 3: $2n = 120–140$ (Sutar *et al.*, 2013).

Germination and dormancy

Out of the 100 seeds kept for germination from each cross, slight differences were observed in the germination percentage. Higher germination percentage (90%) was observed in the cross NBO-9 × IC141032 (Table 2). Dormancy was not observed in seeds obtained from either of the crosses. High percentage of germination was also observed in the study conducted by Reddy (2015).

Hybrid sterility associated with interspecific hybridization

The interspecific F1s exhibited normal growth and flowering, and the fruit formation was normal, but the hybrids were completely sterile. In some fruits, seed set was observed but they were shrivelled and abortive (Fig. 3). The fertility behaviour in F1s is decided by chromosome homology, which is primarily measured by the frequency of bivalent formation. Meshram and Dhapake (1981) reported that meiosis was abnormal in F1 between *A. esculentus* and *A. tetraphyllus* and it showed an average 37 bivalent and 55 univalents at metaphase I. Sterility in the interspecific hybrid can be attributed to this abnormal meiotic behaviour.

Colchicine treatment to overcome the hybrid sterility

The interspecific hybrids were sterile and the seed set was not observed. Restoration of fertility is a prerequisite for advancing interspecific hybrids to further generations and colchicine treatment was done at the two-leaf stage to accomplish this. The seedlings exhibited scorching symptoms on the apical region after the colchicine treatment, but mortality of seedlings was not observed. The interspecific F1s exhibited vigorous sideward growth with normal fruit set (95%) and only a partial seed set (15%) was found (Fig. 4). The obtained fertile F1s were further used for the backcross programme to transfer resistance and other desirable traits.

Backcrossing raw colchiploids to the recurrent parent

Raw colchiploids were used as a pollen parent and NBO-9 was used as a female parent. Normal seed set (around 90%) was observed when raw colchiploids were used as a male parent, whereas a seed set of around 52% was observed when untreated interspecific F1 was used as a male parent. The seed set in the backcross was satisfactory, thus the raw colchiploids can directly be used for backcrossing instead of stabilized colchiploids.

Conclusion

The scarce availability of resistance sources in cultivated okra has led to the search for potential donors in the wild species and its subsequent transfer to the cultivated background. In virus hotspot screening, two *A. tetraphyllus* accessions resistant to both YVMV and ELCV were found and the resistance transfer was initiated. The interspecific crosses attempted for *A. esculentus* × *A. tetraphyllus* were successful. Hybrid breakdown and hybrid lethality were not encountered in F1 hybrid plants. However, the problem of hybrid sterility was identified in the interspecific F1 hybrid plants of *A. tetraphyllus* and *A. esculentus*. The problem of sterility in the interspecific F1 hybrid plants was partially overcome by resorting to colchiploidy. The colchiploids obtained by treating the interspecific F1 seedlings were partially fertile. These colchiploids have to be selfed to ensure complete fertility. Since a good seed set is observed in the first backcross between raw colchiploids and *A. esculentus*, we can initiate backcrossing at this level only if we can ascertain that there is no difference in the seed set using raw colchiploids and stabilized colchiploids by conducting further studies. This study represents the preliminary step in transferring the resistance from wild species *A. tetraphyllus*. Backcrossing followed by generation advancement will be carried out further for the transfer of resistance.

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