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RESEARCH ARTICLE

Unravelling the crossability behavior between cultivated okra (*Abelmoschus esculentus*) and Kasturi okra (*Abelmoschus moschatus*): Occurrence of somatoplastic and hybrid sterility

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Abstract

Crop's wild relatives (CWRs) are the reservoir of various important traits, including resistance to biotic and abiotic stresses. Introgression of YVMV and OELCV-resistant gene(s) from wild relatives plays a crucial role in diversifying the resistant gene in okra and necessitates wide hybridization. Kasturi okra or *Abelmoschus moschatus* produced aromatic seeds and are abundant in the Indian sub-continent and also reported as a source for YVMV and OELCV resistance. A field experiment was conducted using three accessions of *A. esculentus* and seven accessions of kasturi okra to study the cross ability behavior through direct and reciprocal crosses. The fruit set percentage (66.00–88.00%) and average number of seeds/fruit (27.20–40.60) of the direct crosses were found to be significantly higher as compared to reciprocal crosses. Germination percentage of the direct crosses varied from 60.00–88.00%, while none of the seeds from reciprocal crosses were germinated. The accessions VRO-R-8 and IC-039308 were identified as the most compatible parents for interspecific hybridization with the highest crossability index. To find out the reason for the non-germination of seeds in the reciprocal crosses, the growth and development of fruits and seeds were thoroughly examined in the self-pollinated and crossed fruit using *A. moschatus* as the female parent. A shriveled seed with shrunken endosperm & degenerated embryo was found in the crossed fruit at 28 days after cross-pollination with *A. esculentus* pollen, which led to the formation of a non-viable seed. This seed failure might have arisen due to the somatoplastic sterility after interspecific hybridization. In direct crosses *A. esculentus* × *A. moschatus* all the F_1 plants produced fruits without seed and signified the presence of hybrid sterility. The direct crosses of the *A. esculentus* × *A. moschatus* can be utilized in the backcross breeding after restoration of fertility through colchicine treatment.

Keywords: Okra**,** Crossability, Somatoplastic sterility, Hybrid sterility, Disease resistance**.**

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Introduction

Okra, a member of the Malvaceae family, is a well-known genus with significant economic value due to its numerous agriculturally important wild relatives, widely cultivated species and cultivars (Misra et al., 2022). Okra can be grown across the tropical and subtropical regions of the world and in warmer parts of the temperate zone. It is a major vegetable in tropical countries that is highly popular in Ghana, Nigeria, Cameroon, India, Pakistan, and Iraq (Karmakar et al., 2022). The origin of okra remains controversial. Perhaps it originated in Ethiopia and the upper Nile region of Sudan, West Africa, or Tropical Asia (Singh et al., 2023). Cultivation of okra dispersed all over the Middle East and North Africa (Lamont, 1999). Okra has formerly placed in the genus *Hibiscus*, but because of the caducity of the calyx and its adenations to the petals and staminal column, it was later transferred into the genus *Abelmoschus* (Mishra et al., 2021). Okra belongs to the often cross-pollinated group, with outcrossing ranging from 4 to

30%. Due to its large flower size, mono-adelphous stamens and maximum seed production in one pollination, okra is an attractive crop for geneticists and breeders (Verma et al., 2016). Okra fruits' color ranges from green to red and later is the most predominant in the market (Karmakar et al., 2022). India is the richest source of genetic diversity for okra due to geographic distance, genetic barriers to crossability, and distinct evolutionary parents (Kumar et al., 2015). The availability of diverse okra cultivars is necessary to achieve the requirement of regional adaptability to distinct climatic conditions and attend to farmer necessities for different phenotypic characteristics of okra (Reddy et al., 2016). While, *Abelmoschus moschatus* Medik. is an aromatic and medicinal plant in the Malvaceae family, also known as kasturi okra, which originated in India. It is an herb that can grow up to 1.5 to 1.6 meters tall, with an erect, upright hispid stem and a long, slender taproot. Its leaves exhibit a variety of shapes, often polymorphous, typically cordate (heart-shaped). The lower leaves tend to be ovate with pointed or rounded angles, while the upper leaves are divided into 3 to 7 lobes that extend nearly to the base. The lobes are narrow and either pointed (acute) or oblong-ovate in shape, with edges that are wavy (crenate), saw-like (serrate), or irregularly toothed and both surfaces of leaves are hairy. It can be grown as annual or perennial in certain circumstances and reported as tolerant to biotic stresses such as YVMV and OELCV diseases (Kumari et al., 2021) as well as to abiotic stresses like dry conditions and low temperatures prevailing during the winter season in some of the okra growing areas (Akhond et al., 2000). The plant contains a variety of phenolic compounds, flavonoids, carbohydrates, proteins, sterols, tannins, fixed oils, and fats (Pawar et al., 2017). The successful cultivation of okra is often hindered by an array of insect pests, *viz*., shoot and fruit borer, whitefly, aphid, and leafhopper (Dikshit et al., 2001) and diseases like YVMV and OELCV (Singh et al., 2023). In the Indian subcontinent, begomoviruses pose a serious threat to okra crops and are responsible for substantial economic losses to the okra growers. Yellow vein mosaic disease caused by YVMV is one of the major constraints in okra, with an estimated yield loss of about 50 to 94%, depending on the stage of crop growth at which the infection occurs. Similarly, enation leaf curl disease caused by the OELCV is also emerging as a serious threat, rendering a yield penalty of about 30-100%. Whiteflies spread nearly all begomoviruses through their persistent and calculated eating of plants. While adult flies transfer more viruses to the ovary and to their progeny, younger flies transfer fewer viruses to the ovary (Sagar et al., 2020).

In India, exhaustive research efforts were ongoing to develop varieties/hybrids that are resilient to viral diseases, but to date, no stable and durable resistance has been reported in the cultivated gene pool. This is mainly because of the evolution of new strains of viruses or due to recombination in virus strains, which makes them unable to overcome the host-plant defense mechanism. Therefore, the search for resistant gene(s) invariably shifted to crop wild relatives (CWRs). For diversification of the resistant gene(s) in the cultivated background with durable and stable resistance, it is of paramount importance to find new sources of YVMV and OELCV resistance and its transfer to the cultivated okra elite genotypes. Interspecific hybridization succeeds only when there is complete compatibility between the gene complexes of the male and female parents. (Kuboyama et al., 1994). *Abelmoschus* interspecific hybridization may have failed for a number of reasons, including chromosomal and genomic variations as well as pre-and post-fertilization barriers that may have prevented fruit and seed sets. Cross incompatibility and pollen sterility of F_1 hybrids between cultivated and wild okra species resulted in limited success of interspecific hybridization (Nagaraju et al., 2019). For transferring the desirable characters of *A. moschatus* to cultivated okra, it is essential to cross these two species. However, reports of successful hybridization (both direct and reciprocal crosses) between *A. esculentus* and *A. moschatus* are limited. Pre-zygotic barriers such as natural environment (i.e., geographic isolation), floral structure (i.e., form or color), flowering time, or natural pollinator limit interspecific hybridization. Crossability obstacles in interspecific hybridization between *A. esculentus* and *A. moschatus* need to be thoroughly studied. Thus, the objective of this study was to unravel the crossability behavior between *A. esculentus* and *A. moschatus,* which will be useful for transferring desirable characters into cultivated okra.

Materials and Methods

The present experiment was carried out at the research farms of the ICAR-IIVR, Varanasi, which is located at *82.52°E longitude and 25.10°N latitude*. The experimental materials have consisted of seven accessions of *Abelmoschus moschatus viz*. EC-329394, EC-361007, EC-360953, IC-039308, IC-469583, IC-47737, EC-360095, three genotypes of cultivated okra such as Pusa Sawani, VRO-6, and VRO-R-8 along with 21 *A. esculentus* × *A. moschatus* hybrids. The parents (seven accessions of *Abelmoschus moschatus* and three accessions of cultivated okra) were grown during *Kharif* season of 2022-23 to develop both direct and reciprocal crosses. Harvested seeds of these crosses were sown during 2023-24 to generate crossability-related information. Besides, seed growth and development in the self-pollinated fruits of *A. moschatus* and cross-pollinated fruits *A. moschatus* × *A. esculentus* were also studied during 2023-24. Standardized cultural practices were followed periodically to ensure optimum growth of all the species.

Observations were recorded on the number of crosses attempted, the self-pollinated, average number of fruits set, seed set/fruit, F_1 seed germination (%) and % F_1 plant set fruits with or without seeded and these data were used to compute crossability index and hybrid lethality. Crossability index (%) was calculated according to the formula given by (Rao et al., 1979). Pollen viability of parents (3 accessions of cultivated okra & 7 accessions of *A. moschatus*) and 21 interspecific crosses were also studied and compared by using 1% acetocarmine solution.

To study the fruit growth and development in the self-pollinated and cross-pollinated fruit in *A. moschatus*, observation on fruit length and diameter were recorded at 4 days after pollination (DAP), 12 DAP, 20 DAP, 28 DAP and 60 DAP (at seed maturity) in 15 self-pollinated and crossed fruits. Similarly, to monitor the pattern of seed growth and development in the self-pollinated and cross-pollinated fruit in *A. moschatus,* visual observations on the developing seeds and average weight were recorded at 4 days after pollination (DAP), 12 DAP, 20DAP, 28 DAP and 60 DAP. Data regarding the fruit set, the average number of seeds/fruits, germination percentage, fruit length, fruit diameter and pollen viability were statistically analyzed and compared using standard error bars with a probability of less than 5% ($p < 0.05$).

Result and Discussions

Many crops have genetically improved from the frequent usage of wild relatives as sources of quality parameters and resistant to biotic and abiotic stresses (Zamir, 2001). Significant diversity has been produced by effective interspecific crosses in *A. esculentus* L. Moench (Reddy, 2010). However, obstacles within okra interspecific hybrids have impeded the desired gene transmission from wild to cultivated species. Even though successful wide hybridization with *A. moschatus* was earlier reported by Joshi et al., (1974) and many researchers. However, resistant varieties developed through wide hybridization have started showing symptoms of YVVM and OELCV diseases, perhaps due to new virus strains. Hence, it is of utmost essential to search for new variable sources of YVMV and OELCV

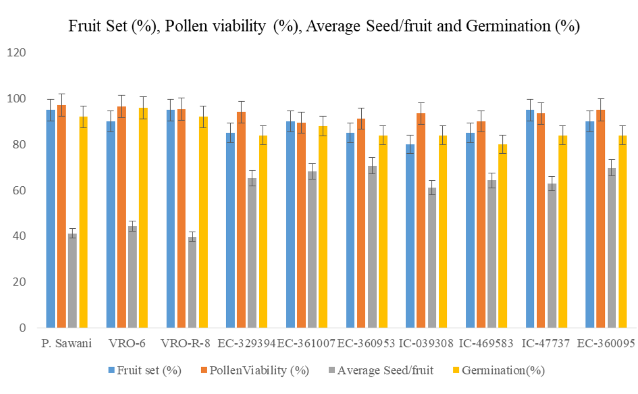

Figure 1: Fruit Set (%), pollen viability (%), average number of seed/fruit, and germination (%) of parents

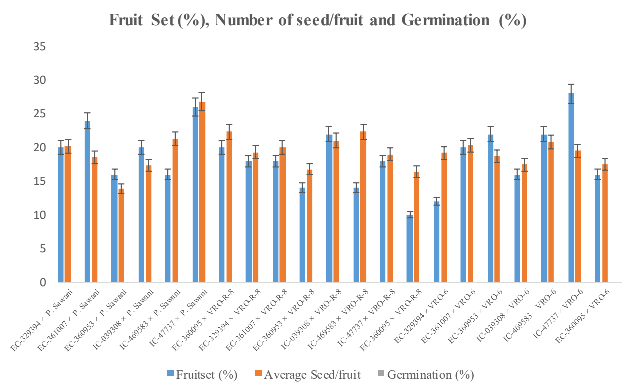

Figure 2: Fruit Set (%), number of seed/fruit and germination (%) of reciprocal crosses between (*A. moschatus* × *A. esculentus*)

resistance with good morphological traits and produce resistant varieties by appropriate gene introgression program. Keeping in view the above facts, an experiment was designed and conducted to explore the possibilities of crossing between *A. esculentus* and *A. moschatus* in both directions and examine the growth of seed and fruit after pollination to maturity stages. This investigation's findings revealed that all three *A. esculentus* accessions and the seven *A. moschatus* accessions used in this study are compatible with one another in direct and reciprocal crosses, providing compelling evidence that there are possibilities of crossing between *A. esculentus* and *A. moschatus*.

Crossability behavior of *A. esculentus* and *A. moschatus*

Estimation selfing & hybridization success

In terms of selfing of three cultivated and seven *A. moschatus* accessions, 50 flowers were self-pollinated for each accession and fruit set (%) was estimated (Figs 1 & 6). In cultivated accessions fruit set percentage varied from 90 to 95% upon selfing and the maximum fruit set was reported in Pusa Sawani and VRO-R-8. On the other hand, fruit set percentage in *A. moschatus* accessions ranged from 80 to 95%, with maximum and minimum reported in IC-47737 (95%) and IC-039308 (80%), respectively. There was negligible variation reported for the fruit set in the cultivated and wild accessions upon selfing. Pollen viability in cultivated and wild accessions varied from 95.38 to 97.22% and 89.52.58 to 95.08%, respectively, which seems to be non-significant (Fig 6 & 7). In relation to an average number of seeds/fruit through self-pollination, the highest number of seeds per fruit (70.72) was observed in EC-360953, which was followed by EC-360095 (69.82) and EC-361007 (68.19) for *A. moschatus* accessions and whereas in *A. esculentus* it was maximum in VRO-6 (44.25) followed by Pusa Sawani (41.23) and VRO-R-8 (39.81). The highest value of ovule-to-seed conversion was reported in self-pollinated fruits than the crossed ones for both cultivated and wild accessions (Fig 6). Additionally, the

Table 1: Crossability behavior of direct crosses between (A. esculentus × A. moschatus)

Crosses	*Fruit set (%)*	*Average No. of seed/fruit*	*Germination (%)*	*Hybrid Lethal (%)*	*Crossability index (%)*	*%F_1 Plant set fruit*	*% F_1 Plant with seeded fruit*	*% F_1 Plant with non- seeded fruit*	*Pollen viability (%)*
P. Sawani × EC-329394	56.00	31.86	45.33	0	62.22	100	0	100	55.36
P. Sawani × EC-361007	40.00	30.25	58.67	0	43.24	100	0	100	62.26
P. Sawani × EC-360953	44.00	31.43	45.33	0	48.89	100	0	100	45.19
P. Sawani × IC-039308	52.00	32.83	49.33	0	59.43	100	0	100	48.57
P. Sawani × IC-469583	46.00	34.29	65.33	0	51.11	100	0	100	56.19
P. Sawani × IC-47737	48.00	33.45	46.67	0	50.53	100	0	100	48.18
P. Sawani × EC-360095	54.00	28.38	66.67	0	58.38	100	0	100	42.21
VRO-R-8 × EC-329394	46.00	36.82	65.33	0	51.11	100	0	100	58.30
VRO-R-8 × EC-361007	58.00	35.85	42.67	0	62.70	100	0	100	39.47
VRO-R-8 × EC-360953	50.00	40.62	38.67	0	55.56	100	0	100	43.67
VRO-R-8 × IC-039308	62.00	31.21	48.00	0	70.86	100	0	100	52.14
VRO-R-8 × IC-469583	50.00	36.94	60.00	0	55.56	100	0	100	56.25
VRO-R-8 × IC-47737	44.00	30.82	41.33	0	46.32	100	0	100	49.02
VRO-R-8 × EC-360095	54.00	29.74	48.00	0	58.38	100	0	100	57.19
VRO-6 × EC-329394	58.00	33.64	65.33	0	66.29	100	0	100	62.22
VRO-6 × EC-361007	44.00	32.61	53.33	0	48.89	100	0	100	58.47
VRO-6 × EC-360953	48.00	27.26	58.67	0	54.86	100	0	100	52.32
VRO-6 × IC-039308	52.00	39.85	40.00	0	61.18	100	0	100	47.25
VRO-6 × IC-469583	54.00	32.67	44.00	0	61.71	100	0	100	42.54
VRO-6 × IC-47737	56.00	33.85	45.33	0	60.54	100	0	100	49.56
VRO-6 × EC-360095	42.00	38.41	40.00	0	46.67	100	0	100	59.29
Mean	50.38	33.47	50.73	0	55.92	100	0	100	51.70
Max	62.00	40.62	66.67	0	70.86	100	0	100	62.26
Min	40.00	27.26	38.67	0	43.24	100	0	100	39.47

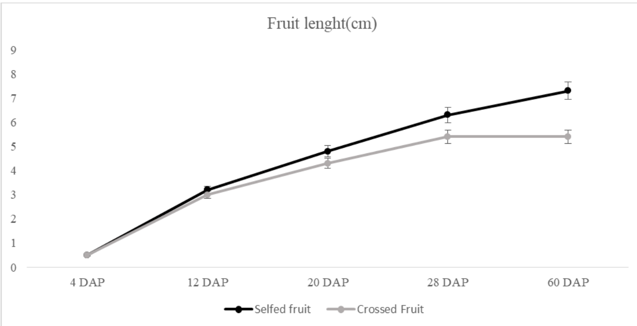

Figure 3(A): Pattern of fruit growth (length) in self-pollinated (*A. moschatus*) and reciprocal crosses (*A. moschatus* × *A. esculentus*)

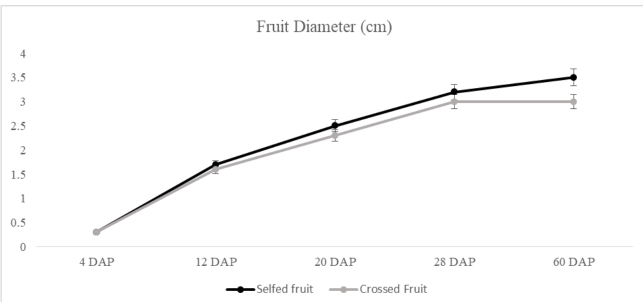

Figure 3(B): Pattern of fruit growth (diameter) in self-pollinated (*A. moschatus*) and reciprocal crosses (*A. moschatus* × *A. esculentus*)

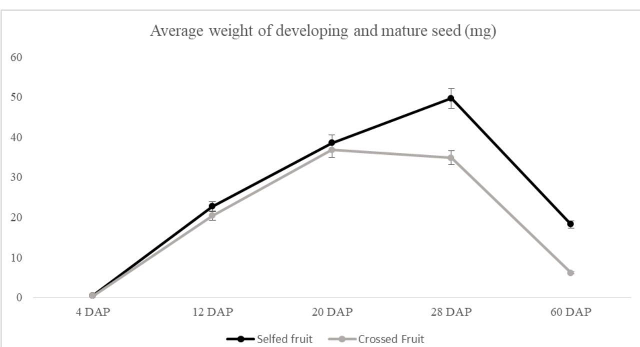

Figure 3(C): Pattern of seed growth (weight of developing & mature seed) in self-pollinated (*A. moschatus*) and reciprocal crosses (*A. moschatus* × *A. esculentus*)

seed germination percentage of *A. esculentus* accessions (92.00–96.00%) was found to be superior over *A. moschatus* genotypes (80.00-88.00%), which might be due to the seed dormancy in the wild species.

With respect to direct interspecific crosses (*A. esculentus* × *A. moschatus*) there was ample variation observed for fruit set percentage and varied from 40.00-62.00% (Table 1 & Fig 6). The highest fruit set of 62.00 % was recorded in VRO-R-8 × IC-039308 followed by VRO-6 × EC-329394 (58.00%), VRO-R-8 × EC-361007 (58.00%), VRO-6 × IC-47737 (56.00%) and Pusa Sawani × EC-329394 (56.00%) while least fruit setting (40.00%) was observed in Pusa Sawani × EC-361007 followed by VRO-6 × EC-360095 (42.00%) and Pusa Sawani × EC-360953 (44.00%), VRO-6 × EC-361007 (44.00%) and VRO-R-8 × IC-47737 (44.00%). As far as the average number of seed/crossed fruit is concerned in direct interspecific crosses, the maximum number of seeds per fruit (40.62) was found in VRO-R-8 × EC-360953, which was followed by VRO-6 × IC-039308 (39.85) and VRO-6 × EC-360095 (38.41) whereas least seed set (27.26) was observed in VRO-6 × EC-360953 followed by Pusa Sawani × EC-360095 (28.38) and VRO-R-8 × EC-360095 (29.74). The fruit and seed set percentage was found to be greater in the direct interspecific crosses than its counterpart.

For reciprocal crosses (*A. moschatus* × *A. esculentus*), the fruit set (%) was very low as compared to direct crosses (Figures 2 & 6) and the highest fruit set (%) was observed in IC-47737 × P. Sawani (28.00%), followed by IC-47737 × Pusa Sawani (26.00%) and EC-361007 × Pusa Sawani (24.00%). Whereas minimum fruit set (%) in reciprocal crosses was observed in EC-360095 × VRO-R-8 (10.00%), EC-329394 × VRO-6 (12.00%), EC-360953 × VRO-R-8 (14.00%) and IC-469583 × VRO-R-8 (14.00%). In terms of average number of seed per fruit for reciprocal crosses, the highest average number of seed/fruit (26.88) was observed in IC-47737 × Pusa Sawani which was followed by EC-360095 × Pusa Sawani (22.38) and IC-469583 × VRO-R-8 (22.34) whereas least average number of seed (13.89) was observed in EC-360953 × P. Sawani followed by EC-360095 × VRO-R-8 (16.42) and EC-360953 × VRO-R-8 (16.78). Though the fruit set and seed

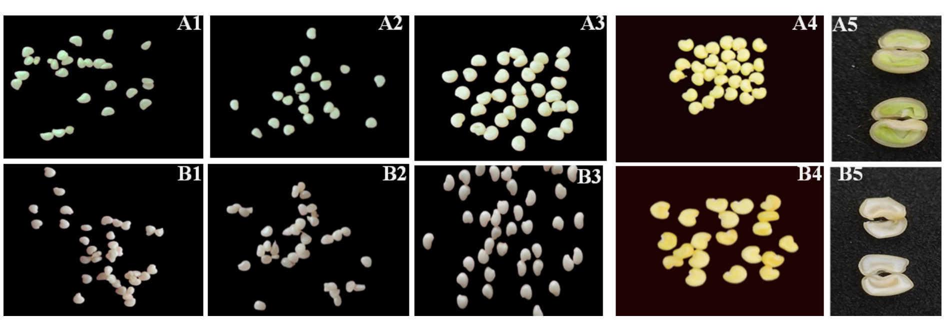

Figure 4: Seed growth and development of self-pollinated *A. moschatus* (A1:4 DAP, A2: 12 DAP, A3:20 DAP, A4 & A5:28 DAP) and cross pollinated (*A. moschatus* × *A. esculentus*) (B1:4 DAP, B2:12 DAP, B3:20 DAP, B4 & B5:28 DAP) fruits.

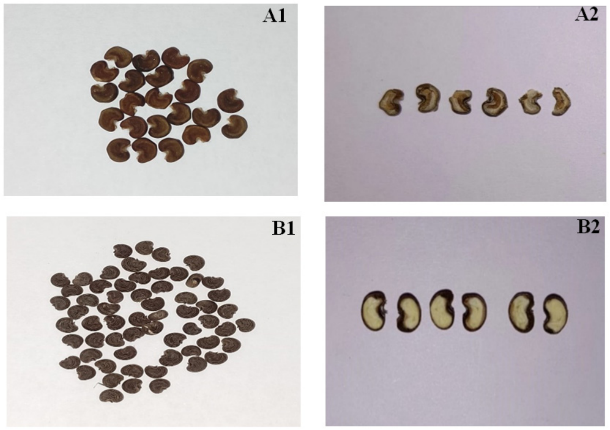

Figure 5: Ripe seeds from interspecific crossed of *A. moschatus* × *A. esculentus* (A1 & A2:60 DAP) and self-pollinated *A. moschatus* (B1 & B2:60 DAP) fruits

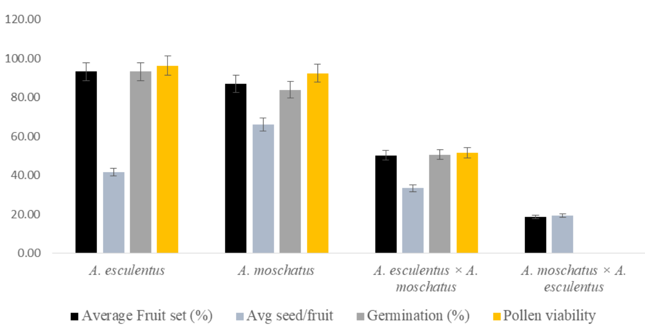

Figure 6: Average fruit set, seed/fruit, germination (%) and pollen viability (%) in parents and interspecific crosses

set seem to be normal in self-pollination. Comparatively, reduction in the fruit set as well as in the seed set was evident in the direct crosses. Besides, the fruit and seed set drastically reduced in the reciprocal crosses. These may be the consequence of the pre-fertilization barrier in the interspecific crosses. Sindhu (1993) reported that less fruit set (%) may be due to the variation in chromosome number in the parental species. These results related to fruit and seed set behavior in the interspecific crosses of *A. esculentus* and *A. moschatus* were also supported by the observation of Prabu and Warade (2013), Kaur et al. (2020) and Sandeep et al. (2022).

Hybrid lethality and crossability index

For estimation of hybrid lethality and crossability index, observation of germination (%) and fruit set (%) in parents and interspecific crosses is essential. Therefore, seeds obtained from direct as well as reciprocal crosses were sown to observe their germinability and viability and significant variation was reported (Table 1). Seed germination percentage were varied from 38.67-66.67% in the direct crosses with highest in Pusa Sawani × EC-360095 (66.67%) followed by VRO-6 × Ec-329394 (64.00%) and Pusa

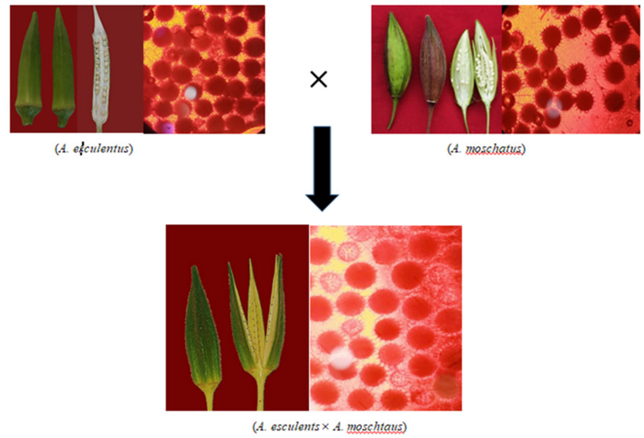

Figure 7: Fruit morphology and pollen viability of parents and interspecific crosses

Sawani × IC-469583 (62.67%); and minimum in VRO-R-8 × EC-360953 (38.67%) subsequently in VRO-6 × EC-360095 (40.00%) and VRO-R-8 × IC-47737 (41.33%). The results are in accordance with the previous findings of Amiteye et al. (2019), Reddy (2015) and Prabu and Warade (2013). Contrary to these results, none of the seeds from reciprocal crosses were germinated (Figures 2 & 6), though the seed coats externally looked normal. A similar type of problem in seed germination was also reported for the interspecific crosses of *Solanum* sp. (Kumchai et al., 2013; Karmakar and Singh, 2023). Hybrid lethality was not encountered in any of the 21 direct crosses and all the seeds germinated were grown in normal plants with flowering and development of fruit without seed. Patel et al. (2021) also reported a nonoccurrence of hybrid lethality in the crosses of *A. esculentus* and *A. tetraphyllus*.

With respect to crossability index (%) there was considerable variation observed, which ranged from 43.24 to 70.86% (Table 1). The accessions VRO-R-8 and IC-039308 were identified as most compatible parents with the highest CI value of 70.86% in its combination followed by VRO-6 × EC-329394 (66.29%), VRO-R-8 × EC-361007 (62.70%), Pusa Sawani × EC-329394 (62.22) and VRO-6 × IC-469583 (61.71). In comparison, the minimum crossability index was estimated in Pusa Sawani × EC-361007 (43.24%) followed by VRO-R-8 × IC-47737 (46.32%) and VRO-6 × EC-360095 (46.67 %). This exhibits their inherent capability as pollen donor and recipient, respectively, since each accessions were crossed under the same conditions. Nevertheless, the identification of a suitable pollen donor and recipient parent is utmost important criterion for the successful interspecific transfer of resistant genes to cultivated backgrounds in *Abelmoschus*. Interspecific cross-compatibility between okra and various wild taxa, viz. *A. moschatus* (Akhond et al., 2000), *A. tuberculatus* (Kuwada, 1966; Pal et al., 1952), *A. manihot* (Kuwada, 1961 & 1974) and *A. tetraphyllus* (Patel et al., 2021) was studied by various workers with the intention of incorporating useful traits and creating genetic diversity.

Occurrence of somatoplastic sterility and hybrid sterility

Somatoplastic sterility

In the 21 reciprocal interspecific crosses (*A. moschatus* × *A. esculentus*), variable numbers of seed sets were observed, which ranged from 13.89-26.88, and the seed coat of these seeds were externally found to be normal. The seed was obtained from reciprocal crossed fruit when sown during the *Kharif* season of 2023 to check their germination and none of the seeds were germinated. To identify the reasons for non-germinability of these, a set experiment was conducted to monitor the growth and development of fruits and seeds in self-pollinated and interspecific cross-pollinated flowers just after the pollination and fertilization. Observations of fruit length, diameter, average weight of developing seeds and their visual appearances in the self-pollinated and cross-pollinated fruit of *A. moschatus* were measured at 4 DAP, 12 DAP, 20 DAP, 28 DAP and at seed maturity (Figure 3A, 3B, 3C,4 & 5). At the time of first observation, *i.e*., 4 DAP (days after pollination), there were no differences observed in the fruit length (0.50cm) and diameter (0.30cm) in selfed and crosses fruit, and up to 20 DAP, an almost similar pattern in the increment for both length and diameter measured. At 12 DAP, the observed fruit length and diameter were 3.2 & 3.0 cm and 1.70 & 1.60 cm, respectively, in selfed and cross-pollinated fruit. Though the maximum fruit length (5.4cm) and diameter (3.0) in cross-pollinated fruit were attained in 28 DAP and there was no further increment observed up to fruit ripening at 60 DAP, but in selfed fruit at 28 DAP length and diameter were found to be 6.3 and 3.2 cm, respectively and which gradually grown to attain maximum length and diameter of 7.3 and 3.5 cm, correspondingly at 60 DAP. This indicated that there was a significant difference in fruit growth and development in selfed and crossed fruit after 28 DAP. With respect to the average weight of developing seeds and their visual appearances in the self-pollinated and cross-pollinated fruit, it had been noticed that there was no significant difference up to 20 DAP and thereafter decreasing trends exhibited by only the cross seeds. The considerable differences for the trait were recorded in selfed (49.61mg) and crossed (34.76 mg) fruit at 28 DAP. Nonetheless, it started declining after 28 DAP in both selfed and crossed fruit. Furthermore, the average weight of the fully mature and ripe seed from interspecific crossed fruit (6.12 mg) was comparatively lower as compared to self-pollinated fruit (18.22 mg). Normal growth and development of the seed coat, cotyledon, endosperm and embryo in seeds of both categories of fruits up to 20 DAP. In the self-pollinated fruit, a normal pattern of seed growth and development was observed just after pollination to seed maturation, while shriveled seed with shrunken endosperm & disintegrated embryo was detected in crossed fruit after 28 days after cross-pollination with *A. esculentus* and lead to the development of non-viable seed. Hindrance in normal growth of fruits and seeds was observed after 28 DAP and 20 DAP, respectively due to degeneration of the embryo and endosperm (Figure 4). Dissolution of the endosperm occurs in the adjacent area of overstimulated maternal tissue due to hypo-function that causes the improper distribution of nutrition in the seeds and the endosperm & embryo get less as compared to the integument which receives more than the normal share. Hypertrophy or overgrowth occurs in these tissues, specifically in the endothelium, and it uses comparatively more nutrients, leaving even a reduced amount for the endosperm and embryo, resulting in their starvation and degeneration, which leads to the formation of non-viable seed due to somatoplastic sterility (Brink and Cooper, 1947). Therefore, the formation of non-viable seeds in the *A. moschatus* × *A. esculentus* crosses might be due to the somatoplastic sterility, and it was also reported in the interspecific crosses of *Vigna* (Gopinathan et al., 1986), *Trifolium* (Williams and White, 1976) and *Ipomea* (Wedderburn, 1967).

Hybrid sterility

Seeds obtained from direct interspecific crosses (*A. esculentus* × *A. moschatus*) were sown during the *Kharif* season of 2023 and after germination, all the interspecific F_1 hybrids showed normal vegetative growth and development with respect to various morphological traits. Though the flowering behavior was found to be normal, like their parents, they developed fruits without seeds. In all the direct interspecific hybrids, 100% of the plants exhibited parthenocarpic fruit set and were devoid of normal growing seed in the developing fruit, which made these hybrids completely sterile (Table 2 & Figure 7). To establish the reason for hybrid sterility in these F_1s, pollen viability was also observed. Considerable variation for pollen viability was reported among the various combinations, which ranged from 39.47 to 62.26% (Table 2; Figures 6 & 7). Nevertheless, the pollen fertility in the interspecific hybrids (51.70%) reduced drastically as compared to their respective parental species *A. esculentus* (96.40%) and *A. moschatus* (92.47%). The chromosome homology, more specifically the prevalence of bivalent formation, determined the extent of fertility & sterility in the interspecific hybrids. Hence, the abnormal meiotic behavior might be the reason for sterility in the *A. esculentus* × *A. moschatus*. Occurrence of hybrid sterility in the *A. esculentus* × *A. moschatus* F_1s will be the key hindrance for successful interspecific introgression of YVMV and OELCV resistance into cultivated okra from *A. moschatus* and this bottleneck can only be overcome by restoring the fertility through colchiploidy. Hybrid sterility in the interspecific hybrids of *Abelmoschus* was earlier also reported by Patel et al. (2021), Reddy (2015) and Sandeep et al. (2022).

Conclusion

In the context of resistance breeding of okra for YVMV & OELCV diseases, whatever the genetic resources in the cultivated gene pool used as a source are very old and also need diversification for durable resistance. Besides, the limited availability of sources of resistance in the *Abelmoschus esculentus* germplasm compelled the plant breeder to look into the kitty of wild genetic resources, including *A. moschatus,* for potential donors of viral disease resistance in okra. From the outcome of this study, it was quite conclusive that *A. moschatus* could be utilized for the transfer of viral disease resistance in to cultivated okra as pollen parent. Direct interspecific crosses of *A. esculentus* × *A. moschatus* did not exhibit any types of pre-zygotic barriers and set viable seeds that are able to grow into normal plants. Though the flower and fruit development was normal, these plants exhibited parthenocarpic fruit set and produced seedless fruit because of hybrid sterility induced due to improper mitotic behavior. On the other hand, the reciprocal crosses of *A. moschatus* × *A. esculentus* set fruits and seeds but failed to germinate. The formation of non-viable seeds in the reciprocal crosses was mainly because of the degeneration of the embryo and endosperm, as they received significantly reduced nutrition as compared to integument and endothelium, resulting in somatoplastic sterility. Therefore, the utilization of direct and reciprocal crosses for introgression of a resistant gene (s) in okra will be possible only through colchiploidy and embryo rescue at an appropriate stage, respectively.

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सारांश

भिंडी की जंगली प्रजातियां जैविक और अजैविक रोगो के प्रतिरोधी होने के साथ-साथ विभिन्न महत्वपूर्ण लक्षणों की भंडार हैं। जंगली प्रजातियो से वाईवीएमवी और ओईएलसीवी प्रतिरोधी जीन का स्थानांतरण भिंडी में प्रतिरोधी जीन को स्थानांतरित करने और व्यापक संकरण में महत्वपूर्ण भूमिका निभाता है। एबेलमोस्कस मोस्कैटस भारतीय उपमहाद्वीप में प्रचुर मात्रा में पाए जाते हैं जिन्हे इनके सुगंधित बीज के लिए उगाया जाता हैं। कस्तूरी भिंडी को वाईवीएमवी और ओईएलसीवी प्रतिरोध के स्रोत के रूप में भी रिपोर्ट किया गया है। कस्तूरी भिंडी की भिंडी की खेती की जाने वाली प्रजातियों के साथ क्रासिंग क्षमता का मूल्यांकन करने के लिए ए. एस्कुलेंटस के 3 एक्सेसन और कस्तूरी भिंडी के 7 एक्सेसन का उपयोग किया गया । फल सेट प्रतिषत (66.00-88.00ः) और बीज/फल की औसत संख्या (27.20-40.60) पारस्परिक क्रॉस की तुलना में प्रत्यक्ष क्रॉस मे काफी अधिक पाई गई। प्रत्यक्ष क्रॉस का अंकुरण प्रतिषत 60.00-88.00ः के बीच था, जबकि पारस्परिक क्रॉस से कोई भी बीज अंकुरित नहीं हुआ। वी.आर.ओ.-आर-8 और आई.सी.-039308 को उच्चतम क्रॉसबिलिटी इंडेक्स के साथ अंतर-विषिश्ट संकरण के लिए सबसे अनुकूल माता-पिता के रूप में पहचाना गया। पारस्परिक क्रॉस के बीजों में अंकुरण न होने का कारण जानने के लिए, ए. मोस्कैटस का उपयोग मादा के रूप में करके स्व-परागीत और पर परागित फलों में बीजों की वृद्धि और विकास की पूरी तरह से जांच की गई। पर परागण के २८ दिन बाद बीजो का मूल्यांकन करने पर सिकुड़े हुए भ्रुणपोश और विकृत भ्रुण के साथ अजीवित बीज पाया गया जो की जमने के योग्य नहीं था। यह अनुमानित है की ऐसा अंतरप्रजातीय संकरण के बाद सोमैटोप्लास्टिक स्टेरिलिटी के कारण हुआ होगा । ए. एस्कुलेंटस × एबेलमोस्कस मोस्कैटस से प्राप्त सभी संकर पौधों ने बिना बीज के फल उत्पन्न किए जो की संकर बंध्यता की उपस्थिति का संकेत है। इन पौधों को कोल्चिसिन से उपचारित करके संकर उर्वरता को पुनः स्थापित किया जा सकता है तथा भविश्य में इनका उपयोग रोग प्रतिरोधी जीन को जंगली प्रजातियों से बैक क्रॉस के माध्यम से हस्तांतरित करने के लिए किया जा सकता है।