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Crossability Behaviour and Fertility Restoration Through Colchiploidy in Interspecific Hybrids of *Abelmoschus esculentus* × *Abelmoschus manihot* subsp. tetraphyllus

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Abstract

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable commercially cultivated in tropical and sub-tropical regions of the world mainly for its immature fruits. Yellow vein mosaic virus (YVMV) transmitted by white fly (*Bemisia tabaci* Gen.) is the most serious disease of okra affecting both fruit yield and quality, for which source of resistance is not available in cultivated okra. Interspecific hybridization followed by backcrossing and selection in the segregating generations is an effective method for developing YVMV resistant varieties. Characterization and evaluation of 28 inbred lines of cultivated okra (RNOYR-1 to RNOYR-28) and two wild species (*A. manihot* subsp. manihot and *A. manihot* subsp. tetraphyllus) in two separate field trials was undertaken at Vegetable Research Station, Rajendranagar during summer 2013. From these field trials, the horticulturally superior but YVMV susceptible cultivated okra RNOYR-19 and YVMV resistant *A. manihot* subsp. tetraphyllus were selected as parental lines for interspecific hybridization, which were crossed during summer 2013. Upon one-way crossing, the species *A. esculentus* (RNOYR-19) as female parent and *A. manihot* subsp. tetraphyllus as male parent were freely crossable and thus self-reproducing. The interspecific F_1 hybrid plants of the above one-way cross were absolutely free from hybrid lethality and hybrid breakdown. Complete sterility was observed in the F_1 hybrid plants of *A. esculentus* and *A. manihot* subsp. tetraphyllus. Hence, we were not able to advance or develop them further by direct backcrossing with the *A. esculentus*. A restoration of partial fertility in the F_1 hybrid plants was achieved by treating the seedlings of the interspecific F_1 plants at two leaf (pseudocotyledonary) stage with 0.1% colchicine on apical meristem through cotton swab method from 4^{th}-7^{th} day after germination 4 times a day at 3 h interval from 6.00 a.m. to 6.00 p.m., resulting in the production of raw colchiploids (C_1). A restoration of complete fertility in the raw colchiploids (C_1) was achieved by single cycle of selfing resulting in the production of stabilized colchiploids (C_2).

Keywords

Colchicines, Colchiploids, Crossability, Hybrid Sterility, Interspecific Hybridization

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1. Introduction

Okra belongs to the genus *Abelmoschus* and family Malvaceae. About 50 species have been described by taxonomists in the genus *Abelmoschus*. Of these, *Abelmoschus esculentus* L. Moench, also called lady's finger, gumbo or bhindi, is one of the world's oldest cultivated crops and is well known for its robust nature. In general, only *Abelmoschus esculentus* L. Moench is grown for commercial cultivation (Charrier, 1984). It is a tropical vegetable, probably of Indian origin (Zeven and Zherkovsky, 1975). It is widely distributed from Africa to Asia, southern Europe and

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America (Oyenuga, 1969; Hamon, 1991; Ariyo, 1993; Oyelade *et al*., 2003). Cultivated okra is an economically important vegetable grown in tropical, intertropical, Mediterranean and sub-tropical regions of the world mainly for its immature fruits. It is a very popular pod vegetable in the Indo-Pak subcontinent. Okra has high nutritional, medicinal and industrial value (Reddy *et al*., 2013) and high financial value (Sawadogo *et al*., 2006). It is a traditional vegetable crop, quite popular and grown all over India because of ease of cultivation, stable price, dependable yield and wider adaptability to varying moisture conditions. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. Being a tropical, hot weather, low land crop and susceptible to low night temperatures, it is extensively cultivated in rainy (June-September) and summer (February-May) seasons in south India (Reddy *et al*., 2013). The major problem underlying its low productivity in central, southern and western regions is low yielding potential of current varieties and reduction in yield due to frequent attacks of pests and diseases, especially the fruit and shoot borer and YVMV (Reddy *et al*., 2012). YVMV transmitted by white fly (*Bemisia tabaci* Gen.) is the main limiting factor in the cultivation affecting both fruit yield causing a loss of 50 to 95 per cent depending on the stage of the crop growth at which infection occurs (Sastry and Singh, 1974) and fruit quality of okra. Since the disease cannot be controlled properly by chemical means, the only practical solution of this problem is to develop tolerant/resistant varieties (Mogili *et al*., 2013).

Interspecific hybridization is considered a possible mechanism of plant diversification. Interspecific hybridization followed by backcrossing and selection in the segregating generations is an effective method for developing YVMV resistant varieties. Hybridization including wild and cultivated species has long been used for transfer of genetic material to the crops. A promising breeding method for creation of new variability is the wild hybridization that became a more common practice after the advancement of hybridization techniques (Mujeeb-Kazi and Rajaram, 2002). Wild relatives of crops have been recognised as an important source of useful genes/traits for breeding programs. The essential prerequisite for improving disease resistance is the availability of a suitable source of resistance within a cultivated species itself or in related wild species; however, for obvious reasons, resistance occurring within cultivated species is more desirable as this can be more easily transferred to an otherwise superior but susceptible variety (Dhankhar *et al*., 2005). For YVMV, source of resistance is not available in cultivated okra. Therefore, an extensive search for YVMV resistance was started by screening germplasm of wild species of *Abelmoschus*. The wild species of *Abelmoschus* of family Malvaceae are source of useful alleles for okra improvement. Several wild relatives of okra showed high degree of YVMV resistance. The species *Abelmoschus caillei, Abelmoschus manihot*, *Abelmoschus manihot* subsp. manihot, and *Abelmoschus manihot* subsp. tetraphyllus were used for interspecific hybridization followed by backcrossing with cultivated species *Abelmoschus esculentus*.

A number of characters present in crop wild relatives have been transferred to cultivated types through wide hybridization at the interspecific or intergeneric levels (Nomura and Makara, 1993). Artificial interspecific hybrids can be obtained in okra (Hamon and Yapo, 1986). It is sometimes possible to obtain first-generation hybrids such as in crosses between *A. esculentus* and *A. tetraphyllus*, but the process is blocked at the second generation (Hamon, 1988). The extent of sexual compatabilities of wild relatives with cultivated okra has been reported previously (Samarajeeva *et al*., 1998). Crossability studies indicated that *A. ficulneus* as a male parent can be crossed with okra to produce fertile progenies (Samarajeeva *et al*., 1998). Colchicine (0.1%) treatment on apical buds at two leaf stage of the seedlings for 65 h in the interspecific hybrids of *A. esculentus* cv. Phule Utkarsha × *A. manihot* subsp. manihot was effective in inducing amphidiploids with fertility (Jatkar *et al*., 2007). Up to now, wild species were frequently utilized for okra improvement and there are several reports for successful hybridization between the two species (Nerkar and Jambhale, 1985). Studies were also undertaken to transfer genes for tolerance to YVMV from related wild species to susceptible cultivated varieties (Sharma, 1982; Dutta, 1984; Nerkar and Jambhale, 1985). Parbhani Kranti (Jambhale and Nerkar, 1986), Arka Anamika and Arka Abhay (Dutta, 1984) were developed using interspecific hybridization followed by backcross breeding and variety Punjab Padmini (Sharma, 1982) by interspecific hybridization but without backcrossing. Several varieties of okra showing resistance to YVMV disease have been developed using wild species *A. manihot* (L.) Medikus subsp. manihot (Thakur, 1976; Nerkar and Jambhale, 1985) and the cultivated variety Parbhani Kranti (Dhankhar *et al*., 1997; Dhankhar *et al*., 1999) as a source of resistance. However, resistant varieties developed by various research organizations by interspecific hybridization have started showing signs of susceptibility, probably due to new virus strains. Hence, it is imperative to find diverse source of resistance to YVMV and evolve resistant varieties by suitable gene introgression program (Prabhu, 2013).

The present study was undertaken to study the crossability behaviour and stage of sterility occurrence and fertility restoration through colchiploidy in interspecific F_1 hybrids of *A. esculentus* and *A. manihot* subsp. tetraphyllus. The present

study is a contribution to the knowledge of crossability and sterility in interspecific hybrids of *Abelmoschus* spp.

2. Materials and Methods

2.1. Choice of Parental Lines for Interspecific Hybridization

Two separate field trials were conducted to characterize, evaluate and screen cultivated and wild species accessions of *Abelmoschus* against YVMV at experimental farm of Vegetable Research Station, Rajendranagar during summer 2013 to identify the horticulturally superior but YVMV susceptible cultivated okra and YVMV resistant wild okra so as to utilize them as parental lines for interspecific hybridization. The screening for YVMV resistance was done by field evaluation under natural epiphytotic conditions by planting highly YVMV susceptible Pusa Sawani all along the borders of entire trial plots to provide adequate virus source to the vector. Of the 28 inbred lines of okra along with one YVMV susceptible check Pusa Sawani and three open pollinated variety (standard) checks Arka Abhay, Arka Anamika and VRO-6 characterized, evaluated and screened, RNOYR-19 was found to be horticulturally superior but highly susceptible (100%) to YVMV as against 100% YVMV incidence in the cross check Pusa Sawani and standard check Arka Anamika. The inbred line RNOYR-19 (*A. esculentus*) was used as female parent for interspecific hybridization (Table 1). Of the 2 wild species of okra (*A. manihot* subsp. manihot and *A. manihot* subsp. tetraphyllus) along with one susceptible check Pusa Sawani characterized, evaluated and screened, both species were found to be resistant (0%) to YVMV as against 100% YVMV incidence in Pusa Sawani. *A. manihot* subsp. tetraphyllus was used as male parent for interspecific hybridization (Table 2).

Table 1. Agro-economic and morphological traits of RNOYR-19 (*Abelmoschus esculentus*).

Agro-economic trait	Mean value	Morphological trait	Descriptor state
Plant height (cm)	139.27	Early plant vigour	Good
Number of branches per plant	2.13	Plant growth habit	Erect
Internodal length (cm)	5.63	Branching habit	Densely branched base
Days to 50% flowering	40.00	Stem pubescence	Slightly rough
First flowering node	5.33	Mature leaf colour	Green
First fruiting node	5.33	Leaf shape	Deeply lobed
Fruit length (cm)	13.07	Leaf rib colour	Green
Fruit width (cm)	1.82	Leaf pubescence	Slightly rough
Fruit weight (g)	17.86	Petiole colour	Green with red colour
Total number of fruits per plant	20.00	Outside petal base colour	Red
Number of marketable fruits per plant	13.47	Immature fruit colour	Green
Total yield per plant (g)	271.32	Position of fruit on stem	Erect
Marketable yield per plant (g)	182.06	Fruit pubescence	Downy
YVMV infestation on plants (%)	89.09	Fruit shape	Angular
YVMV infestation on fruits (%)	3.72	Number of ridges	5

Table 2. Agro-economic and morphological traits of *Abelmoschus manihot* subsp. tetraphyllus.

Agro-economic trait	Mean value	Morphological trait	Descriptor state
Plant height (cm)	120.00	Plant growth habit	Densely branched base
Number of branches per plant	8.50	Stem pubescence	Downy
Days to 50% flowering	50.00	Stem colour	Red
Pod length (cm)	4.50	Leaf type	Cut leaf
Pod width (cm)	2.40	Pod colour	Dark green with red blush
Pod weight (g)	4.00	Pod pubescence	Prickly
YVMV infestation on plants (%)	0.00	Number of ridges	5

2.2. Generating Interspecific F_1 Hybrids

Crossing of horticulturally superior but YVMV susceptible RNOYR-19 (*A. esculentus*) and YVMV resistant *Abelmoschus manihot* subsp. tetraphyllus was carried out during summer 2013 at Vegetable Research Station, Rajendranagar (Fig. 1). Ten flowers of *A. esculentus* were crossed in this one-way cross combination. Crossing was carried out by adopting hand emasculation and pollination. Flower buds of *A. esculentus* which are supposed to open in the next day were chosen for emasculation. Flowers were hand emasculated and bagged to avoid pollination with other okra plants. Hand pollination was carried out using fresh pollen of the wild species *A. manihot* subsp. tetraphyllus the next day morning. Cross pollinated flowers were gently covered by butter paper bag to avoid out crossing. About 35 days after pollination, fully mature and dried crossed fruits were harvested, F_1 hybrid seeds were extracted and the number of seeds set on *Abelmoschus esculentus* was counted. F_1 hybrid seeds were utilized for raising F_1 population to study the seed germinability and dormancy if any, under laboratory conditions and to test the hybrid lethality, hybrid breakdown and hybrid sterility if any, under field conditions.

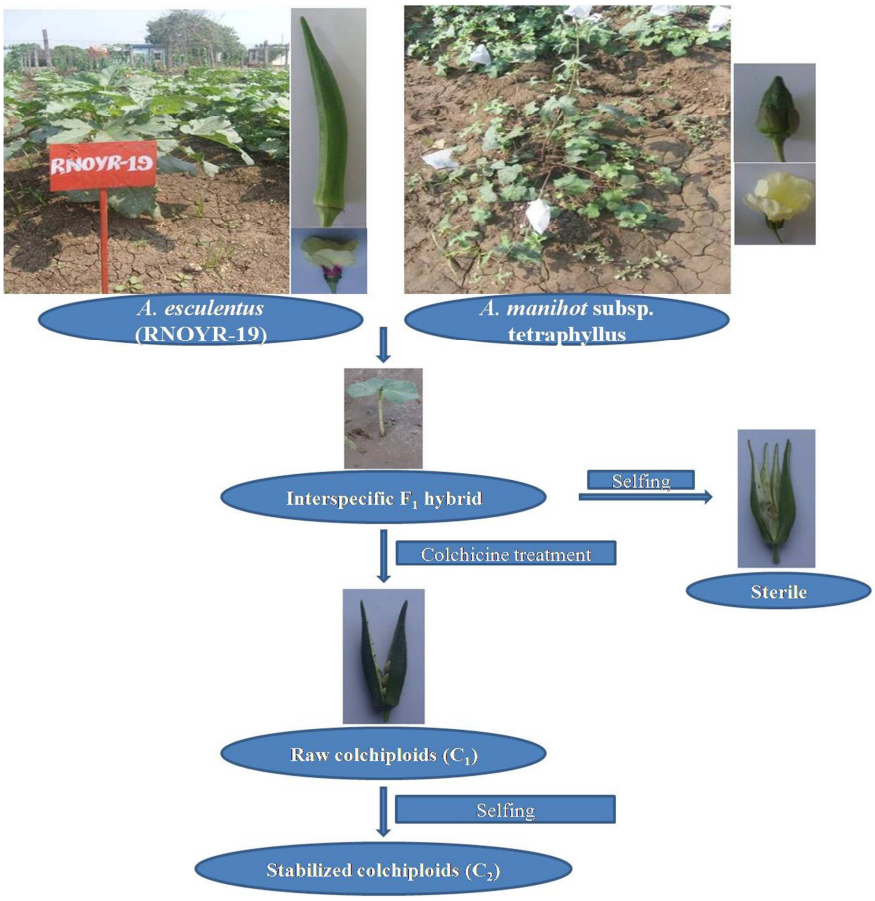

Fig 1. Flow chart of interspecific hybridization and overcoming sterility through colchiploidy in interspecific F_1 hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

2.3. Germinability and Dormancy of Interspecific F_1 Hybrid Seed

In order to test the seeds for germinability and dormancy if any, about one hundred, 25-day old, fully mature and dried F_1 hybrid seeds collected out of the one-way cross attempted were kept in petriplates in the laboratory. Germinability was calculated using the standard formula (Number of seeds germinated/Number of seeds sown×100).

2.4. Hybrid Lethality, Hybrid Breakdown and Hybrid Sterility

After confirming that the F_1 seeds were germinable and non-dormant, ten fully mature and dried F_1 seeds collected out of the one-way cross attempted were raised in a double-row plot of 2.25×1.2 m at a spacing of 60×45 cm in the main field to assess the hybrid breakdown, hybrid lethality and hybrid sterility if any, in the interspecific F_1 hybrid plants during *kharif* 2013. The number of plants survived over germinated seeds was taken to assess the lethality of interspecific F_1 hybrids. Hybrid lethality was calculated using the formula (Number of plants died/Number of seeds germinated×100). Number of plants survived till maturity is hybrid breakdown. Hybrid breakdown was calculated using the formula (Hybrid in viability/Number of seeds germinated ×100).

2.5. Colchiploidy as a Means of Overcoming Sterility in Interspecific F_1 Hybrids

About 35-day old, fully mature and dried F_1 hybrid seeds collected out of the one-way cross attempted were sown to raise 75 seedlings of F_1 hybrids in a multiple-row plot of 2.25×9.0 m at a spacing of 60×45 cm for colchicine treatment. The seedlings of interspecific crosses at two leaf (pseudocotyledonary) stage (Fig. 1) were treated with 0.1% colchicine on apical meristem through cotton swab method from 4^{th}-7^{th}day after germination 4 times a day at 3 h interval from 6.00 a.m. to 6.00 p.m. during *rabi* 2013-14. The number of plants survived out of the total number of plants treated with colchicine to determine mortality, if any due to phytotoxicity of the used dose of colchicine. Upon selfing and after complete pod ripening and drying, the total number of seeds per pod (the number of seeds with an embryo + the number of rudimentary ovules) was counted to arrive at the seed set percentage and thereby fertility status in the raw colchiploids (C_1).

2.6. Selfing as a Means of Restoring Complete Fertility in Raw Colchiploids

After confirming that the raw colchiploids (C_1) were partially fertile, ten fully mature and dried C_1 seeds collected out of the raw colchiploids (C_1) were raised in a double-row plot of 2.25×1.2 m at a spacing of 60×45 cm in the main field and subjected to single cycle of selfing resulting in the production of stabilized colchiploids (C_2) during *kharif* 2014. The status of seed set and fertility in the selfed raw colchiploids was determined.

3. Results and Discussion

Successful intraspecific hybridization in the species *A. esculentus* L. Moench has helped to generate considerable variability (Reddy, 2010). DNA analysis by RAPDs indicated that wild species are related distantly to locally cultivated okra (Samarajeeva and Rathnayaka, 2004). A prerequisite for using wild species as germplasm is a successful interspecific hybridization and backcrossing. However, barriers in the interspecific hybrids of okra have restricted the progress of desired gene transfer from the wild species to the cultivated ones (Dutta, 1984; Hamon, 1988; Nerkar and Jambhale, 1985; Sureshbabu, 1987; Rajamony *et al*., 2006; Jatkar *et al*., 2007). In order to utilize the wild relatives of okra that are resistant to the tested diseases in the crop improvement program, fertile progenies should able to be produced by crosses between the wild *Abelmoschus* species and okra. Although F_1 plants were obtained in certain cases, these plants were highly sterile and it was difficult to produce subsequent generations or even to carry out backcrosses (Singh and Bhatnagar, 1975; Joshi and Hardas, 1976; Siemonsma, 1982a, 1982b; Hamon and Yapo, 1986; Hamon, 1988). Fortunately, most of the well adapted cultivated okra genotypes are crossable with the wild species. Thereby the numbers of breeding lines that can be used for alien introgression are wide. Although considerable cytogenetic evidence for the evolutionary history of the okra has been collected, there are still several hybrid combinations which have not been tried and evaluated for breeding purposes or for genome relationships. The results presented here reveal the possibility of interspecific hybrids between amphidiploid *A. esculentus* possessing good agronomic characteristics and diploid wild species *A. manihot* subsp. tetraphyllus.

3.1. Crossability of *A. esculentus* × *A. manihot* subsp. tetraphyllus

Cultivated okra species, *A. esculentus* and wild species *A. manihot* subsp. tetraphyllus have self compatible flowers. The cultivated okra inbred line RNOYR-19 was hybridized to one accession of wild relative *A. manihot* subsp. tetraphyllus. Nine pods were set out of the 10 flowers that were cross pollinated. The fruit set of this one-way cross combination was 90%. This indicates that the interspecific crossing was successful; resulting in the normal fruit set and seed set (Table 3). The crossability between them expressed as a percent of pollinated flowers produced pods with mature seeds is presented in Table 3. From 10 pollinated flowers we obtained 9 mature pods with fully mature seeds i.e. the crossability for the hybrid combination was 90%. This indicates that *A. esculentus* and *A. manihot* subsp. tetraphyllus are freely crossable and cross compatible with viable seed formation and thus self-reproducing. A high crossability rate of 90% for the given genotypes was achieved. The observed high crossability is in conformity with the law of genetic distance between both the species and their genome homology. Earlier studies also indicated that the interspecific F_1 hybrid plants were obtained from the interspecific F_1 hybrid seeds in certain cases (Singh and Bhatnagar, 1975; Joshi and Hardas, 1976; Siemonsma, 1982a, 1982b; Hamon and Yapo, 1986; Hamon, 1988). The cross of *A. esculentus* and *A. tuberculatus* was successful in both directions of crossing (Joshi and Hardas, 1956). Crossed seeds of interspecific crosses between *A. esculentus* and *A. moschatus* were shrivelled and non-viable due to post zygotic incompatibility to operate between these species (Rajamony *et al*., 2006).

3.2. Germinability and Dormancy in Interspecific F_1 Hybrid Seeds of *A. esculentus* × *A. manihot* subsp. tetraphyllus

Of the 100 F_1 hybrid seeds kept in petriplates for germination, 94 were germinated (Table 4). The hybrid germination of 94% was recorded indicating that the F_I hybrid seeds were absolutely free from any dormancy.

3.3. Hybrid Lethality, Hybrid Breakdown and Hybrid Sterility in Interspecific F_1 Hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus

Cent percent F_I hybrid plants were obtained from the F_I hybrid seeds in this interspecific cross combination indicating that there was absolutely no (0%) hybrid lethality in this cross combination (Table 5). Cent percent of the F_I hybrid seedlings were developed into mature F_I hybrid plants indicating that there was absolutely no (0%) hybrid breakdown in this cross combination (Table 5). The interspecific F_1 hybrid seeds when sown in the field during *kharif* 2013, the seeds germinated normally and hybrid plants were obtained by means of normal seed propagation method. All F_1 hybrid plants were identical to each other. In addition, they exhibited high vigor and manifested traits from both

parents. Although, the hybrid plants were normal with normal flowering and fruit set externally, upon opening of the mature and dried pods, there were abortive seeds (rudimentary ovules). In spite of the 100% fruit set observed in F_1 hybrids, no viable seed formation was ascertained (Table 6). The lack of formation of the seeds in F_1 hybrid plants is an expression of the effect of post-zygotic incompatibility occurred after wild hybridization. The interspecific hybrid plants are fully sterile. The reproductive potential of the F_1 hybrid plants is zero. This indicates that there was sterility in interspecific F_1 hybrids because of formation of no bivalents and irregular gametes and cannot produce subsequent generations. The hybrids of *A. manihot* (L.) Medik × *A. esculentus* cv. Pusa Sawani and *A. manihot* (L.) Medik ssp. manihot × *A. esculentus* cv. Pusa Sawani were partially fertile (Jambhale and Nerkar, 1981a; Dutta, 1984). However, it may be noted that the seeds so obtained after crossing did germinate but failed to produce fertile plants. The sterility is attributable to various reasons such as chromosomal differences as in case of *A. tuberculatus* and *A. ficulneus* and genomic differences leading to irregular gamete formation as in case of *A. manihot*. Joshi and Hardas (1956) obtained a fertile plant from a colchicine treated sterile F_1 hybrid between *A. esculentus* (2n=130) and *A. tuberculatus* (2n=58), which had 21 bivalents (II) and 36 univalents (I). The cultivated okra (*A. esculentus)* has somatic chromosome number 2n=130 and is considered to be an amphidiploid of *A. tuberculatus* (2n=58) and an unknown species with 2n=72 (Datta and Naug, 1968). *A. esculentus* is a ploidy level 2 species with a chromosome number of 2n=72 (Ugale *et al*., 1976), 2n=131-143 (Siemonsma, 1982a, 1982b) and 2n=144 (Datta and Naug, 1968), while *A. manihot* subsp. tetraphyllus is also a ploidy level 2 species with a chromosome number of 2n=130 (Ugale *et al*., 1976) and 2n=138 (Joshi and Hardas, 1976). Both of the species employed in interspecific hybridization in this study were in ploidy level 2 wherein 2n=108-144. Earlier studies also indicated that interspecific crossing between ploidy level 2 species (*A. esculentus* and *A. tetraphyllus*) resulted in the production of viable seeds but sterile hybrids (Joshi and Hardas, 1976; Hamon and Yapo, 1986). Also, the cross of *A. esculentus* and *A. tuberculatus* produced vigorous but sterile hybrids (Joshi and Hardas, 1956). Interspecific F_1 hybrid plants of okra were highly sterile (Singh and Bhatnagar, 1975; Joshi and Hardas, 1976; Siemonsma, 1982a, 1982b; Hamon and Yapo, 1986; Hamon, 1988).

Table 3. Crossability behaviour in interspecific F_1 hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Cross combination	No. of crosses made	No. of fruits set	No. of fruits with seeds	Fruit set (%)	Crossability (%)
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	10	9	9	90	90

Table 4. Germinability and dormancy in interspecific F_1 hybrid seed of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Cross combination	No. of seeds sown	No. of seeds germinated	Germination (%)	Dormancy
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	100	94	94	Absent

Table 5. Hybrid lethality and hybrid breakdown in interspecific F_1 hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Cross combination	No. of seeds sown	No. of seeds germinated	No. of plants died	No. of viableplants	No. of plants reached maturity	Hybrid lethality (%)	Hybrid breakdown(%)
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	10	10	0	10	10	0	0

Table 6. Sterility in interspecific F_1 hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Cross combination	No. of plants grown	No. of plants set fruits	No. of plants set mature seeds	No. of mature seeds per fruit
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	10	10	0	0

Table 7. Colchiploidy as a means of fertility restoration in interspecific F_1 hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Cross combination	No. of plants		Average no. of seeds per fruit				
	Treated	Survived	Set seed	Total no. of seeds	No. of mature seeds	No. of abortive seeds	Seed set(%)
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	75	75	52	32	17	15	53.12

Table 8. Restoring complete fertility in colchiploids of *A. esculentus* × *A. manihot* subsp. tetraphyllus through single generation of selfing.

Cross combination	No. of plants		Average no. of seeds per fruit			
	Selfed	Set seed	Total no. of seeds	No. of mature seeds	No. of abortive seeds	Seed set(%)
A. esculentus (RNOYR-19) × *A. manihot* subsp. tetraphyllus	10	10	32	32	0	100

3.4. Overcoming Sterility in Interspecific F_1 Hybrid of *A. esculentus* × *A. manihot* subsp. tetraphyllus by Colchicine Treatment

Due to complete sterility of these interspecific hybrids, we were not able to advance or develop them further by direct backcrossing with the *A. esculentus* parent as previously experienced by other researchers (Dutta, 1984; Jambhale and Nerkar, 1986). A restoration of fertility in F_1 hybrid plants was achieved through colchiploidy which is prerequisite to utilize backcross strategy for introgression of desirable characters from F_1 hybrid plants into cultivated okra in the future generation. Upon colchicine treatment to the seedlings of the interspecific F_1 plants at two leaf (pseudocotyledonary) stage (Table 7) during *rabi* 2013-14, there was no mortality (0%) in the interspecific F_1 plants with normal fruit set (100%) and partial seed set (53.12%) resulting in the production of raw colchiploids with partial fertility.

3.5. Restoring Complete Fertility in Colchiploids of *A. esculentus* × *A. manihot* subsp. tetraphyllus Through Single Generation of Selfing

These raw colchiploids (C_1) were selfed during *kharif* 2014 and stabilized colchiploids (C_2) with complete fertility (100%) were obtained (Table 8). Artificial and spontaneous amphidiploids between the two species *A. esculentus* and *A. tetraphyllus* had been realized in India in attempts to transfer YVMV to cultivated okra (Jambhale and Nerkar, 1981a, 1981b). Jambhale and Nerkar (1982) induced amphidiploidy in the F_1 hybrids between *A. esculentus* (n=65) and *A. tetraphyllus* (n=69) to overcome the sterility. Jambhale and Nerkar (1982) also reported colchicine-induced amphidiploidy in the cross *A. esculentus* (2n=130) × *A. manihot* subsp. manihot (2n=194). The amphidiploid of *A. esculentus* × *A. manihot* subsp. manihot had 88.12% fertility against 7.07% in the F_1 hybrid (Jambhale and Nerkar, 1982).

3.6. Comparison of Interspecific F_1 Hybrids, Raw Colchiploids and Stabilized Colchiploids of *A. esculentus* × *A. manihot* subsp. tetraphyllus

The salient agro-economic, morphological and genetic traits of interspecific F_1 hybrids, raw colchiploids (C_1) and stabilized colchiploids (C_2) of *A. esculentus* × *A. manihot* subsp. tetraphyllus are presented in Table 9. The wide variation in agro-economic traits among interspecific F_1 hybrids, raw colchiploids (C_1) and stabilized colchiploids (C_2) could probably due to the seasonal variation in which they were developed and grown. There was absolutely no variation in morphological traits among interspecific F_1 hybrids, raw colchiploids and stabilized colchiploids, which could probably due to their non-interaction with seasonal variation in which they were developed and grown. Interspecific F_1 hybrids of *A. esculentus* × *A. manihot* subsp. tetraphyllus were not self-reproducing, while raw colchiploids (C_1) and stabilized colchiploids (C_2) of *A. esculentus* × *A. manihot* subsp. tetraphyllus were partially self-reproducing and completely self-reproducing, respectively. Hence, out of three populations of *A. esculentus* × *A. manihot* subsp. tetraphyllus *viz*., interspecific F_1 hybrids, raw colchiploids (C_1) and stabilized colchiploids (C_2), only stabilized colchiploids (C_2) could be used in further breeding programs.

In breeding programs of okra, the characters that need to be given emphasis include medium tall to tall plants, few branches, short internodes, low position of first flowering and fruiting node, high number of fruiting nodes on main stem, early flowering and early maturity for enhanced productivity; medium long to short, green, smooth (downy), five ridged, angular, straight fruits with no groves and blunt tip for enhanced fruit quality and appearance and tolerance to biotic stresses (pod borer and YVMV) for stable and sustainable production (Reddy *et al*., 2012). The stabilized colchiploids (C_2) were of self reproducing (self fertile), tall growing (150-175 cm), profuse bearing (30-40 fruits /plant), but with unacceptable market quality (thick, short, star shaped and prickly pods) and thus cannot be used directly; however after screening in hotspot, the YVMV resistant plants can be used with or without backcrossing in developing innovative YVMV resistant varieties of okra. Through hybridization and selection among the segregating populations, some resistant lines had been developed which are as early as local varieties and produce long, green, delicate and tasty fruits (Salehuzzaman, 1986). Further advancing lines made from the F_9 generation of interspecific cross between *A. esculentus* and *A. tetraphyllus* will be able to develop high yielding and YVMV resistant okra varieties in the near future (Sureshbabu and Dutta, 1990). In India, interspecific hybridization had been followed in the development of Punjab Padmini (Sharma, 1982), Parbhani Kranti (Jambhale and Nerkar, 1986), Punjab-7 (Thakur and Arora, 1998) and Arka Anamika and Arka Abhay (Dutta, 1984). A vigorous hybrid between *A. esculentus* and *A. manihot* subsp. tetraphyllus var. tetraphyllus was obtained by Sureshbabu (1987).

Table 9. Comparison of interspecific F_1 hybrids, raw colchiploids and stabilized colchiploids of *A. esculentus* × *A. manihot* subsp. tetraphyllus.

Salient feature	Interspecific F_1 hybrids (*Kharif* 2013)	Raw colchiploids (C_1) (*Rabi* 2013-14)	Stabilized colchiploids (C_2) (*Kharif* 2014)
Agro-economic traits			
Plant height (cm)	180.95	176.85	170.26
Number of branches per plant	6.25	5.84	5.46
Days to first flowering	49.00	54.25	50.25
Pod length (cm)	7.85	7.68	7.76
Pod width (cm)	2.65	2.69	2.71
Pod weight (g)	7.75	9.23	11.25
Morphological traits			
Plant growth habit	Densely branched base	Densely branched base	Densely branched base
Stem pubescence	Downy	Downy	Downy
Stem colour	Green with red blush	Green with red blush	Green with red blush
Leaf type	Cut leaf	Cut leaf	Cut leaf
Leaf pubescence	Downy	Downy	Downy
Outside petal base colour	Red	Red	Red
Pod colour	Green	Green	Green
Pod pubescence	Prickly	Prickly	Prickly
Number of ridges	5	5	5
Pod shape	Star	Star	Star
Genetic traits			
Self-reproducibility	Not self-reproducing (Complete)	Self-reproducing (Partial)	Self-reproducing (Complete)

4. Conclusion

A. esculentus × *A. manihot* subsp. tetraphyllus were cross compatible in the one-way cross combination. F_1 hybrid plants were obtained by means of normal seed propagation from the interspecific F_1 hybrid seeds. There was absolutely no hybrid lethality and hybrid breakdown in the F_1 hybrid plants of *A. esculentus* and *A. manihot* subsp. tetraphyllus. The interspecific F_1 hybrid plants of *A. esculentus* and *A. manihot* subsp. tetraphyllus were sterile. The sterility in the interspecific F_1 hybrid plants was successfully overcome by treating the seedlings with colchicine resulting in the production of partially fertile raw colchiploids (C_1). Single cycle of selfing of raw colchiploids (C_1) resulted in the production of fully fertile stabilized colchiploids (C_2). These newly developed fully fertile stabilized colchiploids (C_2) after thorough screening against YVMV can be utilized in okra resistance breeding programs. Here presented results are only initial step of the involvement of wild species *A. manihot* subsp. tetraphyllus in long process of production of *A. esculentus* breeding lines with introgressed alien genes. Successive progenies are going to be screened at morphological, physiological, cytological and molecular level for hybrid identification and enhancing of genetic variation for YVMV resistance and its incorporation into cultivated okra.

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