Vegetable Science (2020) 47(2): 296-299

Crossability studies among cultivated and wild species of okra [(*Abelmoschus esculentus* (L.) Moench]

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Received: January 2021/ Accepted: February 2021

Okra is an important vegetable crop and liked for its green tender fruits. It has high nutritional and medicinal values. Okra seeds contain 15-20% protein and 30-40% of fine quality edible oil. Among fresh vegetables okra has prominent position due to its multiple uses, easy cultivation practices, good export return, wider adaptability and possibility of year-round production (Reddy et al. 2012). Although, India rank's top in the world for the production of okra but its productivity is far less than Ghana (22 t/ha) (FAO Stat 2018). Vulnerability of cultivated okra varieties to various insect pests and diseases is the main reason for poor pod quality and low yield potential. Therefore, it has become imperative to broaden the gene pools of the cultivated okras through interspecific hybridization for the development of new cultivars possessing high yield and resistance to disease and insect-pests. Among biotic factors, Yellow Vein Mosaic Virus (YVMV) & Okra Enation Leaf Curl Virus (OELCV) transmitted through whitefly i.e. *Bemesia tabaci* are major constraint to the successful cultivation of okra in India. It was first reported in the Bombay presidency by Kulkarni in 1924. Later, its viral origin was reported by Uppal et al. (1940). Depending upon the factors like environment conditions, white fly population, crop characteristics and crop growth stage at which infection occurs, it may cause 50 to 94 % yield losses (Solankey et al. 2014). Management of this disease is only possible way of reducing the whitefly population using insecticides. Many researchers have exploited the wild germplasm to transfer the resistance against YVMV into the cultivated species (Jambhale and Nerkar 1981, Sharma 1982, Sureshbabu and Dutta 1990). Earlier, interspecific hybridization resulted in the development of YVMV resistant okra varieties. YVMV disease resistant okra variety Punjab-7 was developed using wild species *A. manihot* subsp. manihot (Thakur and Arora 1988). Variety Punjab Padmini developed by interspecific hybridization but without backcrossing (Sharma 1982) whereas, Parbhani Kranti (Jambhale and Nerkar 1986), Arka Anamika and Arka Abhay (Dutta 1984) were developed using interspecific hybridization followed by backcross breeding. But with the emergence of new biotype of whitefly and development of new strains of virus these varieties become susceptible to YVMV (Sanwal et al. 2014). Therefore, it is of utmost importance to find new sources of YVMV resistance and its transfer to the cultivated okra elite genotypes. Cross incompatibility (Sindhu 1993) and pollen sterility of F_1 hybrids between cultivated and wild okra species resulted limited success of interspecific hybridization (Nagaraju et al. 2019). Keeping this in view, the present investigation was initiated to study the crossability relationship among cultivated okra species *A. esculentus* and five different entries of two wild species namely *A. angulosus* and *A. manihot*.

For the present experiment, experimental plant material comprised of cultivated okra variety Punjab Padmini and five YVMV resistant daughter stocks belonging to five different entries (*A. manihot* PAUAcc-1, *A. manihot* accession IC-90339, *A. angulosus*, *A. angulosus* accession IC-203833, *A. angulosus* accession IC-470751 of two wild species namely *A. manihot* and *A. angulosus*). YVMV resistant daughter stocks were developed by performing YVMV field screening followed by two years of artificial screening and further confirmation with Begomovirus specific primers (Wyatt and Brown 1996, Deng et al. 1994) at Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Cultivated okra is a summer season crop under North Indian conditions and can be sown during February-March and June –July. The rainy season sown

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crop ends by October, because it is highly sensitive to low temperature. On the other hand, in wild types flowering start in the month of November. Therefore, to get the synchronization of flowering for cultivated and wild okra species for crossing purpose, staggered sowing for cv. Punjab Padmini was done at 15 days interval starting from 1st October 2017 to 30th November, 2017 under polyhouse conditions. For crossing purpose, wild species entries belonging to *A. manihot* and *A. angulosus* were used as male parents and *A. esculentus* cv. Punjab Padmini was used as female parent. All the five entries of two wild species namely *A. manihot* PAUAcc-1, *A. manihot* accession IC-90339, *A. angulosus*, *A. angulosus* accession IC-203833, *A. angulosus* accession IC-470751 were used as male parent and cultivated *A. esculentus* species variety Punjab Padmini as female parent. Interspecific crosses were attempted at Vegetable Research Farm, PAU, Ludhiana during 2017 and 2018. Buds of optimum size of female parent were emasculated in evening a day before anthesis and pollinated in the next morning. Emasculated buds were covered with the butter paper bags. For the male parent, buds ready to open in the next morning were also covered with butter paper bags. Next day in the morning the male flowers were plucked and dusting of pollen on stigma of female parent was done. The cross-pollinated flowers were tagged and again covered with butter paper bags to avoid out crossing. Cross pollinated flowers were gently covered by butter paper bag to avoid out crossing. Fully mature and dried crossed fruits were harvested to extract F_1 hybrid seeds and afterwards number of seeds set per fruit was counted. F_1 hybrid seeds were used for growing F_1 plants to study the percentage of seed germination. Observations were recorded on the number of crosses attempted, pollinated, average number of fruits set, fruit set (%), Crossability index (%), seed set/fruit, F_1 seed germination (%). Percent fruit setting was obtained from [Number of pods set/ Number of buds pollinated] × 100. Germination percentage was calculated from [Number of seeds germinated/ Number of seeds sown] × 100 and seeds were dipped into fresh water for 24 hours before sowing in plug trays. Crossability index was calculated as suggested by Rao (1979).

Interspecific F_1 hybrid development is challenging in okra due to crossability barriers. However, Hamon and Yapo (1986) reported the development of interspecific hybrids. The prime requisite of introgression is the successful development of interspecific F_1 hybrids and their further backcrossing. Development of fertile progenies has been reported between *A. esculentus* and *A. ficulneus* by Samarajeeva et al. (1998). Interspecific hybridization barriers restrict the use of wild species in okra (Jatkar 2007). In the present study it was evident that upon self pollination *A. manihot* accession IC-90339 recorded maximum fruit set (94.4%) followed by Punjab Padmini (90.8 %), *A. manihot* PAUAcc-1 (86.7%), *A. angulosus* accession IC-203833 (84.6%), *A. angulosus* (76.9%) and *A. angulosus* accession IC-470751 (63.6%) (Table 1). Whereas for interspecific crosses highest fruit setting (63.7 %) was recorded for cross for *A. esculentus* cv. Punjab Padmini × *A. manihot* accession IC-90339 followed by *A. esculentus* cv. Punjab Padmini × *A. manihot* PAUAcc-1 (63.3%), *A. esculentus* cv. Punjab Padmini × *A. angulosus* (54.4%), *A. esculentus* cv. Punjab Padmini × *A. angulosus* accession IC-470751 (51.9%) and *A. esculentus* cv. Punjab Padmini × *A. angulosus* accession IC-203833 (31.7%)(Table 2). Similar, results were reported by Prabu and Warade (2013) and Kaur (2018). Development of partial fertile F_1 hybrids was reported between *A. esculentus* cv. Pusa Sawani and *A. manihot* ssp. manihot (Dutta 1984).

The crossability index ranged from 18.1% to 35.7%. It was found maximum in cross Punjab Padmini × *A. manihot* PAUAcc-1 (35.7%) followed by. Punjab Padmini × *A. manihot* accession IC-90339 (34.4%), Punjab Padmini × *A. angulosus accession* IC-470751 (33.6%), Punjab Padmini × *A. angulosus* (32.5%) and. Punjab Padmini × *A. angulosus* accession IC-203833 (18.1%). These findings are in conformity with the study conducted by Prakash and Pitchaimuthu (2010), showing the production of normal fruits in cross combination of local variety Kiran and wild species *A. manihot*. However, cross combination Punjab Padmini x *A. angulosus* accession IC-470751 produced empty fruits without seeds. It indicated the role of post-zygotic incompatibility between during inter-specific hybridization (Prabu and Warade 2013, Sheela 1986).

Table 1: Fruit set (%) and germination (%) of cultivated and wild okra species

Entries	Total number of flowers selfed	Number of fruits set	Fruit set (%)	Number of seeds/ fruits	Germination (%)
A. esculentus cv. Punjab Padmini	130	118	90.8	28-35	95
A.manihot (PAUAcc-1)	15	13	86.7	25-30	55
A.manihot (IC-90339)	18	17	94.4	35-38	60
A. angulosus	13	10	76.9	45-60	45
*A. angulosus (*IC-203833)	26	22	84.6	28-32	40
*A. angulosus (*IC-470751)	11	7	63.6	35-40	30

Table 2: Crossability index (%) and germination (%) for different interspecific okra crosses

Interspecific Cross	Total no. of crosses attempted	Number of fruits set	Fruit set (%)	Crossability index (%)	No. of seeds per fruit	Germination (%)
A. esculentus cv. Punjab Padmini × *A. manihot* (PAUAcc-1)	139	88	63.3	35.7	1.8	5.2
A. esculentus cv. Punjab Padmini × *A. manihot* (IC-90339)	386	246	63.7	34.4	1.6	5.0
A. esculentus cv. Punjab Padmini × *A. angulosus*	283	154	54.4	32.5	0.4	4.4
A. esculentus cv. Punjab Padmini × *A. angulosus* (IC-203833)	773	245	31.7	18.1	1.4	1.2
A. esculentus cv. Punjab Padmini × *A. angeluses* (IC-470751)	81	42	51.9	33.6	empty fruit	-

The maximum number of seeds per fruit were obtained from cross between Punjab Padmini × *A. manihot* PAUAcc-1 (1.8) followed by *A. esculentus* cv. Punjab Padmini × *A. manihot* accession IC-90339 (1.6), *A. esculentus* cv. Punjab Padmini × *A. angulosus A. esculentus* cv. Punjab Padmini × *A. angulosus* (IC-203833 (IC-203833 (1.4) and *A. esculentus cv. Punjab Padmini* × *A. angulosus* (0.4) seeds/fruit. In general, many of the interspecific F_1 seeds were empty, smaller in size and shriveled in shape. The recovery of empty seeds, which appeared normal, may be ascribed to endosperm degeneration (Sindhu 1993). Crossed seeds extracted for interspecific crosses between *A. esculentus* and *A. moschatus* were shriveled and non-viable due to post zygotic incompatibility between these species (Rajamony et al, 2006). Germination percentage of interspecific F_1 hybrid seeds developed with different wild species under study ranged from 1.2 to 5.2 *per cent*. On contrary, selfed seed of cultivated okra cv. Punjab Padmini reported 95 *per cent* germination followed by *A.manihot* (IC-90339) (60%), *A. manihot* (PAUAcc-1) (55%), *A. angulosus* (45%), *A. angulosus* (IC-203833) (40%) and *A. angulosus* (IC-470751) (30%). Similarly, Nagaraju et al. (2019) also observed lower seed germination of inter-specific F_1 hybrids than the parents. The production of viable F_1 hybrid seeds using wild okra entries namely *A. manihot* (PAUAcc-1), *A. manihot* (IC-90339), *A. angulosus*, *A. angulosus* (IC-203833) as pollen parent and *A. esculentus* cv. Punjab Padmini as female parent accentuated the use of these four wild donors in future okra breeding programmes.

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