

## INTERSPECIFIC CROSSES BETWEEN *A. esculentus* L. AND *A. angulosus* L.

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### ABSTRACT

Interspecific hybridization was carried out between the cultivated okra, *Abelmoschus esculentus* and one of its wild relatives, *A. angulosus*. Hybridization was successful in both directions. When *A. angulosus* was used as the female parent incompatibility was observed resulting in failure of embryo formation. In such cases embryo rescue was successful to raise viable hybrids. When the cultivated type was used as the female parent, progeny plants were produced without incompatibility difficulties. Hybrid nature of all the F<sub>1</sub> plants was confirmed by morphological analysis and they had characters mostly intermediate between parent plants. F<sub>1</sub> plants were found to show both low pollen viability and very rare seed fertility. F<sub>1</sub> plants could be raised after embryo culture of the fertile seeds.

**KEY WORDS:** *Abelmoschus*, Okra, Wide cross, Embryo culture.

### INTRODUCTION

A number of useful characters present in wild crop relatives has been transferred to cultivated crops through wide hybridization at the interspecific or intergeneric levels (Nomura and Makara, 1993). Resistance to yellow vein mosaic virus (YVMV) has been transferred to okra from *A. manihot* (Thakur, 1976; Data, 1984). However, there are several other wild relatives of okra such as *A. angulosus* that could serve as an important source of resistance to insect pests and diseases. This variety is one of the wild relatives that has been collected, conserved and characterized at the Plant Genetic Resources Centre (PGRC) of Sri Lanka. The preliminary evaluation work on *A. angulosus* has revealed a number of useful agronomic traits, including resistance to virus diseases (personal communication). To use this germplasm in the improvement of okra, it is essential to understand the crossability relationship between okra and the wild species in order to produce viable F<sub>1</sub> hybrids.

Our studies on crossability of *A. esculentus* with *A. angulosus* indicated that using conventional breeding techniques F<sub>1</sub> hybrids could be obtained only when *A. esculentus* was used as the female plant (Samarajewa *et al.*, 1996). In the present work we report the production of F<sub>1</sub> hybrid plants in both directions. The detailed characteristics and the levels of fertility of F<sub>1</sub> hybrids obtained in the crosses are also presented.

### MATERIALS AND METHODS

The seed materials of *Abelmoschus esculentus* and *A. angulosus* were obtained from the PGRC gene bank, Gannoruwa, Peradeniya, Sri Lanka. They were planted in 98/99 Maha season in the PGRC greenhouse to coincide their flowering times (*A. esculentus* takes 56 days to flower whereas *A. angulosus* takes 78 days). Reciprocal crosses were carried out according to a standard pollination procedure for okra. The

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emasculated flowers were tagged after hand pollination and were covered with oil paper bags.

A total of 60 reciprocal crosses was carried out. The success of each cross was determined by pod formation and was represented as a percentage. At about 25 days ten mature pods were removed. They were opened and 10 young seeds /pod were dissected under a stereomicroscope and the presence or absence of embryos was recorded. Fertility of seeds was also tested by germinating mature seeds of the two parents and the hybrids on paper towels in petri dishes. In crosses where empty seeds were observed young pods were picked at different times, 6, 7, 8, 9, 10 and 11 days after tagging for examination of embryo formation. Ten-day-old embryos from immature seeds, which were visible as tiny green spots after dissection of young seeds, were transferred immediately to different culture media. The basic culture media used were White (White, 1937), B<sub>5</sub> (Gamborg *et al.*, 1968), MS (Murashighe and Skoog, 1962) and 1/2 MS. Each culture media was supplemented with different levels 0, 0.2, 0.5, 1.0 and 2.0 mg/l of BAP. The plants established *in vitro* were transferred to pots filled with soil three weeks after the culture.

Five to ten plants of hybrids, each obtained with or without embryo culture, were grown in a greenhouse together with their parents. Seeds of different pods were allowed to germinate individually. Morphological characters were recorded with reference to the PGR Descriptor for Okra. Data on days to flowering, fruit size, fruit length and the number of ridges per fruit were recorded by averaging 10 measurements per character.

Observations on pollen were made both under a light and a UV microscope after the pollen were stained with fluoresceine diacetate

(FDA). Data were recorded on the total number of pollen grains per microscopic field. Observations were also made on the pollen with deep yellow fluorescence of *A. angulosus*, *A. esculentus* and F<sub>1</sub> hybrids. Selfing was carried out on F<sub>1</sub> plants. The pods were tagged after the bud break and collected at different growth stages of 10, 12, 15, 18, 20, 30 days. The number of seeds in each pod and the seeds with healthy embryos were recorded. Three embryos were cultured in B<sub>5</sub> medium.

## RESULTS

Pod formation was more successful when *A. esculentus* was used as the female parent than when *A. angulosus* was used as the female parent (Table 1). On examination of mature seeds for the prescence of embryos all the seeds were found to be empty without embryos in the cross where *A. angulosus* was used as the female parent. This was further confirmed by the fact that these seeds did not germinate. According to seed germination tests, F<sub>1</sub> seeds obtained in the cross using *A. esculentus* as the female parent had as high a rate of germination as the seeds of the parent, *A. esculentus*.

Since no fertile seeds could be obtained in crosses where *A. angulosus* was used as the female parent embryo formation was analyzed. The embryo growth was visible from 8 - 10 days after pollination and subsequently they began to degenerate leading to formation of empty seeds. Embryo culture was successful in B<sub>5</sub> medium provided with 0.2 mg/l BAP (Fig. 1a). The F<sub>1</sub> hybrid plants obtained by embryo rescue were transferred to soil and grown in a greenhouse. Morphological characters of F<sub>1</sub> plants obtained in reciprocal crosses followed by or without embryo culture were recorded along with those of their parents (Table 2).

**Table 1.** Degree of success of crosses between okra (*Abelmoschus esculentus*) and a wild relative *A. angulosus*.

| Cross                                         | No.of crosses | Success of pod formation (%) | Seed germination (%) |
|-----------------------------------------------|---------------|------------------------------|----------------------|
| <i>A. esculentus</i> x<br><i>A. angulosus</i> | 20            | 50                           | 95                   |
| <i>A. angulosus</i> x<br><i>A. esculentus</i> | 40            | 45                           | 0                    |
| <i>A. angulosus</i>                           | -             | -                            | 85                   |
| <i>A. esculentus</i>                          | -             | -                            | 98                   |

**Table 2.** Morphological characters of parents [*A. esculentus* (E) and *A. angulosus* (A)] and their hybrids.

| Character          | <i>A. esculentus</i> | <i>A. angulosus</i> | F <sub>1</sub> hybrids (A x E)<br>(embryo rescued) | F <sub>1</sub> hybrid (E x A) |
|--------------------|----------------------|---------------------|----------------------------------------------------|-------------------------------|
| Branching          | orthotropic          | moderate            | moderate                                           | moderate                      |
| Stem pubescence    | absent               | slight              | slight                                             | slight                        |
| Flower             | medium,<br>yellow    | large,<br>yellow    | medium,<br>yellow                                  | medium,<br>yellow             |
| Days to flowering  | 56                   | 72                  | 60                                                 | 60                            |
| Fruit length (cm)  | 21                   | 5                   | 12                                                 | 9                             |
| Fruit width (cm)   | 2.5                  | 2.5                 | 2.5                                                | 2.5                           |
| No.of ridges/fruit | 5-7                  | 5                   | 5-7                                                | 5-7                           |
| Fruit pubescence   | downy                | slightly rough      | prickly                                            | prickly                       |

When the morphological characters of F<sub>1</sub> progeny obtained after embryo culture were compared with those of the parents, they mostly showed intermediate features. F<sub>1</sub> plants had more branching habit compared to cultivated type (Fig. 1b). Days to flowering and pubescence are some of the other characters which are deviating from parents (Table 2). Long fruit stalk and long slender intermediate fruit size were some prominent morphological differences (Figs. 1c and 1d).

In F<sub>1</sub> plants obtained from the seeds of the cross between *A. esculentus* and *A. angulosus*, with *A. esculentus* as the female plant, plants which are morphologically different could be observed. The plants derived from some crosses resembled almost

completely those obtained by embryo culture. Table 2 compares the different characters of those F<sub>1</sub> plants with their parents. The pollen grains produced by F<sub>1</sub> hybrid plants are given in Fig.2. Only some pollen grains which appeared to be normal under the light microscope (2a) were showing fluorescence (2b) under the UV microscope.

When the pollen were counted as indicated by their fluorescence only 54% of the total pollen counted under light microscope were recorded to be viable (Table 3). This was compared with the data on parent plants where *A. esculentus* and *A. angulosus* recorded respectively 87% and 80% pollen viability.

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In  $F_1$  plants although every flower bud gave rise to a pod, different pods had varying numbers of seeds (Table 4). The average numbers of seeds per pod was 6.8 seeds compared to seeds in *A. angulosus* and *A. esculentus*. When a total number of 126 seeds was examined for embryos only six seeds were found with embryos. The pods

with a higher number of seeds usually had one or two embryos. Out of the 6 embryos 4 could be cultured. Two of them turned out to be albino plants which died after 3-4 weeks in culture. Two other embryos could be raised into healthy looking plants. After one month in culture the plants were transferred to soil.

**Table 3.** Observation of pollen of  $F_1$  hybrids and parents (P1, P2) under light microscope (a) and fluorescence microscope (b) for pollen viability.

|                      | Total # of<br>pollen (a) examined | # of UV fluorescent<br>pollen (b) | % fluorescent<br>pollen (average) |
|----------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| <i>A. esculentus</i> | 16<br>13<br>18                    | 16<br>10<br>15                    | 87                                |
| <i>A. angulosus</i>  | 22<br>25<br>16                    | 20<br>18<br>13                    | 80                                |
| $F_1$ hybrid         | 13<br>07<br>11                    | 9<br>3<br>5                       | 54                                |

**Table 4.** Presence of seeds and embryos in pods formed in  $F_1$  hybrids between *A. angulosus* and *A. esculentus*.

| Age of pods<br>(days after pollination) | No. of pods<br>observed | Number of<br>seeds in each pod | Presence of<br>embryos |
|-----------------------------------------|-------------------------|--------------------------------|------------------------|
| 5                                       | 6                       | -                              | -                      |
| 12                                      | 4                       | 25<br>06<br>15                 | 1                      |
| 18                                      | 3                       | 6<br>2<br>-                    | -<br>-<br>-            |
| 20                                      | 3                       | 18<br>-<br>-                   | 2<br>-<br>-            |
| 30                                      | 3                       | 28<br>-<br>26                  | 2<br>-<br>1            |
| Total                                   | 19                      | 126                            | 6                      |

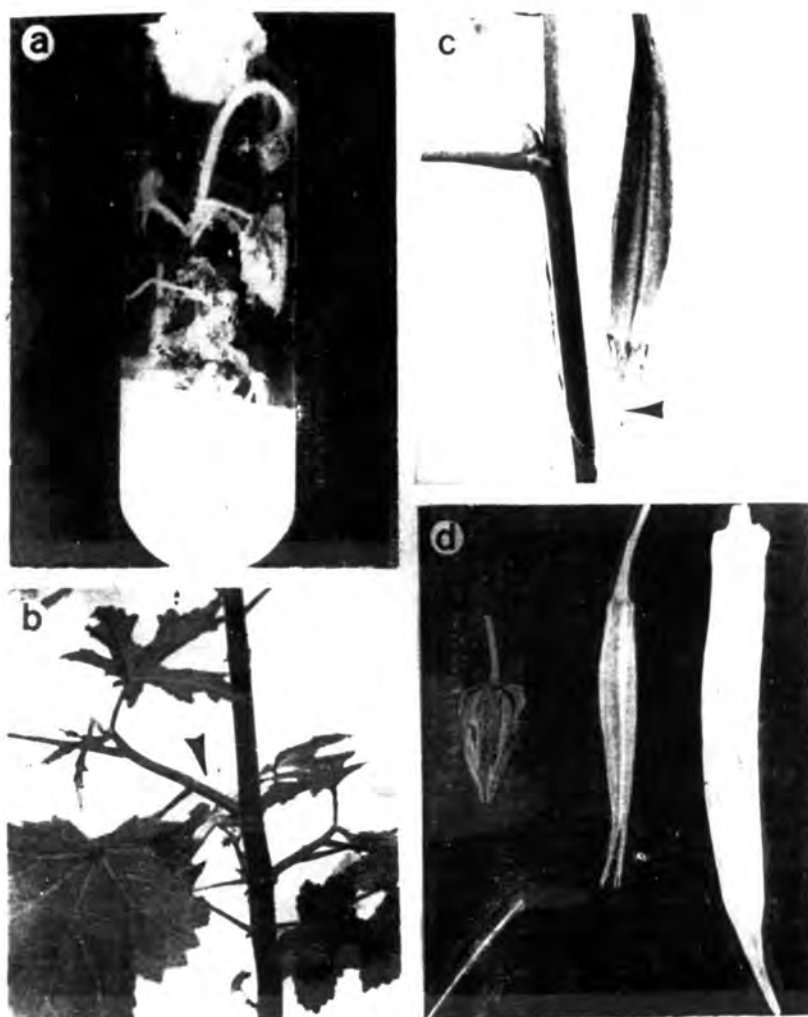


Fig. 1. Characteristics of  $F_1$  hybrids in a cross  
*A. angulosus* (wild) x *A. esculentus* (cultivated).

- a).  $F_1$  plant *in vitro* after embryo culture
- b). Branching habit of the mature plant
- c). Fruit formation with a long fruit stalk
- d). Comparison of pods with those of parents.  
(Left *A. angulosus*, Centre  $F_1$  hybrid,  
Right *A. esculentus*).

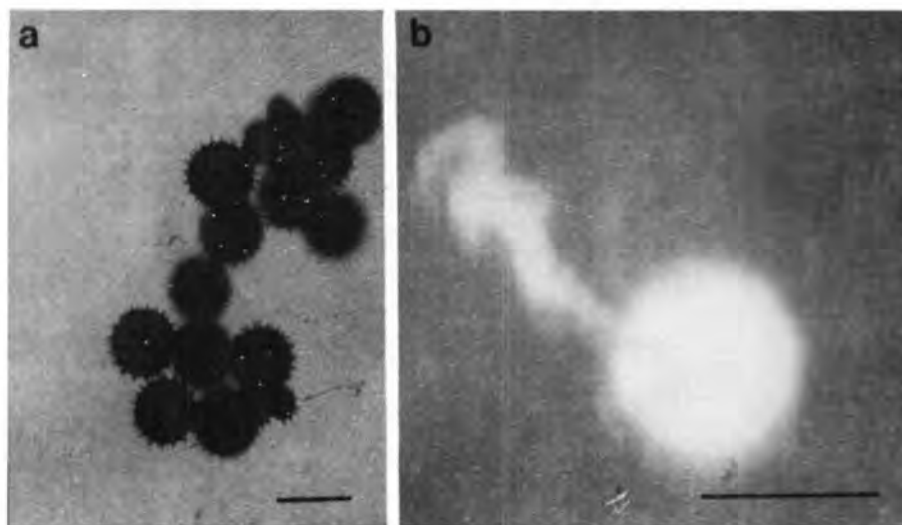


Fig. 2. Formation of viable pollen in  $F_1$  hybrids following the reciprocal cross between *A. esculentus* x *A. angulosus*.

- a. Pollen under light microscope
- b. Fluorescence of a viable pollen grain under UV microscope after FDA treatment (bar indicated 200  $\mu$ m).

### DISCUSSION

The present investigation indicates that  $F_1$  hybrid plants can be produced by making crosses between the cultivated okra and its wild relative *A. angulosus* in either direction. To our knowledge this is the first attempt to make hybrids between okra and its wild relative *A. angulosus*. Low level of success in crosses as indicated by pod formation suggests that there is an incompatibility problem when the wild relative is used as the female plant. This is further confirmed by lack of fully developed embryos in developing seeds and germination tests with mature seeds. These tests also suggest that the incompatibility could operate at postzygotic level. Often this has been the case with many examples of wide

hybridization where a wild relative is used as the mother plant (Hasan and Lester, 1990). In such cases it could be possible to apply embryo or ovule culture to raise hybrids (Gadwal *et al.*, 1968).

We successfully applied the embryo culture technique to produce  $F_1$  hybrids in crosses where the degeneration of embryos occurred, using  $B_5$  as the basal medium supplemented with 0.2 mg/l of BAP. Comparison of morphological traits between the parents and the putative  $F_1$  hybrids confirmed the hybrid nature of  $F_1$  plants. Most of the characters inherited were from the female parent while some mixed or intermediate characters were also shown.

It was evident that when using *A. esculentus* as the female parent some crosses produced plants with intermediate characters suggesting that they were true hybrids. The  $F_1$  plants resembling the parent could be considered as doubtful crosses. There is evidence to indicate that in *Solanum* wide crosses progeny showed characters close to the female parent (Schaff *et al.*, 1982). However, these results also suggest that the crosses in this direction can be performed without any obstacle to produce  $F_1$  hybrids.

In the present study the reciprocal crosses between *A. esculentus* and *A. angulosus* occurred, but the resulting  $F_1$  plants were mostly sterile. This could be due to incomplete chromosome pairing of the combined genome at meiosis (Siemonsma, 1982). Low pollen viability could be attributed to this. One would require evidence from further cytological studies to clarify these events. However, to carry out further crossing in both directions fertility has to be restored in  $F_1$  plants. It was evident from the results that very few seeds had formed embryos. Although two  $F_2$  plants could be raised one should be able to produce several  $F_2$  plants as a selfed progeny if a large number of embryos are cultured. Since  $F_1$  plants are producing viable pollen there is a possibility of producing a back cross population.

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