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# Production of Cucurbita Interspecific Hybrids Through Cross Pollination and Embryo Rescue Technique

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**Abstract:** Interspecific hybridization is an important method to widen variations and to develop novel cultivars. Interspecific cross-pollination was carried out among three species of Cucurbita (*C. moschata*, *C. ficifolia* and *C. martinezii*) as male parents with ten cultivars of *C. pepo* as female parents. Only four cultivars of *C. pepo* (Eskandarani cv., Queen $F_1$, Jedida $F_1$ and MHTC77 $F_1$) were successful in producing interspecific hybrids with *C. moschata*. Traditional sexual hybridization technique was unsuccessful in producing hybrids between *C. ficifolia* and *C. pepo* as well as *C. martinezii* and *C. pepo*. However, among the ten cultivars of *C. pepo*, only Queen $F_1$ with *C. ficifolia* and MHTC77 $F_1$ with *C. martinezii* were able to give fertile set fruits but failed to produce seeds. Therefore, hybridity was accomplished when embryo rescue technique was employed. The immature embryos of *C. ficifolia* × *C. pepo* (Queen $F_1$) and *C. martinezii* × *C. pepo* (MHTC77 $F_1$) recorded regeneration efficiency of 40% and 15%, respectively.

**Key words:** Breeding · Cucurbitaceae · Regeneration · Squash · Tissue culture

## INTRODUCTION

The family Cucurbitaceae includes 96 genera and 75 species. The genus Cucurbita is one of the most economically important genera, which includes 27 species. *Cucurbita pepo* L. is the most widely grown and polymorphic of the cucurbita species. Interspecific hybridization is used to improve crops by transferring specific traits to crops from their wild relatives [1]. In the genus Cucurbita, several attempts have been made to produce interspecific hybrids among five cultivated species (*C. pepo*, *C. maxima*, *C. moschata*, *C. argyrosperma* and *C. ficifolia*) and a number of non-cultivated ones. In general, success of hybridization within this genus is a species-dependant. A limited success in obtaining fully developed seeds that germinated, forming viable plants has been reported for a few interspecific combinations such as *C. andreana* × *C. ficifolia* [2], *C. lundelliana* × *C. moschata* [3] and *C. maxima* × *C. ecuadorensis* [4]. However, interspecific hybridization may not succeed due to failure of double fertilization or early embryo abortion. Embryo-rescue method has been reported to be successful in *C. moschata* × *C. pepo* [5], *C. moschata* × *C. maxima* [6], *C. maxima* × *C. pepo* [7, 8], *C. ecuadorensis* × *C. pepo* [9] and *C. martinezii* × *C. pepo* [10]. The aim of the present study was to produce interspecific hybrids through cross pollination of three Cucurbita species i.e. *Cucurbita moschata, Cucurbita ficifolia* and *Cucubrita martinezii* as male parents with ten cultivars of *Cucurbita pepo* as female parents.

## MATERIALS AND METHODS

Seeds of each Cucurbita species [*Cucurbita moschata* L. (Nigerian local cv; $2n = 2x = 40$), the wild species *Cucurbita ficifolia* ($2n = 2x = 40$) and *Cucubrita martinezii* ($2n = 2x = 40$)] and seeds of 10 cultivars all belonging to the cultivated species *Cucurbita pepo* L. ($2n = 2x = 40$), i.e., Eskanadarani, Queen $F_1$, Diamant $F_1$, Hurricane $F_1$, Jedida $F_1$, Cavili $F_1$, Arlika $F_1$, Revenue $F_1$, Eskandarani × Arlika and MHTC77 $F_1$ were sown under unheated plastic tunnel. The seeds of each species were sown in hills on the north side of ridges (6 × 1 m) and planting space of 30 cm. The field was immediately irrigated after sowing. Fertilizers were added to the soil as follows: 1) old cattle manure was added at the rate of 47.6 $m^{-3}$ per ha before planting. 2) commercial fertilizers were applied as a mixed fertilizer having 1: 2: 1 ratio with a rate of 238 kg per ha. Ammonium sulphate, super

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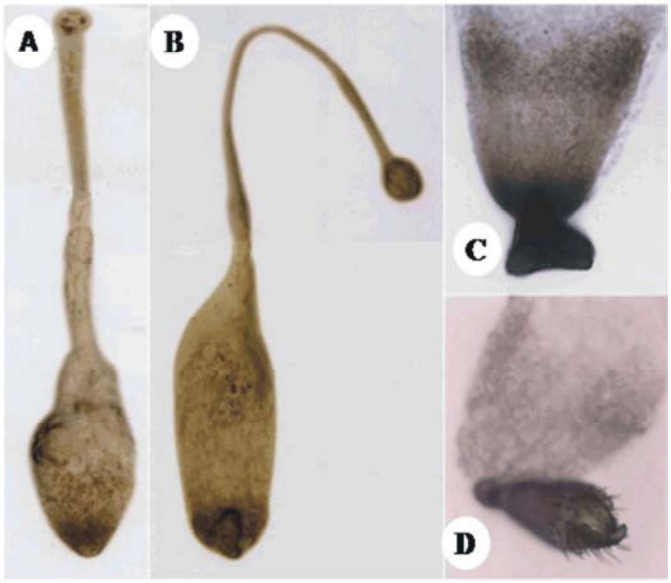


Fig. 1: Embryo development of the interspecific hybrid *C. martinezii* L. × *C. pepo* L. (MHTC77 $F_1$) through embryo rescue technique A) Embryo at excision time; B) embryo at 7 days-old C) embryo at 14 days-old D) embryo at 30 days-old

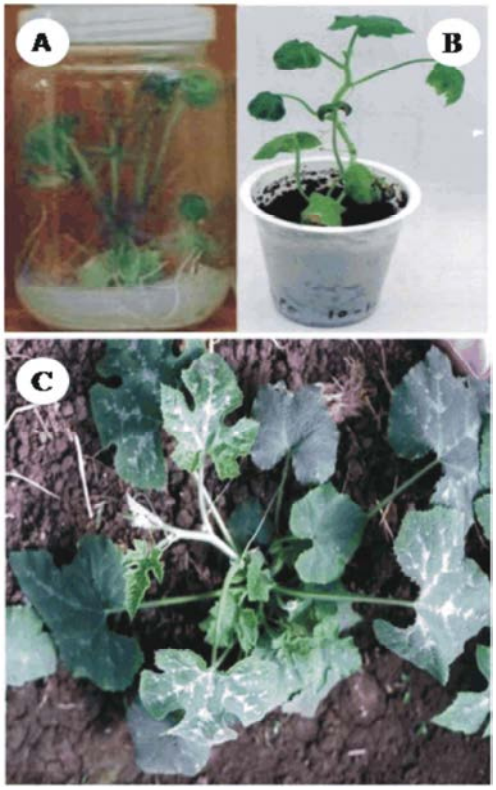


Fig. 2: *In vitro* growth and acclimatization of the interspecific hybrid *C. martinezii* L. × *C. pepo* L. (MHTC77 $F_1$) A) Plantlets grown in tissue culture jar B) Acclimatized plantlet C) Mature plant grown in greenhouse.

phosphate and potassium sulphate were used as sources of N, P and K, respectively. One half of the fertilizers' mixture was applied 14 and 21 days post-sowing. At flowering time, crosses were made between (*C. moschata, C. ficifolia* and *C. martneziii*) as male parents and the ten cultivars of *C. pepo* as female parents.

For embryo rescue, the set fruits of the hybrid *C. martinezii* × *C. pepo* L. (MHTC77 $F_1$) and *C. ficifolia* × *C. pepo* (Queen $F_1$) were picked in the morning 14 days post-pollination. They were surface sterilized on a gyratory shaker at 150 rpm for 30 s in 70% ethanol followed by 30 min in a solution of 20% (*v/v*) Clorox bleach (containing 5.2% sodium hypochlorite) and 2 - 3 drops of Tween-20. Then, they were washed thrice with sterile distilled water. The immature embryos (14 days old at heart shape stage) were carefully excised using stereomicroscope and two sharp dissection needles. The excised embryos (Fig. 1 A) were cultured into petri dish (7 × 1 cm) containing 15 ml of MS medium [11] supplemented with 0.1 mg $l^{-1}$ kinetin and 0.01 mg $l^{-1}$ indol acetic acid (IAA). The pH of the medium was adjusted to 5.8 ± 0.1 before autoclaving (at 121°C and 1.1 kg $cm^{-2}$ pressure for 20 min). All media were solidified with 0.8% agar. The cultures were incubated at 25 ± 1°C, with 16 h light at 40 µ mol $m^{-2}$ $s^{-1}$ photosynthetic photon flux (PPF) provided by cool white fluorescent tubes. Embryo was developed (Fig. 1 B, C) and the hybrid plantlet was visible (Fig. 1 D) after 4 weeks of culture. The hybrid plantlets were transferred into jars each containing 25 ml of MS

medium without plant growth regulators for 4 weeks (Fig. 2 A). Regenerated plantlets were harvested, washed under tap water and cultured in pots containing sterilized peat moss. The pots were covered with clear polyethylene bags and hardened in growth chamber for 1 week (Fig. 2 B) followed by 1 week in greenhouse prior to their transplanting in the field (Fig. 2 C).The data were analyzed by Student's unpaired *t*-test and the treatment mean values were compared at $P \leq 0.05$.

## RESULTS AND DISCUSSION

The interspecific crosses between *C. moschata* and *C. pepo* were dependant on the cultivars of *C. pepo*. Only four cultivars (Eskandarani cv., Queen $F_1$, Jedida $F_1$ and MHTC77 $F_1$) were successful in producing interspecific hybrids while all other cultivars were unsuccessful (Table 1). It has been reported that traditional sexual hybridization technique is successful in producing hybrids between *C. pepo* and *C. moschata* [12]. Interspecific cross between *C. moschata* and *C. pepo* "Eskandarani cv" was successful and viable seeds were obtained [13]. However, in the present study, the interspecific hybridization is greatly influenced by the genotypes of *C. pepo*. Traditional sexual hybridization technique was unsuccessful in producing hybrids between *C. ficifolia* and *C. pepo* as well as *C. martinezii* and *C. pepo*. These crosses have been reported as difficult to be made even when the embryo culture technique is employed [14, 15]. However, in the present study, among the ten cultivars of *C. pepo,* only Queen $F_1$ with *C. ficifolia* and MHTC77 $F_1$ with *C. martinezii* were able to give fertile fruits but failed to produce seeds. Therefore, embryo *in vitro* cultures were employed to rescue *C. martinezii* × *C. pepo* L. (MHTC77 $F_1$) and *C. ficifolia* × *C. pepo* (Queen $F_1$) hybrids. Significant difference in embryo regeneration of these hybrids,were observed (Table 2). Regeneration efficiency at 40% and 15% was obtained from *C. ficifolia* and *C. martinezii,* respectively. These results suggest that plant genotype is an important factor which can have a profound effect on the efficiency of plant regeneration system. In *Cucurbita martinezii*, it has been reported that only 10% of cultured embryos were developed to plantlets [10] while in *Cucumis melo*, 0.09% to 90% were recorded [16]. The embryo rescue technique has been useful to enhance hybridization, reduce generation time of elite germplasm and obtain valuable haploid plants in crops such as melon [16], onion [17] and squash [18, 19]. Not all attempts to obtain a plant materialvia embryo rescue technique, such as squash and melon, have been successful because embryos generally failed to undergo complete differentiation [20-23]. In cucurbits, embryo rescue techniques have been used to recover normal seedlings from anther, ovule and zygotic-embryo cultures in order to obtain interspecific hybrids or dihaploid lines [17-19, 21, 23, 24]. Similar to other plant species, embryo-rescue culture for cucurbits is affected by genotype [17, 25, 26] and formulation of the culture media [23, 25]. The production of pure lines in vegetable crops especially from open pollinated plants such as Cucurbita requires several years of conventional plant breeding program [18, 26]. Therefore, crosses between distant elite genotypes are thus required. To date, *C. pepo* is highly susceptible to diseases especially those caused by viruses. Therefore, disease resistance has been considered an important goal of summer squash breeding [27]. As no sources of resistance to these pathogens are available in *C. pepo*, breeders have resorted to introgression of resistance from other species. For virus resistance, *Cucurbita moschata* Duchesne has been the primary source, as sparingly fertile progeny can be obtained from the interspecific cross of *C. pepo* L. with this species [27, 28]. *C. moschata* 'Nigerian Local' has been the common source of nearly all virus-resistant, commercially deployed *C. pepo* L. [29]. *Cucurbita martinezii* Bailey possesses a completely dominant resistance to CMV [30]. In addition, *Cucurbita ficifolia* has been reported to be resistant to certain

Table 1: List of crosses between *C. moschata* L. and ten cultivars of *C. pepo* L

| | |
|---|---|
| | *Successful interspecific hybdridization* |
| 1 | *C. moschata* L. × *C. pepo* L. Eskandarani |
| 2 | *C. moschata* L. × *C. pepo* L. Queen F1 |
| 3 | *C. moschata* L. × *C. pepo* L. Jedida F1 |
| 4 | *C. moschata* L. × *C. pepo* L. MHTC77F1 |
| | *Unsuccessful interspecific hybdridization* |
| 5 | *C. moschata* L. × *C. pepo* L. Diamant $F_1$ |
| 6 | *C. moschata* L. × *C. pepo* L. Hurricane $F_1$ |
| 7 | *C. moschata* L. × *C. pepo* L. Cavili $F_1$ |
| 8 | *C. moschata* L. × *C. pepo* L. Arlika $F_1$ |
| 9 | *C. moschata* L. × *C. pepo* L. Revennue $F_1$ |
| 10 | *C. moschata* L. × *C. pepo* L. Eskandarani × Arlika |

Table 2: Regeneration efficiency of immature embryos that developed normal plants through embryo rescue technique

| Interspecific hybrids | Regeneration efficiency (%) |
|---|---|
| *C. ficifolia* × *C. pepo* (Queen $F_1$) | 40 ** |
| *C. martinezii* × *C. pepo* (MHTC77 $F_1$) | 15 |

** = significantly different at $P = 0.05$ according to Student's unpaired *t*-test

*Fusarium* species [31]. Therefore, *C. moschata*, *C. martinezii* and *Cucurbita ficifolia* has been used in our breeding program to improve the cultivated species of Cucurbita.

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