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Interspecific Hybridization between *Cucurbita Maxima* Duch. and *Cucurbita Moschata* Duch. for High Efficient Development of Cucurbit Rootstocks

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ABSTRACT

Interspecific hybrids between *Cucurbita maxima* and *C. moschata* have been commonly used as a rootstock for watermelon, melon, and cucumber, as well as to develop new Cucurbit types and widen the genetic variation. In this study, 218 interspecific hybridizations were performed between *C. moschata* (Alseiny) and ten lines of *C. maxima*. *C. moschata* (Alseiny) was successfully crossed with all ten lines of *C. maxima* resulted 20 interspecific hybrid combinations (including reciprocal) and fruit set, hybrid normal seed and seed germination was obtained. Fruit set and hybrid seed was generally higher in interspecific hybrid combinations when *C. moschata* (Alseiny) used as maternal parent, with the exception of hybridizations involved *C. maxima* MAX5 and MAX7. The highest fruit set percentage with normal seeds was obtained in interspecific hybrid MAX5 X Alseiny (86.6%), followed by Alseiny X MAX14 (83.3%). In addition, interspecific hybrid combinations Alseiny X MAX20, MAX2 X Alseiny, MAX5 X Alseiny gave the higher hybrid seed number and weight per fruit. Overall, interspecific hybrid MAX5 X Alseiny was the most promising combination for fruit setting and hybrid seed yield. Success in production of interspecific hybrid seeds and germination could be promising for cucurbit rootstock production in Egypt.

Keywords: abiotic and biotic stresses, breeding, cucurbits, hybrids, pumpkin

INTRODUCTION

The winter squash (*Cucurbita maxima* Duch.) and pumpkin (*C. moschata* Duch.) are the most commonly cultivated species of *Cucurbita* genus which also includes other domesticated species *C. argyrosperma* Huber, *C. ficifolia* Bouché, and *C. pepo* L. A wide genetic diversity have been reported among these species for morphology, disease and insect resistance, and adaptation to abiotic stresses (Whitaker and Bemis 1964; Saade and Hernandez, 1994; Loy, 2004; Hajjar and Hodgkin, 2007). Interspecific hybridization between cucurbit species is an effective way to develop new *Cucurbit* types and also for crop improvement by transferring important traits such as disease and insect resistance into cultivated crops from their wild relatives (Zhang *et al*., 2012). However, challenges such as crossing barriers, male sterility, and incompatibility of the interspecific F_1 complicate transfer of useful traits among *Cucurbit* species (Rhodes 1959; Hiroshi 1963; Shifriss 1987; Zhang *et al*., 2012). Crosses between *Cucurbit* species were successfully made to obtain fruit and fertile seeds through additional techniques such as embryo rescue, protoplast fusion, mixed pollen pollination, and bud pollination (Hiroshi, 1963; Bemis, 1973; Cheng *et al*., 2002; Plazas *et al*., 2016; Rakha *et al.,* 2012).

Interspecific hybrids between *C. maxima* and *C. moschata* have widely used rootstocks for watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and cucumber (*Cucumis sativus* L.) for the control of biotic and abiotic stresses and to enhance scion vigor (Ruiz *et al*., 1997, Hassel and Daley 2015). High compatibility was found between these interspecific hybrids and watermelon. Breeding of appropriate rootstocks is still lacking in Egypt. The aim of this study was to develop interspecific hybrids from the crosses between *C. maxima* and a locally adapted variety of *C. moschata*, and evaluate hybrid seed production and germination of obtained interspecific hybrids for rootstocks use in cucurbits.

MATERIALS AND METHODS

This study was conducted at experimental farms of Horticulture Department, Faculty of Agriculture, Kafr elsheikh University in Kafr El-Sheikh Governorate, Egypt. Ten lines of *C. maxima*, MAX2, MAX3, MAX5, MAX6, MAX7, MAX8, MAX13, MAX14, MAX19, MAX20, and a known local pumpkin cultivar grown in Egypt *C. moschata* (Alseiny) were used in this experiment. Both *C. maxima* and *C. moschata* genotypes were used as the female and male parents in reciprocal crosses to develop interspecific hybrids.

Seeds of *C. maxima* lines and *C. moschata* (Alseiny) were sown in 84-cell seedling trays with 60 ml peat moss:vermiculite mixture (1:1 volume) per seedling and watered daily. Seedlings were fertilized weekly using NPK 19-19-19. Two weeks after sowing, 10 seedlings (1-2 true leaves) from each line of *C. maxima* and 100 seedlings of *C. moschata* (Alseiny) were transplanted in a plastic greenhouse (25 - 36°C, 16/8 h day/night), spaced 2.5 m × 3.0 m between and within rows, respectively. Plants were grown under drip irrigation and all horticultural practices including fertilizer, weed, disease and insect control were implemented as indicated in Rakha *et al*., (2012) and recommendations of the Egyptian Ministry of Agriculture.

Pollinations between *C. maxima* lines and *C. moschata* (Alseiny) were made by hand in the morning (0700 hr and 0900 hr) on the day of anthesis. All flowers used in pollinations were covered by paper bags one day before anthesis, and female flowers were covered again directly after pollinations. All unused female flowers in pollinations were removed to prevent pollen contamination. Pollinated female flowers were tagged for identification and for calculation of the percentage of fruit set. Fruits obtained from the interspecific crosses were harvested 65 to 85 days after pollination when the fruits were physiologically ripe. Fruit set (%) and percentage of fruit with normal seeds were measured. Seeds from individual fruit were extracted and placed on paper to dry under laboratory conditions and seed number and weight were measured.

Weight of 100 seeds of each of 20 hybrid combinations between ten lines of *C. maxima* and *C. moschata* (Alseiny) was also measured. A sample of 168 seeds of each of 20 hybrid combinations between *C. maxima* and *C. moschata* were germinated in two 84-cell seedling trays as described above. Germinated seeds were counted as emerged seedlings to calculate the seed germination percentage (%). Data of seed yield (seed number and weight per fruit, and 100 seed weight), and fruit weight were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (p = 0.05) using SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

A total of 218 interspecific crosses were performed between *C. moschata* (Alseiny) and ten lines of *C. maxima* (Table 1). Fifty-six percent of these crosses were performed when *C. maxima* lines used as female parent. The number of the crosses performed between each *C. maxima* line and *C. moschata* (Alseiny) were variable because of the differences in flowering among the ten lines of *C. maxima*. For instance, *C. maxima* MAX19 produced late and few flowers, which resulted in fewer number of the crosses made with *C. moschata*. Twenty interspecific combinations between *C. maxima* and *C. moschata* (including reciprocal) were made successfully and were produced fruits and normal seeds (Tables 1 and 2). These results confirm that interspecific crosses could be obtained between *C. maxima* and *C. moschata* (Karaagac and Balkaya, 2012), which can be used as rootstocks for grafting watermelon and cucumber scions, providing high level of tolerance and resistance against salinity, low temperatures soil, and soil-borne disease (Lee, 2003; Passam, 2003; King *et al*., 2008; Huitron-Ramırez *et al.,* 2009; Karaagac and Balkaya, 2012).

Table 1. Number of pollinations, fruit set (%), fruit set with normal seeds (%), seeds weight per fruit (g/fruit), and number of seeds per fruit in interspecific hybridizations between *C. maxima* and *C. moschata* based on the direction of the hybridizations

C. maxima	*C. moschata* (female parent)				
	Pollinations(no.)	Fruit set (%)	Fruit set with normal seeds (%)	Seeds/fruit (g)	Seeds/fruit (no.)
MAX2	14	100.0	71.1	69.2±12.8 bcd	307.0±32.4 abc
MAX3	8	75.0	62.5	89.2±14.7 abcd	331.0±39.0 abc
MAX5	7	85.5	71.1	34.8±13.9 d	124.0±48.5 d
MAX6	15	66.6	66.6	89.8±20.5 abcd	326.0±66.2 abc
MAX7	11	54.5	45.4	86.0±17.1 abcd	301.0±75.3 abc
MAX8	7	57.1	57.1	86.5±9.6 abcd	291.2±37.4 abcd
MAX13	10	60.0	50.0	98.4±19.1 abcd	280.2±68.8 abcd
MAX14	6	83.3	83.3	98.6±14.4 abcd	371.0±37.3 ab
MAX19	9	55.5	55.5	120.4±26.2 abc	374.0±55.7 ab
MAX20	8	50.0	50.0	142.0±15.4 a	402.5±47.1 ab
	C. moschata (male parent)				
MAX2	14	35.7	21.4	137.0±23.9 ab	451.6±38.7 a
MAX3	18	33.3	5.5	70.0±0.0 bcd	165.0±0.0 cd
MAX5	15	86.6	86.6	135.8±17.3 abc	393.0±40.5 ab
MAX6	10	60.0	50.0	67.6±17.2 cd	278.0±43.6 bcd
MAX7	14	57.1	50.0	93.6±19.7 abcd	271.0±46.6 bcd
MAX8	10	50.0	40.0	84.2±16.5 abcd	266.7±20.9 bcd
MAX13	9	55.5	44.4	95.2±10.7 abcd	295.0±35.2 abcd
MAX14	12	50.0	50.0	73.2±5.5 bcd	276.6±29.6 abcd
MAX19	9	66.6	33.3	87.6±25.6 abcd	286.6±8.8 abcd
MAX20	12	50.0	41.6	123.4±27.4 abc	242.0±32.6 bcd

Means followed by ± standard error; means with the same letter within the same column are not significantly different at ($P \leq 0.05$) according to Duncan's multiple range test.

Fruit set percentage was variable in interspecific combinations depending on the direction of the hybridization of *C. maxima* lines involved. Both fruit set (%) and fruit set with normal seeds (%) were generally higher in interspecific hybrid combinations when *C. maxima* lines were used as male parent, with the exception of hybridizations involving *C. maxima* MAX5 and MAX7 (Table 1 and 2). For instance, fruit set (%) and fruit set with normal seeds (%) were increased >60% when *C. maxima* MAX3 was used as male parent and crossed with *C. moschata* (Alseiny) as female parent. The highest fruit set percentage (100%) was obtained when *C. moschata* (Alseiny) used as female parent and crossed with *C. maxima* MAX2. However, the highest fruit set percentage with normal seeds (86.6%) was obtained when *C. moschata* (Alseiny) was used as male parent and crossed with *C. maxima* MAX5 as female. Conversely, very low fruit set percentage (33.3%) and fruit set percentage with normal seeds (5.5%) was obtained when *C. moschata* (Alseiny) was used as male parent and crossed with *C. maxima* MAX3. Other studies have also succeed to obtain interspecific hybrid plants from the crosses between *C. pepo*, *C. maxima,* and *C. moschata* (Baggett, 1979; Korakot *et al*., 2010; Karaagac and Balkaya, 2012; Rakha *et al*. 2012; Zhang *et al*., 2012). Whitaker and Davis (1962) summarized that *C. moschata* occupies a central position among *Cucurbita* species and can be crossed with some difficulties with *C. pepo*, *C. maxima*, and *C. mixta*. Other *Cucurbita* species such as *C. ficifolia* and *C. martinezii* Bailey have been successfully crossed with *C. pepo* through embryo rescue (Robinson and Shail, 1987, Rakha *et al*., 2012).

When using *C. moschata* (Alseiny) as a female parent, the largest amount of seeds in the fruits was obtained in the crosses with *C. maxima* MAX20, which had 142 g per fruit and more than 402 seeds per fruit (Table 2). In contrast, the lowest amount of seeds in the fruits was obtained in the crosses with *C. maxima* MAX5, which had 34.8 g per fruit and 124 seeds per fruit. When using *C. moschata* (Alseiny) as a male parent, the largest amount of seeds in the fruits was obtained in the crosses with *C. maxima* MAX2, with an average values 137 g per fruit and more than 451 seeds per fruit. In contrast, the lowest amount of seeds in the fruits was obtained in the crosses with *C. maxima* MAX6 for seed weight per fruit (67.6 g) and in the crosses with *C. maxima* MAX3 for the seed number per fruit (165). For 100 seeds weight, the highest seed weight was obtained when *C. moschata* (Alseiny) as a female parent and crossed with MAX3 (52 g/ 100 seeds).

Table 2. Number of pollinations, fruit weight (kg), 100 seeds weight (g), seed germination in interspecific hybridizations between *C. maxima* and *C. moschata* based on the direction of the hybridizations

C. maxima	***C. moschata* (female parent)**			
	Pollinations (no.)	**Fruit weight(kg)**	**100 seeds weight (g)**	**Germination (%)**
MAX2	14	5.4±1.0 ab	25.9±0.5 j	72.0
MAX3	8	7.4±1.0 ab	52.0±0.7 a	69.0
MAX5	7	8.2±1.6 a	30.0±0.3 g	80.0
MAX6	15	5.7±0.4 ab	25.7±0.3 j	95.2
MAX7	11	6.5±1.7 ab	32.6±0.5 f	91.1
MAX8	7	8.2±2.2 a	33.4±1.3 e	87.1
MAX13	10	3.9±0.7 bc	26.4±0.7 j	93.5
MAX14	6	1.7±0.4 c	24.2±0.5 k	88.1
MAX19	9	6.6±0.8 ab	44.7±0.9 b	80.0
MAX20	8	6.9±1.4 ab	35.5±0.7 d	80.0
	C. moschata (male parent)			
MAX2	14	4.6±0.4 abc	28.7±0.5 h	85.1
MAX3	18	5.7±0.5 ab	29.1±0.3 gh	81.0
MAX5	15	7.1±0.3 ab	29.4±0.5 gh	80.0
MAX6	10	7.0±1.1 ab	29.4±0.7 gh	68.5
MAX7	14	7.1±0.8 ab	27.9±0.9 i	79.9
MAX8	10	5.5±1.2 ab	23.0±0.5 l	87.5
MAX13	9	7.2±0.4 ab	38.1±0.9 c	92.9
MAX14	12	4.7±3.0 abc	24.1±0.3 k	92.3
MAX19	9	4.5±1.1 abc	37.9±0.9 c	95.6
MAX20	12	5.4±0.2 ab	27.7±0.3 i	92.3

Means followed by ± standard error; means with the same letter within the same column are not significantly different at ($P \leq 0.05$) according to Duncan's multiple range test.

The highest seed germination in interspecific hybrids was obtained with *C. maxima* MAX19 X *C. moschata* (Alseiny), whereas *C. moschata* (Alseiny) X *C. maxima* MAX3 gave the lowest value. Interestingly, seed germination rates were ≥ 80% in the most promising interspecific hybrids for hybrid seed production. Conversely, Karaagac and Balkaya (2012) have reported seed germination rates of the promising interspecific hybrids were not high which could be due to embryo or endosperm failure in the interspecific hybrid combinations. These results suggest that plant genotype is an important factor which can have a profound effect on the success of interspecific crosses, seed production and germination (Karaagac and Balkaya, 2012; Plazas *et al*., 2016).

Linear correlations were calculated between parameters resulted in the interspecific hybridizations between *C. maxima* and *C. moschata* (Table 3). Highly and positive significant correlations were obtained between fruit set percentage and fruit set percentage with normal seeds. Seed number and weight per fruit are desirable traits for commercial seed production. In our study, seed weight per fruit was positive and highly correlated with seeds per fruit and fruit weigh. Moderate and negative significant correlations were obtained between 100 seeds weight and fruit set percentage and fruit set percentage with normal seeds. Conversely, moderate and positive significant correlations were obtained between 100 seeds weight and fruit weight. Correlations between seed germination and other parameters were weakly statistically significant or not significant.

Our results indicate that interspecific hybrids between *C. maxima* and a locally adapted *C. moschata* developed in this study are promising rootstocks for grafting watermelon and cucumber in Egypt and other neighboring countries. The use of *C. maxima* and *C. moschata* hybrid rootstocks has been shown to enhance fruit yield and firmness in watermelon (Salam *et al*., 2002; Yilmaz *et al*., 2007; Huitron-Ramırez *et al*., 2009), providing significant economic returns to farmers. Interspecific hybrids between *C. maxima* and

C. moschata could also provide a solution for resistance to serious soilborne pathogen including Fusarium and Verticillium wilts in both open field and greenhouses (Passam, 2003; King *et al*., 2008; Huitron-Ramırez *et al*., 2009). Our data also suggest that, in general, using *C. moschata* as a female parent results in higher fruit set and seeds per fruit. This can make an effective contribution to improving the production of cucurbits in Northern Africa countries using local *C. moschata* resources.

Table 3. Linear correlations among fruit set, fruit set with normal seeds, seed weight per fruit, and number of seeds per fruit, fruit weight, 100 seeds weight, seed germination in interspecific hybridizations between *C. maxima* and *C. moschata*

Parameters	Fruit set (%)	Fruit set with normal seeds (%)	Seeds/fruit (g)	Seeds/fruit (no.)	Fruit weight (kg)	100 seeds weight (g)	Germination (%)
Fruit set (%)		0.85 **	-0.20 ns	-0.04 ns	0.03 ns	-0.28 **	-0.10 ns
Fruit set with normal seeds (%)			-0.06 ns	0.08 ns	-0.04 ns	-0.54 **	0.08 ns
Seeds/fruit (g)				0.77 **	0.30 **	0.09 ns	0.21 *
Seeds/fruit(no.)					0.06 ns	-0.12 ns	0.26 *
Fruit weight(kg)						0.28 **	-0.25 *
100 seed weight (g)							-0.19 *
Germination (%)							

*** and ** indicate significance at $P \leq 0.05$ and $P < 0.001$, respectively; ns indicates not significant.**

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التهجين بين نوعي القرع العسلي ***Cucurbita maxima*** **Duch.** و ***Cucurbita moschata*** **Duch** لإنتاج أصوال لتطعيم القرعيات بكفاءة عاليه

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تستخدم التهجينات النوعيه بين *Cucurbita moschata* Duch و *Cucurbita maxima* Duch لإنتاج أصوال لتطعيم البطيخ والقاون والخيار، وكذلك زيادة التباينات الوراثية. تم إجراء ٢١٨ تهجين نوعى فى هذه الدراسة بين (Alseiny) *Cucurbita moschata* وعشرة سلالات من *Cucurbita maxima*. تم إنتاج عشرون هجين نوعي بنجاح (متضمنا الهجن العكسيه)، وتم العقد الثمرى والحصول على بذور هجينية جيدة الإنبات. وقد كان العقد الثمرى وإنتاج البذور الهجينية أفضل عند استخدام ال Alseiny كأم بإستثناء التهجين النوعي مع MAX5 و MAX7 . وأعطى الهجين Alseiny x MAX5 أعلى نسبه عقد ثمرى محتويا على بذور طبيعية (٨٦.٦%) متبوعا بالهجين ال MAX14 **x** Alseiny (٨٣.٣%). كما أعطت الهجن MAX20 x Alseiny **و** x MAX2 Alseiny و Alseiny x MAX5 أعلى عدد ووزن بذور للثمرة. وإجمالا كان الهجين Alseiny x MAX5 أفضل الهجن الواعدة بالنسبه لعقد الثمار وإنتاج البذور الهجينية . ويعد نجاح إنتاج بذور الهجن النوعيه ذات الإنبات الجيد مبشرا لتربية إنتاج أصوال للقرعيات بمصر.