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# Fruit, Seed and Seedling Characteristics of Eight Newly-developed Interspecific Hybrids of Citrus

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**Abstract:** This study was carried out during the years 2005, 2006 and 2007 at a private farm in Wardan village and greenhouses and laboratories of Faculty of Agriculture, Cairo university, Giza, Egypt. The objectives were to develop and evaluate characteristics of fruits, seeds and seedlings as well as leaf endogenous hormones of eight newly-developed citrus interspecific hybrids. Five Egyptian cultivars of citrus viz. Sour orange (SO), Volkamer lemon (VL), Lime (LI), Balady mandarin (BM) and Valencia orange (VO) were used as parents; the 1st two, i.e SO and VL were used as seed parents to be crossed with the pollen parents, so eight interspecific citrus hybrids were developed. Selfing was also done for the two seed parents. Germination tests of the seeds indicated that two hybrids (VLLI and VLBM) failed to develop embryos (embryo abortion) and six hybrids developed embryos and seedlings, namely SOVL, SOVO, SOLI, SOBM, VLVO and VLSO. Sour orange cultivar was better than Volkamer lemon as a seed parent in giving hybrids with better fruit, seed and embryo traits and could therefore be consider a good seed parent in hybridization programs of citrus breeding. However, hybrids made on Volkamer lemon showed higher means of most studied seedling characteristics, higher contents of endogenous cytokinins and lower contents of ABA than those made on Sour orange. More specifically, the hybrid VLSO combined the greatest number of desirable traits of seedlings, the highest content of leaf cytokinins and gibberellins and the lowest content of ABA; the hybrid VLVO ranked second in this regard and its seed contained a large number of nucellar embryos. Moreover, the hybrid SOLI was the earliest in fruit maturity and the highest in seed germination%, polyembryonic seeds% and embryos/seed; the hybrid SOBM ranked second in this regard. These hybrids were more vigorous than others and than VL and SO selfed parents and could therefore be considered of good potential as genetically improved rootstocks.

**Key words:** Interspecific hybrids, Citrus, Fruit set, Fruit maturity, Polyembryony, Seedling, Endogenous hormones.

## INTRODUCTION

Citrus constitutes a major group of fruits that is comprised of orange, mandarin, lime, grapefruit, shaddock (pummelo), tangelo, lemonine, kumquat, trifoliate orange, citron, citrange etc. Thus citrus fruits belong not only to the genus *Citrus* but also to *Poncirus* and *Fortunella* besides interspecific and intergeneric hybrids. The basic chromosome number is X=9 in all *citrus* species and related genera so far examined. The majority are diploid. The fact that citrus and related genera readily hybridize has created an interesting group of unusual plant forms and names.

Hybridization in citrus has been under practice for over 70 years with objective to breed superior cultivars and suitable rootstocks. In general, much of the hybridization in citrus has been interspecific rather than intraspecific; this still continues to be the pattern [17]. Some of the most common interspecific hybrids are of greatest commercial importance primarly as rootstocks[6]. The diversity available in citrus species provides tremendous potential for developing interspecific hybrids with desirable characteristics. The objectives for breeding a suitable rootstock should include better stock-scion compatibility, reduction of the size without affecting yield or scion health and resistance to pests and diseases or hardiness to adverse climatic and soil conditions.

Most citrus cultivars are very heterozygous. Therefore, selfing would appear to be a useful technique for breeding. However, selfing has produced mostly weak or otherwise inferior progeny. Selfing is also restricted by the high degree of nucellar embryony of many cultivars and by self-incompatibility in others. There is little information on general or specific combining ability on which to base the selection of parents. There is evidence for hybrid vigor and inbreeding depression in citrus, although critical data are difficult to obtain. Wider crosses within the genus citrus often produce favorable proportions of vigorous

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offspring. Mandarin X grapefruit hybrids frequently average better in vigor than mandarin X mandarin or mandarin X orange [17].

Several hybrids have been released as new cultivars since those reported by Soost and Cameron [18] and Soost [18]. A mandarin hybrid Encore has exciting possibilities [15]. It was bred by crossing mandarin (*Citrus reticulate*) cv. King X Willow leaf at California. The pummelo (*C. maxima*) Hayasaki from the cross Matou X Hirado Buntan, has oblate to pyriform fruits weighing 600-1000g with light yellow rind and greenish yellow fresh with 12-15% TSS [12]. A new late maturity high quality mandarin named "Wanmi" has been developed by Chen *et al.,* [5]. It is a hybrid between *C. unshiu* cv. Weizhou Satsuma X *C. sinenses* cv. $S_8$ with average fruit weight of 120 g and TSS range of 1108-17.5%. A tangor cv. Ariake has been developed as a cross between Seike Navel orange X Clementine mandarin (*C. clementina*). Its tree shows poor vigour, spreading habit, parthenocarpic and nearly seedless. Fruits are low in acid with average fresh weight 170-200g and TSS 12-13% [22]. More recently, new mandarin hybrids (e.g. Desiderio= Miho x Comune, and Bellezza= Okitsu x Carvalhais) and selections (e.g., Edelgard) have been reported to be highly promising [21,4].

The objectives of the present study were to develop new interspecific hybrids among five Egyptian cultivars of citrus, and characterize their fruit, seed and seedling morphological traits and endogenous hormones in their leaves, in an attempt to select a new genetically improved rootstock.

## MATERIALS AND METHODS

The experimental work of this study has been carried out during the years 2005, 2006 and 2007 at a private farm in Wardan village and green houses and laboratories of the Faculty of Agriculture, Cairo University, Giza, Egypt.

**Genetic Material:** Five Egyptian cultivars of citrus (Table 1) were used in this study as parents of interspecific crosses. These parents belong to five different species of the genus *citrus*. Eight interspecific hybrids were made and two parents were selfed.

**Developing Hybrid and Selfed Seeds:** The above mentioned citrus parents were used to obtain eight interspecific hybrids. The two parents SO and VL were used as females (seed parents) to be pollinated by the other four parents so two groups of interspecific hybrids were produced, each group shared one common seed parent (Table 2).

Selfing was also done in VL and SO parents. The self pollination was made by bagging the inflorescences after selecting good flowers just before opening without emasculating. The artificial hybridization work was carried out during the period from 15-30 March of both 2005 and 2006 years. Six random homogenous trees of the mother parental cultivars (Volkamer lemon and Sour orange) were used (each two trees were considered as one replicate). The completely randomized design was used. A sufficient number of the inflorescences selected on each tree of the mother parents were used. Three hundred flowers were selected on the female parents in each replicate prior to opening, emasculated and bagged. Unselected (unemasculated) blooms from the surrounding area were removed to improve setting of fruits. Flowers to provide pollen were collected from male parents just before opening, petals and pistil were removed, and anthers were allowed to dehisce for one day at room temperature. Pollen grains were applied with a brush on the stigmas of the emasculated flowers, when the stigma was fully receptive with sticky secretion. Paper bags were placed soon after pollination and removed approximately two weeks later when fruit set has been accomplished.

**Evaluation of Fruit Characters:** The hybrids and selfed fruits on the female parent Sour orange were harvested in the 1st nine days of February, whilst those on Volkamer lemon were harvested in the last two weeks of January of 2006 and 2007 years. The fruits were checked to be mature and free of any insects or diseases. The following traits were recorded on the fruits harvested from mother plants during the two studied seasons: (i) the period from pollination to fruit maturity (in days),(ii) the fruit set %, (iii) number of seeds per mature fruit and (iv) number of seeds per gram.

**Testing Seed Germination and Embryos:** Three replicates each of 120 seeds of each of the eight hybrids and the two selfed parents produced previously, have been sown randomly at the last week of March in 2006 and 2007 seasons in three germination beds (1X3 m) that included a mixture of 1 peat moss: 3 sand under the green house conditions. The extracted seeds from fruits of each hybrid and selfed parent produced were sown in individual rows by strewing the seeds in each row and giving a regular irrigation during the whole experiment period. The completely randomized design was also used. After completing the germination i.e., after 60 days from sowing, the following characteristics have been measured for each hybrid and selfed genotype: (1) germination %, (2) polyembryonic seeds % and (3) number of germinated embryos per seed.

**Morphological Characterization of Seedling:** The hybrid and selfed (sexual) seedlings could be distinguished by selecting the variant seedling in leaf size and shape (according to the methods of Rifaat [13] among the seedlings produced by each seed, while the vegetative seedlings (asexual) had been removed for saving good growth conditions for the sexual seedlings. So the seedlings which differ in leaf size and shape, and differ in growth (sexual plants), were selected, but the nucellaur (asexual) seedlings that were homogenous in their growth were removed. This step was made after one month from completing the germination. Two months after completing the germination, the following characteristics were measured on the seedlings for all hybrids and selfed parents, except VLLI and VLBM hybrids, where seeds could not germinate: (1) seedling height (cm), (2) number of shoots on the stem of seedling (shoots/seedling), (3) number of leaves per seedling (leaves/seedling), (4) diameter of the seedling stem (cm) (stem thickness), (5) leaf area ($cm^2$), (6) number of roots per seedling, (7) depth of the root system (cm), (8) average length of the roots (cm), (9) width of the root system (cm) and (10) fresh weight of the seedling (g) without any injuries during its life.

**Assessment of Leaf Endogenous Hormones:** Leaf hormonal analyses had been carried out for explaining the relationship between the endogenous hormones of the hybrids and selfed parents produced and their seedlings growth behaviour. These analyses were made on samples of about 2 grams of mature leaves; as two leaves were collected from the middle part of each seedling. The hormonal estimation was made as follows: (1) total indoles (µg/g f.w.) were estimated according to the method of Larson *et al.* [11], (2) total cytokinins (µg/g f.w.) were measured according to the methods of A.O.A.C. (1980) and (3) total gibberellins and Abscisic acid (µg/g f.w.) were estimated according to the methods described by El-Ezaby [7].

Data of the completely randomized design were statistically analyzed and means were compared by using LSD test at 0.05 level of probability according to Snedecor and Cochran [16].

## RESULTS AND DISCUSSION

**Characteristics of Fruits Following Artificial Pollination:** Means of fruit maturity, fruit set percentage, seeds per fruit and seeds per gram in 2005/2006 and 2006/2007 seasons for the eight interspecific crosses of citrus and two selfed parents are presented in Table (3). Results indicated that number of days from pollination to fruit maturity was generally greater in the selfed parents than that in the hybrids. In other words, the interspecific hybrids showed earlier fruit maturity than their selfed seed parents. The selfed VL parent exhibited the latest fruit maturity (310 and 325 days) while the hybrid SOLI showed the earliest maturity (285 and 300 days) in the first and second season, respectively. The range of fruit maturity was 25 days in both seasons among all studied hybrids and selfed parents (Table 3). It is interesting to mention that the hybrids shared a common seed parent generally followed their selfed seed parent in fruit maturity; those share SO parent were on average earlier in maturity than those share VL parent.

Percentage of fruit set ranged from 0.95 to 4.3% in 2005/2006 season and from 0.82 to 4.0 % in 2006/2007 season (Table 3). The highest mean percentage of fruit set was exhibited by the hybrid SOVO while the lowest one was shown by the hybrid VLLI in both seasons. The selfed seed parent of low fruit set percentage (VL) gave hybrids with low average fruit set percentage, while the seed parent with higher percentage (SO) gave hybrids with higher average fruit set percentage in both seasons.

Number of seeds per fruit varied from 24.2 to 54.6 in 2005/2006 season and from 21.4 to 51.8 in 2006/2007 season (Table 3). The highest number of seeds/fruit was shown by SOMB hybrid while the lowest number was exhibited by VLVO in both seasons. Hybrids shared the seed parent SO showed in average higher number of seeds/fruit than the hybrids sharing the seed parent VL in both seasons.

Mean number of seeds/gram differed from 9.8 to 20.7 and from 10.3 to 23.1 in 2005/2006 and 2006/2007 season, respectively (Table 3). The lowest number of seeds/gram was exhibited by the hybrid SOVO, while the highest number was shown by the hybrid VLSO in both seasons. In general, selfed seed parent of lower number of seeds/gram (SO) resulted in hybrids of lower average number of seeds in both seasons.

It is worthy to note that, hybridization among *citrus* species resulted in some new hybrids that showed significant earliness in fruit maturity (SOLI, SOVL, VLLI, VLBM and VLSO), higher fruit set percentage (SOVO) and higher number of seeds/gram than their corresponding seed parents in both seasons. In general, hybrids followed their corresponding selfed parents in the studied fruit characters. The hybrids shared Sour orange as a seed parent showed on average lower number of days to fruit maturity, higher fruit set percentage, higher number of seeds/fruit and lower number of seeds/gram than hybrids shared VL as a seed parent. Interspecific hybrids were generally earlier in fruit maturity than their selfed female parents; with the earliest hybrids developed by using Sour orange as a seed parent. The hybrid SOVO showed

maximum fruit set, indicating good cross compatibility between SO and VO, while the opposite was true for the hybrid VLLI, where cross compatibility could be low between VL and LI.

**Hybrid Seed Germination and Embryo Traits:** Out of eight interspecific hybrids of citrus, six were able to germinate and two were not able, viz. VLLI and VLBM; these two hybrids showed zero % seed germination, zero % polyembryonic seeds and zero embryos/seed (Table 4). This suggests that neither sex nor nucellar embryos were developed in these two hybrids and embryo abortion might be happened. These two hybrids have the seed parent Volkamer lemon as a common female. Moreover, the hybrid VLSO and the selfed seed parent VL showed very low means of seed germination and number of embryos /seed and zero % polyembryonic seeds in both seasons.

All hybrids shared Sour orange as a common seed parent, except SOVL showed high estimates of seed germination %, polyembryonic seed % and embryos /seed; with the highest estimates shown by the hybrid SOLI. It showed 16.0 and 14.5% seed germination, 57.0 and 52.0% polyembryonic seed and 3.2 and 2.9 embryos/seed in 2005/2006 and 2006/2007 seasons, respectively (Table 4). It was followed by the hybrids SOBM and SOVO for the three studied germination and embryo traits.

Significant superiority were shown by the hybrids SOLI and SOMB for seed germination % and polyembryonic seed % in both seasons over their seed parent Sour orange; this could be attributed to good cross compatibility between SO and each of LI and BM as well as to hybrid vigor. Hybrids made on the seed parent of Sour orange were on average much higher than hybrids made on Volkamer lemon seed parent for seed germination, polyembryonic seed and embryos/seed in both seasons. It seems that the seed parent had a great effect on the fruit, seed and embryo traits of the hybrid resulted from it. The previous results are in favor of utilizing the Sour orange cultivar as a seed parent for giving citrus interspecific hybrids with better fruit, seed and embryo traits, provided that there are an effective method of distinguishing between sexual and nucellar (asexual) embryos.

It is worth noting that results on fruits, seeds and embryo traits presented in Tables 3 and 4 for the two seasons were in the same trend, but estimates were little higher in 2005/2006 season than in 2006/2007 season for all traits, except for days to fruit maturity, where the opposite was true. This could be attributed to that the trees in the second season seemed to be in the off-year season, and consequently they had not adequate nutrition storage for feeding the flower formation and growth, which may be resulted in decrease in fruit set and number of seeds /fruit and increase in number of seeds/gram (light weight of seed) and weak formation of the strong tissues of the seed (cotyledons and nucellar tissues) which feed embryos during the growth. In this respect, Staford [20] reported an increase in number of seeds/fruit of the mandarin cultivar Ellendal when cross pollinated with pollen of Valencia orange and mandarin Emperor and Imperial. Rifaat [13] reported that number of seeds/fruit was affected greatly by the pollinators; it was decreased when Clementine or the Grape fruit cultivar Duncan was pollinated by the Sweet orange or for the reciprocal cross. He attributed that to the partial incompatibility between the two previous species. Ibrahim and Hagag [9] stated that most cultivars of citrus included variant degrees of sterility, either in the pollen or in ovules and some self or cross sexual incompatibilities were found in Valencia orange.

**Morphological Characters of Hybrid Seedlings:** Means of seedling characteristics of the six hybrids that were succeeded in germination along with their selfed seed parents are presented in Tables (5 and 6). Results of stem characters of the seedling (Table 5) showed that the hybrid VLSO was the most vigorous for seedling height (54.4 and 46.7 cm), number of shoots/seedling (5.0 and 5.4), number of leaves/seedling (23.0 and 22.8) and stem thickness (0.30 and 0.24 cm) in 2005/2006 and 2006/2007 seasons, respectively.

The superiority of this cross (VLSO) in seedling shoot characters might be attributed to the lowest percentage of seed germination and to non-existence of nucellar embryos (Table 4) which permitted good environmental conditions to the few sexual embryos to germinate and develop into seedlings with better characteristics (Table 5). The largest leaf area was shown by the hybrid SOLI followed by SOBM. In the second rank came the hybrid VLVO for seedling height and SOLI for shoots/seedling, leaves/seedling and stem thickness in both seasons.

It is interesting to mention that in contrast to fruit and seed characteristics (Tables 3 and 4) the average of all studied seedling characters, except leaf area was higher for hybrids share the seed parent Volkamer lemon (VL) than that for hybrids share the seed parent Sour orange (SO) in both studied seasons.

Results on root characteristics of the seedlings (Table 6) indicated also superiority of the hybrid VLSO for root system depth and root length, VLVO for root system width and SOVL for number of roots/seedling. In the second rank came VLSO for root system width, VLVO for root system depth and root length and SOLI for number of roots/seedling. It was noticed that VLSO and VLVO hybrids had the highest means of root system depth, root length and root system width, but

had the lowest number of roots/seedling. On the contrary, the hybrids SOVL and SOLI had the highest number of roots/seedling, but the lowest mean of root system depth and width and root length. This indicates to the negative relationship between number of roots/seedling and root length or depth.

In general, hybrids made on Volkamer lemon female showed higher values than hybrids made on Sour orange female for all studied seedling shoot and root characteristics, except number of roots/seedling, where the opposite was true.

Mean weight of fresh seedling (shoots and roots) of the hybrid VLSO was the highest (19.0 and 19.6 g) while that for the seed parent VL was the lowest (8.0 and 7.7 g) in 2005/2006 and 2006/2007 seasons, respectively (Table 6). The hybrid VLVO came in the second rank for fresh seedling weight.

It is worthy to note that hybrid vigour, expressed as a significant superiority of the hybrid over its respective seed parent was exhibited in the crosses SOLI and SOVL for earliness of fruit maturity, percent of polyembryonic seed, number of shoots and leaves/seedling and VLVO and VLSO for seedling fresh weight, seedling height, stem thickness, number of leaves and shoots/seedling, root system depth and width, root length and number of roots/seedling. In this respect, some reports indicated the existence of hybrid vigour in many intrespescific hybrids among citrus species for fruit, seed and seedling characteristics [17].

**Seedling Leaf Hormonal Status:** Seedling leaf endogenous hormones content of the developed citrus interspecific hybrids and their selfed female parents are presented in Table (7). Contents ranged from 4.8 and 5.3 µg/g f.w. (SOVO) to 31.50 (VLVO) and 35.90 µg/g f.w. (VLSO) for total cytokinins, from 90.01 and 90.91 µg/g f.w. (VLVO) to 145.51 and 150.30 µg/g f.w. (SOVL) for total gibberellins, from 2.15 and 2.12 µg /g f.w. (VLSO) to 5.56 and 5.31 µg/g f.w. (SOBM) for ABA and from 136.7 (VLSO) and 138.3 µg/g f.w. (SOVL) to 271.8 and 259.3 µg/g f.w. (SOBM) µg/g f.w. for total indoles in 2005/2006 and 2006/2007 seasons, respectively. In general, total gibberellins and total indoles showed the highest contents, while ABA exhibited the lowest in seedling leaves. The hybrids showed on average higher contents of endogenous hormones than their respective selfed female (seed) parents; this was more pronounced for cytokinins and indoles. There was a negative relationship between ABA and each of cytokinins and gibberellins and a positive association between ABA and total indoles and between cytokinins and gibberellins. The hybrid VLSO showed the highest leaf cytokinins and gibberellins and the lowest ABA and total indoles. The hybrid VLVO had also high leaf content of total cytokinins but had the lowest contents of gibberellins. The hybrid SOVL showed the highest leaf content of gibberellins, but ranked the second highest in ABA. The hybrid SOBM exhibited the highest contents of ABA and total indoles. The lowest contents of cytokinins were shown by the hybrid SOVO. In this respect, several investigators indicated that orange trees on vigorous rootstocks such as Volkamer lemon had higher cytokinin levels than those on the less vigorous rootstock such as Troyer [14,10]. Moreover, results of Bertling and Lovatt [3] suggested that a vigorous rootstock is characterized by a relatively greater concentration of IAA and lower concentration of ABA; a dwarfing one is characterized by a reverse trend. Awad [2] found that ABA concentrations were very low in the Volkamer lemon rootstock whereas they were as much as 5.5 and 14.5 fold greater in Sour orange and Troyer citrange; the highest contents were shown by Troyer citrange. Her results also suggested that a vigorous citrus rootstock (i.e. Volkamer lemon) is characterized by a relatively greater concentrations of IAA and $GA_3$ than the less vigorous one (Troyer citrange); Sour orange, as a semi-vigorous rootstock manifests the intermediate levels. Results of the present study are in agreement with the previous reports in that Volkamer lemon (selfed seed parent) exhibited higher contents of indoles than the selfed parent Sour orange. The hybrids derived from Volkamer lemon seed parent had higher contents of endogenous cytokinins and lower contents of ABA than those hybrids derived from Sour orange parent.

It is interesting to mention that the most vigours hybrids developed in this study, expressed in seedling fresh weight such as VLSO and VLVO manifested higher contents of leaf endogenous total cytokinins (about 4 to 5 fold greater) than the least vigorous hybrids, namely SOVO and SOBM. The hybrid SOBM showed about 2.5 and 2 fold greater contents of ABA and indoles than VLSO, respectively. The poor vigour of hybrid SOBM might be attributed to the high level of inhibitors such as ABA in the shoot. The most vigorous hybrid (VLSO) exhibited the highest leaf endogenous cytokinins and gibberellins and the lowest ABA, and therefore is predicted to be a good rootstock in citrus plantings in Egypt. The hybrid VLSO combined the greatest number of desirable morphological traits of its seedlings, besides high contents of leaf endogenous cytokinins and gibberellins and low content of ABA and its seed contain nucellar embryos; the hybrid VLVO ranks second in this regard. Moreover, the hybrid SOLI combined traits of early fruit maturity good seed germination and large number of nucellar embryos; the hybrid SOBM rankes second in this regard. All these hybrids are therefore of good potential as improved rootstocks and could prove superiority over Volkamer lemon and Sour orange.

**Table 1:** The five citrus parents used in this study.

| Designation | Citrus group | Parent cultivar and botanical name |
|---|---|---|
| SO | Sour orange | Sour Orange (*Citrus aurantium* L.) |
| VL | Lemons | Volkamer Lemon (*Citrus volkamariana* L.) |
| VO | Sweet Orange | Valencia Orange (*Citrus Sinensis* L. Osbeck) |
| LI | Limes | Lime (*Citrus aurantifolia* L.) |
| BM | Mandarins | Balady Mandarin (*Citrus deliciosa* Tenore) |

**Table 2:** Interspecific hybrids of citrus made in this study.

| No. | Hybrid designation | Parents | | Interspecific hybrid name |
|---|---|---|---|---|
| | | Female | Male | |
| 1 | SOVL | SO | VL | *C. aurantium* X *C. volkamariana* |
| 2 | SOVO | SO | VO | *C. aurantium* X *C. Sinensis* |
| 3 | SOLI | SO | LI | *C. aurantium* X *C. aurantifolia* |
| 4 | SOBM | SO | BM | *C. aurantium* X *C. deliciosa* |
| 5 | VLSO | VL | SO | *C. volkamariana* X *C. aurantium* |
| 6 | VLVO | VL | VO | *C. volkamariana* X *C. Sinensis* |
| 7 | VLLI | VL | LI | *C. volkamariana* X *C. aurantifolia* |
| 8 | VLBM | VL | BM | *C. volkamariana* X C. deliciosa |

**Table 3:** Means of studied characteristics of fruits resulted after interspecific hybridization and selfing in citrus at 2005/2006 and 2006/2007 seasons.

| Hybrids and selfed females | 2005/2006 season | | | | 2006/2007 season | | | |
|---|---|---|---|---|---|---|---|---|
| | Days from pollination to fruit maturity | Fruit Set % | Seeds / fruit | Seeds / gram | Days from pollination to fruit maturity | Fruit Set % | Seeds / fruit | Seeds / gram |
| Hybrids: | | | | | | | | |
| SOVO | 295 | 4.30 | 52.3 | 10.3 | 310 | 4.00 | 48.6 | 9.8 |
| SOVL | 290 | 2.30 | 50.2 | 11.2 | 306 | 2.40 | 46.2 | 10.0 |
| SOLI | 285 | 1.20 | 50.3 | 10.5 | 300 | 1.10 | 45.3 | 9.9 |
| SOBM | 295 | 3.20 | 54.6 | 11.4 | 312 | 2.95 | 51.8 | 10.2 |
| VLVO | 305 | 2.90 | 24.2 | 19.1 | 320 | 2.63 | 21.4 | 17.5 |
| VLLI | 295 | 0.95 | 24.5 | 20.3 | 308 | 0.82 | 22.2 | 18.6 |
| VLBM | 300 | 2.50 | 24.6 | 19.0 | 316 | 2.21 | 23.2 | 17.5 |
| VLSO | 290 | 2.00 | 25.4 | 23.1 | 305 | 2.12 | 24.3 | 20.7 |
| Average | 294.3 | 2.40 | 38.2 | 15.6 | 308.8 | 2.2 | 35.3 | 14.2 |
| Selfed females: | | | | | | | | |
| SO | 300 | 350 | 53.60 | 11.20 | 317 | 332 | 50.00 | 10.10 |
| VL | 310 | 1.70 | 31.30 | 18.20 | 325 | 1.92 | 26.30 | 17.20 |
| LSD (0.05) | 8.69 | 0.90 | 16.36 | 4.48 | 8.10 | 0.81 | 14.91 | 4.05 |

**Table 4:** Means of studied characteristics of seeds resulted from interspecific hybridization and selfing in citrus at 2005/2006 and 2006/2007 seasons.

| Hybrids and selfed females | 2005/2006 season | | | 2006/2007 season | | |
|---|---|---|---|---|---|---|
| | Seed Germination % | Polyembryonic Seeds % | Germinated Embryos / seed | Seed Germination % | Polyembryonic Seeds % | Germinated Embryos / seed |
| Hybrids: | | | | | | |
| SOVO | 7.4 | 15 | 2.1 | 6.3 | 13.2 | 1.9 |
| SOVL | 1.2 | 0.0 | 0.3 | 0.9 | 0.0 | 0.3 |
| SOLI | 16.0 | 57 | 3.2 | 14.5 | 52 | 2.9 |
| SOBM | 11.2 | 33 | 2.5 | 9.8 | 30 | 2.1 |
| VLVO | 4.3 | 16 | 2.5 | 3.9 | 14 | 2.3 |
| VLLI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| VLBM | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| VLSO | 1.1 | 0.0 | 0.3 | 1.0 | 0.0 | 0.3 |
| Average | 5.15 | 15.12 | 1.05 | 4.57 | 13.65 | 1.22 |
| Selfed females: | | | | | | |
| SO | 65 | 13.4 | 2.2 | 5.2 | 10.5 | 1.9 |
| VL | 1.1 | 0.0 | 0.3 | 1.1 | 0.0 | 0.3 |
| LSD (0.05) | 2.90 | 14.31 | 1.96 | 2.61 | 12.96 | 1.61 |

**Table 5:** Means of studied seedling shoot growth characteristics of some citrus interspecific hybrids compared with selfed female parents in 2005/2006 and 2006/2007 seasons.

| Hybrids and selfed females | Seedling height (cm) | Shoots/ seedling | Leaves/ seedling | Stem thickness (cm) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|
| | | | 2005/2006 season | | |
| Hybrids: | | | | | |
| SOVO | 19.2 | 0.0 | 11.2 | 0.23 | 8.2 |
| SOVL | 15.5 | 1.2 | 17.3 | 0.23 | 9.2 |
| SOLI | 15.3 | 4.1 | 20.4 | 0.29 | 0.8 |
| SOBM | 25.2 | 2.2 | 16.5 | 0.22 | 9.5 |
| VLVO | 28.2 | 2.2 | 14.1 | 0.27 | 7.8 |
| VLSO | 45.4 | 5.0 | 23.0 | 0.30 | 7.5 |
| Average | 24.80 | 2.45 | 15.41 | 0.25 | 8.8 |
| Selfed Females: | | | | | |
| SO | 29.3 | 1.0 | 12.2 | 0.25 | 9.5 |
| VL | 16.8 | 3.0 | 12.0 | 0.24 | 7.6 |
| LSD (0.05) | 10.16 | 1.89 | 6.11 | 0.02 | 0.19 |
| | | | 2006/2007 season | | |
| Hybrids: | | | | | |
| SOVO | 20.0 | 0.3 | 10.5 | 0.20 | 7.9 |
| SOVL | 15.8 | 1.4 | 16.4 | 0.21 | 9.0 |
| SOLI | 15.5 | 3.9 | 21.0 | 0.27 | 9.8 |
| SOBM | 27.3 | 2.4 | 15.9 | 0.20 | 9.1 |
| VLVO | 29.5 | 2.0 | 14.0 | 0.27 | 7.5 |

**Table 5:** Continue

| | | | | | |
|---|---|---|---|---|---|
| VLSO | 46.7 | 5.4 | 22.8 | 0.32 | 7.0 |
| Average | 25.80 | 2.56 | 16.76 | 0.24 | 8.3 |
| Selfed Females: | | | | | |
| SO | 30.80 | 1.00 | 11.80 | 0.24 | 9.0 |
| VL | 18.20 | 3.20 | 12.60 | 0.23 | 7.3 |
| LSD (0.05) | 9.99 | 1.81 | 5.99 | 0.02 | 0.19 |

**Table 6:** Means of studied seedling root characteristics and fresh weight of whole seedling of some citrus interspecific hybrids and their selfed female parents in 2005/2006 and 2006/2007 seasons.

| Hybrids and selfed females | Depth of root system (cm) | No. of roots/ seedling | Root length (cm) | Root system width (cm) | Seedling fresh weight (g) |
|---|---|---|---|---|---|
| Hybrids: | | | 2005/2006 season | | |
| SOVO | 14.80 | 19.6 | 5.50 | 4.78 | 11.0 |
| SOVL | 8.20 | 32.6 | 2.85 | 5.92 | 13.0 |
| SOLI | 7.80 | 24.7 | 4.00 | 4.92 | 14.5 |
| SOBM | 5.90 | 13.2 | 2.22 | 3.98 | 10.0 |
| VLVO | 27.30 | 20.7 | 6.40 | 8.80 | 17.7 |
| VLSO | 28.60 | 18.4 | 6.70 | 7.40 | 19.0 |
| Average | 15.43 | 21.53 | 4.61 | 5.96 | 14.20 |
| Selfed females: | | | | | |
| SO | 18.50 | 22.40 | 4.25 | 7.30 | 13.20 |
| VL | 7.90 | 9.00 | 3.80 | 4.50 | 8.00 |
| LSD (0.05) | 6.13 | 8.01 | 1.70 | 2.00 | 2.95 |
| | | | 2006/2007 season | | |
| Hybrids: | | | | | |
| SOVO | 15.2 | 18.7 | 5.8 | 5.2 | 11.4 |
| SOVL | 8.4 | 30.5 | 3.0 | 6.3 | 13.6 |
| SOLI | 7.6 | 23.6 | 4.4 | 5.4 | 15.0 |
| SOBM | 6.2 | 12.9 | 2.5 | 4.2 | 10.5 |
| VLVO | 28.2 | 20.8 | 6.7 | 9.3 | 18.0 |
| VLSO | 29.3 | 18.2 | 7.2 | 7.8 | 19.6 |
| Average | 15.81 | 20.78 | 4.93 | 6.36 | 14.68 |
| Selfed females: | | | | | |
| SO | 17.9 | 22.5 | 4.5 | 7.8 | 13.8 |
| VL | 8.2 | 8.9 | 4.2 | 4.8 | 7.7 |
| LSD (0.05) | 6.05 | 7.98 | 1.68 | 1.99 | 2.81 |

**Table 7:** Seedling leaf endogenous hormone contents of some citrus interspecific hybrids and their selfed female parents in 2005/2006 and 2006/2007 seasons.

| Hybrids and selfed females | Total cytokinins (µg /g f.w.) | Total Gibberellins (µg/g f.w.) | Abscisic Acid (µg/g f.w.) | Total indoles (µg /g f.w.) |
|---|---|---|---|---|
| Hybrids: | | 2005 / 2006 season | | |
| SOVO | 4.8 | 100.67 | 3.43 | 165.6 |
| SOVL | 20.30 | 145.51 | 3.97 | 140.8 |
| SOLI | 19.70 | 103.71 | 3.48 | 149.7 |
| SOBM | 8.00 | 115.80 | 5.56 | 271.8 |
| VLVO | 31.50 | 90.01 | 3.47 | 149.6 |
| VLSO | 31.00 | 135.37 | 2.15 | 136.7 |
| Average | 19.20 | 115.17 | 3.67 | 169.0 |
| Selfed females: | | | | |
| SO | 8.20 | 140.19 | 2.24 | 148.0 |
| VL | 5.00 | 100.51 | 3.46 | 136.2 |
| | | 2006 / 2007 season | | |
| Hybrids: | | | | |
| SOVO | 5.30 | 103.10 | 3.02 | 184.2 |
| SOVL | 21.40 | 150.30 | 3.90 | 138.3 |
| SOLI | 20.60 | 107.50 | 3.4 | 140.5 |
| SOBM | 8.60 | 110.30 | 5.31 | 259.3 |
| VLVO | 34.90 | 90.91 | 3.05 | 146.0 |
| VLSO | 35.90 | 140.10 | 2.12 | 140.5 |
| Average | 17.50 | 117.03 | 3.466 | 168.1 |
| Selfed females: | | | | |
| SO | 8.80 | 146.09 | 2.17 | 141.2 |
| VL | 6.10 | 98.11 | 3.4 | 135.2 |

It could be concluded that some interspecific citrus hybrids developed in the present study combined several desirable traits and could maximize genetic diversity of citrus rootstocks. Hybrids with the most potential can be efficienlly and rapidly identified for subsequent field evaluation research which should result in the release of improved hybrid-derived rootstocks.

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