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Efficient propagation of citrus rootstocks by stem cuttings

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ABSTRACT

A simple multicomponent system is described that is effective for rapid propagation of a diversity of citrus rootstock genotypes by single node cuttings, including new hybrids and cultivars that are most commonly used as rootstocks. Efficiency of this system for rooting shoot explants of six important rootstock cultivars, Swingle, Cleopatra, US-802, US-812, US-897, and US-942 is compared in a repeated study. Many of the cuttings began to grow within 2 weeks after planting into potting mix. Growth for the resultant plants of different genotypes was compared through 20 weeks, and significant differences were observed. US-802 had the highest success in establishing growing plants at 8 weeks, with 82–91% of single node cuttings successfully rooted and growing, while Cleopatra was the least efficient with a 42–45% recovery of growing plants. Comparison of plant weight for cuttings and seedlings indicated that 12 week old nucellar and true-to-type seedlings are similar in size to 12–16 week old cuttings of the same cultivars. Plants of all rootstocks, whether cuttings or seedling-propagated, were observed to approach a dry weight ratio of 80% shoot and 20% root. Pronounced differences in the number and length of roots were identified among the rootstocks, indicating large differences in root structure that might be important in relation to eventual field plant health and growth. The commercial utility of the described methods is discussed.

1. Introduction

The citrus rootstock is regarded as a critical component of successful citrus production in the modern era (Bowman et al., 2016a, Bowman et al., 2016b; Castle et al., 2011, 2015a, 2015b; McCollum and Bowman, 2017). Although commercial citrus rootstocks historically have been propagated almost exclusively by apomictic seed, there are four important reasons why a simple and efficient cutting propagation system for citrus rootstocks is now of significant value: 1) cutting propagation allows replicated testing of rootstock germplasm many years before new hybrids first produce seed, 2) cutting propagation allows the use of rootstock germplasm which never or infrequently produces nucellar seed, 3) cutting propagation allows large-scale propagation of rootstocks for which inadequate amounts of nucellar seed is available, and 4) cutting propagation from disease-free source material avoids the potential risk of disease transmission by the seed.

As new disease and pest problems have spread, and severe economic stress has followed, breeding programs over the last 20 years have increasingly been expected by the industries they serve to develop and release new improved rootstock selections at a faster pace than ever before. Waiting 5–15 years for new citrus hybrids to begin fruiting before replicated testing can begin is incompatible with this need for accelerated rootstock development. Propagation of new hybrid citrus rootstocks by cuttings allows replicated greenhouse and field testing for new rootstock hybrids to begin within a year after the original hybrid seedling begins to grow. This is many years before those hybrids will reach sexual maturity and produce their first seeds. Five new rootstocks released by the USDA breeding program in 2014 for improved tolerance to huanglongbing (HLB) disease (Bowman and McCollum, 2015), were tested and released much more rapidly than rootstocks in the past because they were entered into replicated field testing by the use of cuttings many years before the hybrid seedlings produced their first fruit.

Large portions of the citrus germplasm do not produce nucellar seed, or produce zygotic seedlings at a high frequency that is unacceptable for efficient uniform commercial propagation (Soost and Roose, 1996; Xiang and Roose, 1988). For citrus germplasm with a high level of zygotic embryony, some form of vegetative propagation like cuttings, is necessary to enable potential use as a rootstock. Even for rootstocks which produce nucellar seed and for which there are mature sources, it takes many years to increase the number and size of fruiting trees, and often there can be a severe seed shortage for new rootstocks which are in high demand. This has been the case for the new rootstocks US-802, US-812, US-897, and US-942 in Florida (Bowman et al., 2016a, 2016b; Bowman and Rouse, 2006). Demand for nursery trees on

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the rootstocks US-897 and US-942, in particular, has far exceeded the available seed supply because of reported HLB tolerance for these clones (Albrecht and Bowman, 2011, 2012; Bowman and Albrecht, 2015).

Finally, concerns about seed transmission of HLB and other diseases have interfered with seed movement between different citrus growing regions and countries (Albrecht and Bowman, 2009; Hilf, 2011). Similarly, problems with transmission of citrus canker on the seed of some rootstocks that are highly susceptible, has resulted in major nursery losses. Even when seed of a rootstock is available in one region, it often is prohibited or strongly discouraged from transport and use in another region because of phytosanitary concerns. Clean shoot material of rootstock selections that can be used for cuttings is often available from regional budwood programs, and avoids the risk of disease transmission in or on the seed.

Cutting propagation of citrus has been previously described, but in most cases, the described methods are not suitable for large-scale commercial use because they make use of relatively large amounts of tissue per explant (Bhusal et al., 2001; Mourão Filho et al., 2009; Pio et al., 2006), use a specialized rooting chamber and a long-duration rooting period (Mourão Filho et al., 2009; Pio et al., 2006), or were noted to be ineffective with many genotypes (Bhusal et al., 2001; Sagee et al., 1992; Villas-Boas et al., 1987). Generally, these studies did not define simple, rapid, and broadly applicable methods that would be suitable for both small scale use in rootstock breeding programs, and large scale use in a commercial nursery.

Methods for propagation of new hybrid citrus rootstocks by cuttings have been refined over several years in the USDA citrus rootstock breeding program in Ft. Pierce, Florida, to fill this need. In this paper, we describe the use of the simple cutting system that has been developed, to compare efficiency for propagation of six commercially important citrus rootstocks (four of them new hybrids for which there have been severe shortages of seed to satisfy the commercial demand), document growth and development of those cuttings through 5 months, and compare plants produced by stem cuttings with those produced from seed.

2. Materials and methods

Two experiments were conducted in the greenhouse at USHRL (Ft. Pierce, Florida, USA) in summer 2016. The greenhouse was unheated during the course of these experiments, and made use of ventilation fans and evaporative cooling pads to moderate temperature during the day. Average daily high and low temperatures in the greenhouse during the course of these experiments were 34.8 °C and 27.1 °C, respectively. Shade cloth in the house was closed from 9 am to 6 pm daily during the first six weeks after the cuttings were placed into soil. Maximum photosynthetic photon flux (PPF) in the greenhouse at midday when the shade was closed was 180 μmol s^{-1} m^{-2}, while maximum PPF in the greenhouse at midday when the shade was open (beginning the seventh week for each experiment) was 1070 μmol s^{-1} m^{-2}. The other details of methods used for the two experiments are as described below.

2.1. Experiment 1

Six commercially important citrus rootstocks were used, Swingle citrumelo (*Citrus paradisi* Macf. × *Poncirus trifoliata* [L.] Raf.) and Cleopatra mandarin (*C. reticulata* L. Blanco), which have had long-term commercial use worldwide, and US-802 ('Siamese' pummelo [*C. grandis* Osbeck] × trifoliate orange [*P. trifoliata*], US-812 ('Sunki' mandarin [*C. reticulata*] × 'Benecke' trifoliate orange [*P. trifoliata*]), US-897 ('Cleopatra' mandarin × 'Flying Dragon' trifoliate orange [*P. trifoliata*]), and US-942 ('Sunki' mandarin × 'Flying Dragon' trifoliate orange), four new hybrid rootstocks released by USDA that have gained major commercial importance in Florida (Bowman et al., 2016a, 2016b). One to two year-old plants derived from nucellar seed of each cultivar and maintained in the USHRL greenhouses, were used as a source of shoots for the cuttings. Source plants in the greenhouse received alternating water irrigation and liquid fertilizer application, and periodic insecticide applications, as needed.

2.1.1. Making the cuttings

Single node cuttings (average length 2.6 cm) were taken in April from sections of 2–5 month-old branches of the greenhouse source trees, leaving the leaf attached to each node, but trimming to reduce the leaf size to about 20–30% its original area. In this paper, this original stem piece is referred to as the explant, to distinguish it from the shoot and roots which subsequently grow out of the cutting. The basal end of each cutting explant was dipped in *a* commercial rooting powder (Hormodin 2, E.C. Geiger, Inc., Harleysville, PA, USA) containing 0.3% IBA, and immediately inserted into pre-moistened soilless potting mix (Pro Mix BX; Premier Horticulture, Inc., Quakertown, PA, USA), using racks of 3.8 cm x 21 cm cone cells (Cone-tainers; Stuewe and Sons, Tangent, OR, USA). One single-node cutting was inserted into each cone cell deeply enough to secure the cutting, but allowing the leaf base and node to remain above the soil surface. Four replicates of 98 cuttings were made for each of the six rootstock cultivars.

2.1.2. Care of cuttings

The racks of cones were placed on a mist bench, and arranged in a completely randomized design. Misting was applied at 40 cm above the height of the cuttings by brass nozzles (Flora-mist nozzle, Growerssolution.com, Cookeville, TN, USA), and was switched on periodically by an automated system controlled by both a wet leaf sensor and timer (Mist-a-matic controller, Growerssolution.com). Typically, the misting ran about 6 s every 5–10 min during full daylight, but ran rarely at night.

During the fifth week, the plants received a liquid fertilizer application of water-soluble fertilizer (20N-10P–20 K; Peters Professional, The Scotts Company, Marysville, OH, USA) applied with a proportioner through a hose and breaker at a rate of 400 mg per liter N. The misting was discontinued after 6 weeks and the plants received another liquid fertilizer application which included chelated iron (Sequestrene 138 Fe; Ciba-Geigy Corp., Greensboro, N.C., USA). At the beginning of the seventh week, the shade cloth was left open continuously. Subsequently, plant care was the same as applied to normal citrus greenhouse plants, with alternating water irrigation and liquid fertilizer application, and periodic insecticide applications, as needed.

2.1.3. Scoring and measurements

The individual explants were scored for presence or absence of shoot growth at 2 weeks and again at 8 weeks from when the cuttings were placed in potting mix. Explants were also scored for death at 8 weeks. Occasional explants that remained green but did not have shoots by 8 weeks were not scored as having growing shoots or dead, but were removed from further evaluation. Thirty random growing plants from each rootstock cultivar were chosen for further study, and six from each were destructively measured at 8 weeks, 12 weeks, 16 weeks, and 20 weeks. Measurements were made for shoot length and diameter, shoot fresh and dry weight, explant length and diameter, explant fresh and dry weight, total root length (8 weeks only) and root fresh and dry weight.

2.1.4. Root structure analysis

A more detailed analysis of root structure was conducted on another group of six random plants from each rootstock at 20 weeks. For this analysis, Image J (Schneider et al., 2012) and Assess 2.0 (Lakhdar Lamari; American Phytopathological Society image analysis software) were used to calculate length of individual adventitious roots and total root length, respectively. Other parameters measured were stem length, number of leaves, leaf area, and dry mass of leaves, stems and roots.

2.1.5. Seedlings for comparison

Seeds of the cultivars Cleopatra, US-802, and US-942 were planted in the same potting mix and the same cone cells on the neighboring bench and maintained without misting, but otherwise under the same conditions and fertilization as the cuttings. At 12 weeks from planting, six random true-to-type nucellar seedlings from each of these three cultivars were destructively measured in the same way as the cuttings described above.

2.1.6. Statistical analysis

Data for experiment 1 was analyzed using Statistica 10 software (Dell Statistica, Tulsa, OK, USA) for ANOVA and Tukey HSD test, as indicated.

2.2. Experiment 2

In July, another group of single node cuttings were prepared from the regrowth on the same nucellar seedling plants used in experiment 1. The same methods were used as described for experiment 1 (2.1.1–2.1.3), except that six replications of 49 cuttings were made for each of the rootstock cultivars and randomized on the bench. Data was collected on number of explants with growing shoots at 2 and 8 weeks and number of explants which had died at 8 weeks. Statistical analysis of the data from experiment 2 was as described for experiment 1 (2.1.6).

3. Results

3.1. Success of cuttings at 2–8 weeks

Shoot growth was apparent on 51% of all explants from experiment 1 at 2 weeks after the stem cuttings were inserted into the potting mix (Fig. 1) and on 27% of all explants from experiment 2 (Table 1). The rootstock that had the lowest number of explants with growing shoots at 2 weeks in experiment 1 (Cleopatra) was the rootstock with the highest number of growing shoots at that stage in experiment 2. By 8 weeks, US-802 and US-942 had the highest proportion of explants with growing shoots in both experiments (80–91%), with US-897 and US-942 having an equal percent of explants with growing shoots (81%) in experiment 2. The rootstock with the lowest proportion of growing shoots at 8 weeks in both experiments was Cleopatra, with 42 and 45 percent growing, respectively. The proportion of explants dead at 8 weeks indicated a similar difference among rootstocks, with Cleopatra having the most explant death, and US-802 having the least explant death across both experiments. Since misting was discontinued at 6 weeks and shade was removed at 7 weeks, most explants either had roots and developed shoots by 7–8 weeks, or they died. The average proportion of explants across all six rootstocks that developed growing shoots by 8 weeks ranged from 66% to 71% for experiment 1 and 2, respectively.

Table 1
Success of rootstock propagation by cuttings in experiments 1 (April) and 2 (July).

Rootstock	Experiment 1			Experiment 2		
	Percent growing @ 2 wks	Percent growing @ 8 wks	Percent dead @ 8 wks	Percent growing @ 2 wks	Percent growing @ 8 wks	Percent dead @ 8 wks
US-802	77	91 a	7 b	39 ab	82 a	9 b
US-942	64	80 ab	17 ab	27 a–c	81 a	16 b
Swingle	51	63 ab	36 ab	15 bc	67 ab	23 b
US-897	41	61 ab	33 ab	22 bc	81 a	13 b
US-812	37	57 ab	41 ab	8 c	71 ab	12 b
Cleopatra	36	42 b	54 a	53 a	45 b	50 a
P > f	0.0557	0.00612	0.00768	0.00122	0.0044	0.00027

Mean separations for significant ANOVA within columns were by Tukey HSD test at $P < 0.05$.
Means with the same letter in a column are not significantly different at $P < 0.05$

3.2. Cutting size at 8 weeks

In experiment 1, cuttings for the six rootstocks were healthy (Fig. 2), but differed significantly at 8 weeks in all measures of size, including shoot length, shoot and root fresh weight, explant diameter, and total root length (Table 2). At 8 weeks, Cleopatra cuttings were the smallest plants in all dimensions, while US-802 cuttings were the largest for many traits.

Fig. 1. Single node stem cuttings of US-802 rootstock from experiment 1, showing good shoot growth at 2 weeks of age.

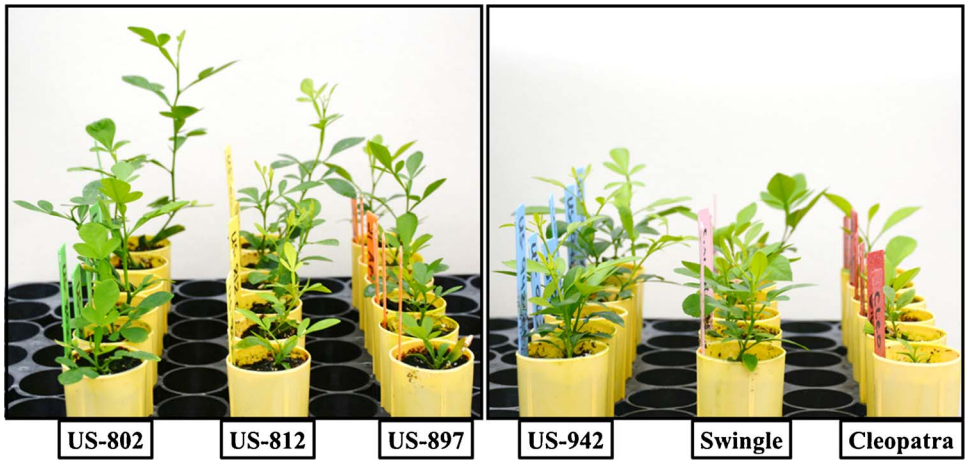

Fig. 2. Six growing cuttings of each rootstock at 8 weeks of age.

Table 2
Size of growing cuttings from experiment 1 for the six rootstocks at 8 weeks.

Rootstock	Shoot length (mm)	Shoot fresh weight (mg)	Explant diameter (mm)	Root total length (mm)	Root fresh weight (mg)
US-802	71 a	587 a	4.05 a	422 a	174 a
US-812	59 a	429 ab	3.47 ab	488 a	133 ab
US-942	58 ab	476 ab	3.05 ab	412 a	103 a–c
Swingle	41 ab	419 ab	3.82 a	275 ab	126 a–c
US-897	36 ab	274 ab	3.12 ab	253 ab	55 bc
Cleopatra	17 b	156 b	2.67 b	69 b	36 c
P > f	0.0053	0.0072	0.0042	0.0008	0.0010

Mean separations for significant ANOVA within columns were by Tukey HSD test at P < 0.05.
Means with the same letter in a column are not significantly different at P < 0.05

3.3. Cuttings at 12–20 weeks

Roots and shoots for rooted cuttings of all six rootstocks grew rapidly from 8 weeks to 20 weeks. Cuttings for the six rootstocks differed significantly in all measures of size at 20 weeks, including shoot length and diameter, shoot and root fresh weight, and explant diameter (Table 3). For all dimensions at 20 weeks, Cleopatra cuttings were the smallest plants, while US-812, US-942, and US-897 had the longest shoots of the six rootstocks. Although US-812 had significantly more root fresh weight at 20 weeks than US-942 and US-897, the latter two rootstocks had similar shoot length, shoot fresh weight, and shoot diameter.

Table 3
Size of growing cuttings from experiment 1 for the six rootstocks at 20 weeks.

Rootstock	Shoot length (mm)	Shoot fresh weight (mg)	Shoot diameter (mm)	Explant diameter (mm)	Root fresh weight (mg)
US-812	571 a	8379 ab	3.58 a	5.10 ab	3013 a
US-942	563 a	8904 a	3.36 a	4.52 bc	1945 cd
US-897	534 a	7462 a–c	3.21 a	4.44 c	2074 b–d
US-802	427 b	6400 cd	3.23 a	5.22 a	2742 a–c
Swingle	347 c	7289 b–d	3.32 a	4.92 a–c	2870 ab
Cleopatra	326 c	5862 d	2.40 b	3.32 d	1507 d
P > f	0.000000	0.000003	0.000000	0.000000	0.000014

Mean separations for significant ANOVA within columns were by Tukey HSD test at P < 0.05.
Means with the same letter in a column are not significantly different at P < 0.05

Table 4
Total dry weight (mg) per plant of growing cuttings from experiment 1 for the six rootstocks at 12–20 weeks, and total dry weight of growing seedlings for three rootstocks at 12 weeks.

Rootstock	Seedlings	Cuttings		
	Total dry weight at 12 weeks	Total dry weight at 12 weeks	Total dry weight at 16 weeks	Total dry weight at 20 weeks
US-802	1285 a	796 a	2200 a	3008 b
Swingle	ND	776 a	1796 a	3015 b
US-812	ND	759 a	1884 a	3870 a
US-942	768 b	698 a	2008 a	3834 a
US-897	ND	695 a	1781 a	3378 ab
Cleopatra	446 c	294 b	1024 b	2143 c
P > f	0.000000	0.002095	0.000411	0.000002

Mean separations for significant ANOVA within columns were by Tukey HSD test at P < 0.05.
Means with the same letter in a column are not significantly different at P < 0.05

3.4. Comparison of cutting dry weights 12–20 weeks

Between 12 weeks and 20 weeks, total plant dry weight for cuttings of the six rootstock cultivars increased from an average 670 mg to 3208 mg per plant (Table 4), ranging from 378 percent biomass increase for US-802 to 729 percent biomass increase for Cleopatra. Although US-802 plants had the largest biomass at 12 weeks, both US-812 and US-942 had significantly more biomass than US-802 at 20 weeks.

3.5. Comparison of seedling to cutting dry weights

Seedlings of US-802, US-942, and Cleopatra at 12 weeks, were larger than cuttings of the same genotypes at 12 weeks, although the 12 week seedling biomass was less than the biomass of cuttings for the same genotype at 16 weeks. Seedling biomass for US-942 at 12 weeks was 768 mg, while US-942 cutting biomass at 12 weeks and 16 weeks was 698 mg and 2008 mg, respectively.

3.6. Root-shoot biomass partition for cuttings and seedlings

The partition of biomass between shoot, explant, and root was examined for cuttings of Cleopatra and US-942 at 12–20 weeks, and for seedlings at 12 weeks. For Cleopatra cuttings, this biomass partition was about 65% shoot, 20% explant, and 15% root at 12 weeks (Fig. 3A). For seedlings of Cleopatra at 12 weeks, biomass partition was about 80% shoot and 20% root. Generally, during the ensuing 8 weeks, root biomass of the Cleopatra cuttings increased faster than shoot biomass, while explant biomass increased less. Consequently, at 20 weeks the

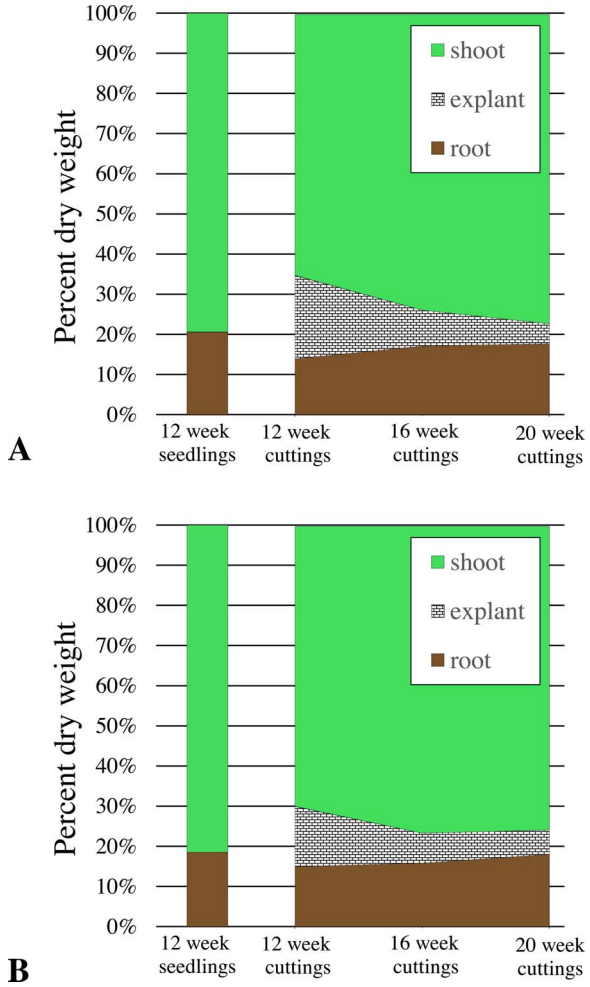

Fig. 3. Dry weight partition of seedlings at 12 weeks and cuttings at 12–20 weeks for A) Cleopatra, B) US-942.

Cleopatra cutting partition into root and shoot components approached very closely to the approximate 80% shoot: 20% root ratio observed for Cleopatra seedlings at 12 weeks. The relative biomass partitions for cuttings and seedlings of US-942 (Fig. 3B) were similar to those observed for Cleopatra during the same time period.

3.7. Detailed evaluation of plant structure

A more detailed study was conducted on a second group of cuttings from experiment 1 at 20 weeks of age, using image analysis software

Table 5
Number of leaves, leaf area, stem length, and dry weight of leaves, shoots and roots of 20 week-old cuttings from experiment 1.

Rootstock	Number of leaves	Leaf area (cm^2)	Stem length (cm)	Leaf dry weight (mg)	Shoot dry weight (mg)	Root dry weight (mg)
US-942	36 a	321.2 a	53.6 a	1547 a	1450 a	732 ab
US-812	30 b	221.0 b	52.1 a	1152 c	1405 a	753 a
US-897	31 b	229.2 b	49.2 a	1212 bc	1127 ab	505 bc
US-802	25 c	252.7 b	43.1 ac	1255 bc	1170 ab	702 ab
Swingle	24 c	310.5 a	33.5 bc	1450 ab	973 b	828 a
Cleopatra	25 c	213.9 b	29.6 b	1130 c	523 c	417 c
P	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000

Mean separations for significant ANOVA within columns were by Tukey HSD test at $P < 0.05$.
Means with the same letter in a column are not significantly different at $P < 0.05$

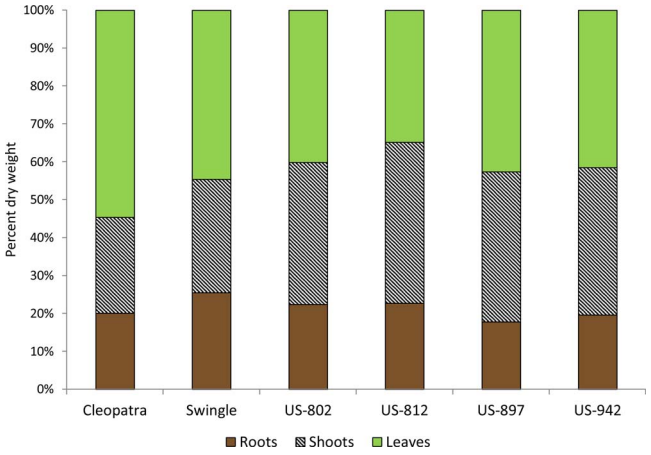

Fig. 4. Biomass partition of cuttings from six rootstocks at 20 weeks.

(Table 5). For this group of plants, total shoot and root dry weights for each rootstock were similar to that measured for the first group of cuttings of the same cultivars at 20 weeks (Section 3.4) and summarized previously in Table 4. The average number of leaves per cutting varied significantly between rootstocks and was lowest for Swingle (24), followed by Cleopatra and US-802 (25), and highest for US-942 (36). Leaf area was significantly higher for Swingle, and US-942 (310–321 cm^2) compared with the remaining four rootstocks (213–253 cm^2). Plant height (stem length) of the second group of cuttings ranged from 29.6 cm for Cleopatra to 53.6 cm for US-942, similar to the height measured for the first group of cuttings. Significant differences for leaf-, shoot-, and root biomass were observed between the six different rootstocks. The percent partition of the three tissue types for all six rootstock cultivars is displayed in Fig. 4. It is apparent that, despite variation in the percentage of leaf and shoot biomass, the ratio between above-ground tissue and below-ground tissue is similar between the different rootstocks. A photo of four of the six rootstocks from this set of cuttings is presented in Fig. 5.

3.8. Root structure

Analysis of the root architecture of the six rootstocks revealed a noticeably ($P = 0.0581$) different number of adventitious (1st order) roots arising from the base of each cutting (Table 6). The highest average number of 1st order roots was observed for US-812 (11) and the lowest number was observed for Cleopatra (5). The length of these adventitious roots was 19 cm on average and was limited by the length of the cone cell in which the plants were grown. The average number of 2nd order roots arising from each 1st order root varied significantly ($P < 0.05$) between rootstocks and was lowest for Swingle (22) and highest for US-897 and US-942 (37–39). The latter two rootstock cultivars also had the highest total number of 1st and 2nd order roots combined (309–346). The total root length ranged from 517.1 cm for Cleopatra to 1462.6 cm for US-942. This significant difference in total root number and length between rootstocks can clearly be seen in Fig. 6.

4. Discussion

Early shoot growth (within the first two weeks) was striking in some cases, especially for US-802 in experiment one, where 77% of cuttings had growing shoots at 2 weeks (Fig. 1). Most of these cuttings with early shoots continued to grow in the following weeks and were included in those established cuttings with shoots counted at 8 weeks. The experiment did not attempt to systematically determine whether shoot growth or root growth was initiated first from explants after planting in the soil. However, there were some explants in a separate

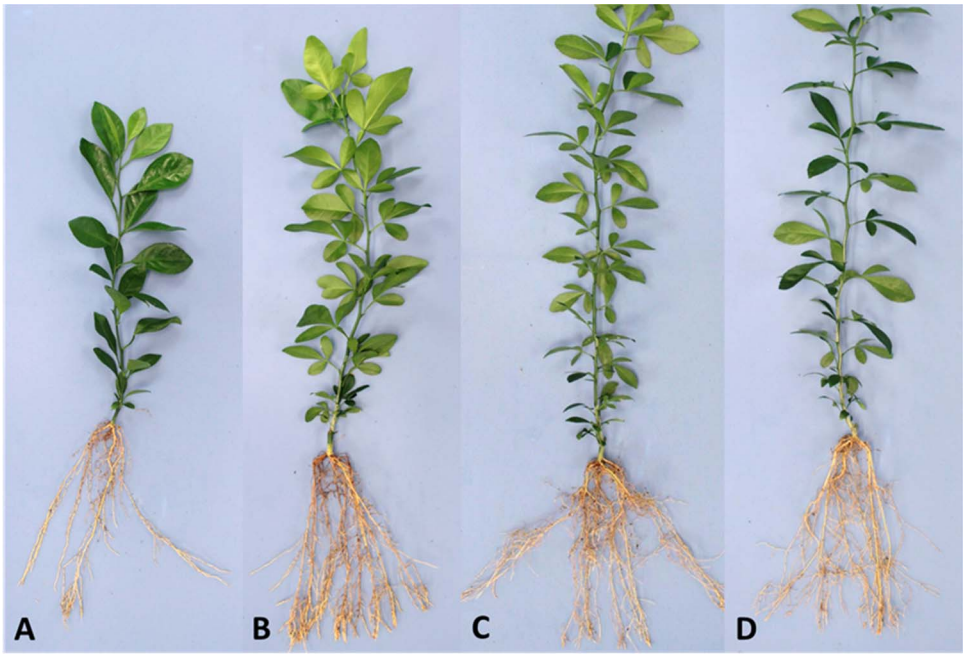

Fig. 5. Shoot and root development of cuttings from four rootstocks at 20 weeks. A) Cleopatra, B) Swingle, C) US-897, D) US-942.

Table 6
Root structure of cuttings from six rootstocks at 20 weeks. First order roots designate adventitious roots arising from the base of the internode cutting. Second order roots designate roots arising from the 1st order roots. Total number of roots is the sum of all 1st and 2nd order roots.

Rootstock	Number of 1st order roots	Length of 1st order roots (cm)	Number of 2nd order roots per 1st order root	Total number of roots	Total root length (cm)
US-942	10 ab	19.7	37 ab	346 a	1462.6 a
US-897	8 ab	19.9	39 a	309 a	1035.5 b
US-812	11 a	18.2	29 ab	282 ab	1013.5 b
US-802	7 ab	18.5	32 ab	216 b	936.6 b
Swingle	9 ab	18.6	22 b	201 b	930.3 b
Cleopatra	5 b	18.1	28 ab	112 c	517.1 c
P	0.0581	0.2062	0.0416	0.0000	0.0000

Mean separations for significant ANOVA within columns were by Tukey HSD test at $P < 0.05$.
Means with the same letter in a column are not significantly different at $P < 0.05$

similar experiment with small growing shoots at 2 weeks, which were examined and did not have roots present. It was also noted that some explants in experiments one and two, alive at 8 weeks but lacking shoot growth (and thus discarded from further study) did have roots. Based on these anecdotal observations, it can be seen that either shoot or root growth may occur first.

Success of plant establishment and shoot growth by 8 weeks, within the two experiments ranged from 42 to 45% for explants for Cleopatra, to 82–91% for explants for US-802. Generally, by 8 weeks most cuttings were either growing, or the explant was dead. However, at 8 weeks a small proportion (1–17%) of cuttings was observed, which were still green, but had not initiated shoot growth. These plants were discarded from further study, but as indicated above, it was noted that some had formed roots. Based on previous work with cuttings, it was expected that some of these cuttings that were alive, but without shoots, would have initiated shoot growth given more time. However, trying to recover growing plants from this group of lagging explants should probably be considered impractical for efficient cutting management and were ignored for the purposes of this study.

Significant differences between the six genotypes were noted for the size of growing cuttings at eight weeks, both in shoot and root measures. In general, these differences appeared associated with relative growth of those genotypes, regardless of propagation type. Plants propagated by cuttings of US-802 were significantly larger than cuttings of Cleopatra at the same age, and this relative size difference between the two cultivars was also observed with seedlings (Table 4). Similar differences in size were measured among the rootstocks propagated by cuttings for size at 12, 16, and 20 weeks (Tables 3–5). Although Cleopatra remained the smallest of the plants at all time points, there was a shift among the larger plants, with cuttings of US-802 being the largest at 8 weeks, and cuttings of US-812 and US-942 significantly larger than US-802 by 20 weeks. There was no apparent reason for this differential growth of US-802, US-942, and US-812, and plants of all the rootstock cultivars appeared healthy throughout.

It was noted that dry biomass partition between root and shoot tissues was similar for seedlings and cuttings of the two rootstock cultivars compared, appearing very close to 80% shoot and 20% root. It can be noted that as plants grow, the cutting explant becomes a part of the shoot base which continues to grow, in girth and mass, while the seed remnants in a seedling separate from the seedling plant (and cease to be a part of the growing plant). There was no indication from this study, that this difference between cuttings and seedlings in development had any effect on plant health or growth.

It is interesting to note that the root structure differed noticeably between some of the rootstock cultivars. Cuttings prepared from US-897 and US-942 had a considerably larger number of 2nd order roots and total length of roots at 20 weeks of age compared with the other four rootstocks. This difference in root architecture is likely to have an effect on tree physiology, particularly as it relates to nutrient and water uptake, and is the subject of continuing research.

In addition to cuttings, *in vitro* micropropagation has been demonstrated an effective alternative to seed for citrus rootstock propagation (Bowman et al., 1997; Carimi and De Pasquale, 2003; Chiancone and Germanà, 2013). The disadvantage of *in vitro* propagation is the extended time – typically one year or more – to refine culture conditions and establish source material in culture for each rootstock selection. In comparing *in vitro* propagation to greenhouse shoot cuttings for citrus rootstocks, it appears that greenhouse cuttings provide the best alternative when smaller quantities (10–1000) of many different rootstocks are needed quickly, while *in vitro* propagation becomes a better choice when the quantity of plants needed for a particular rootstock is much

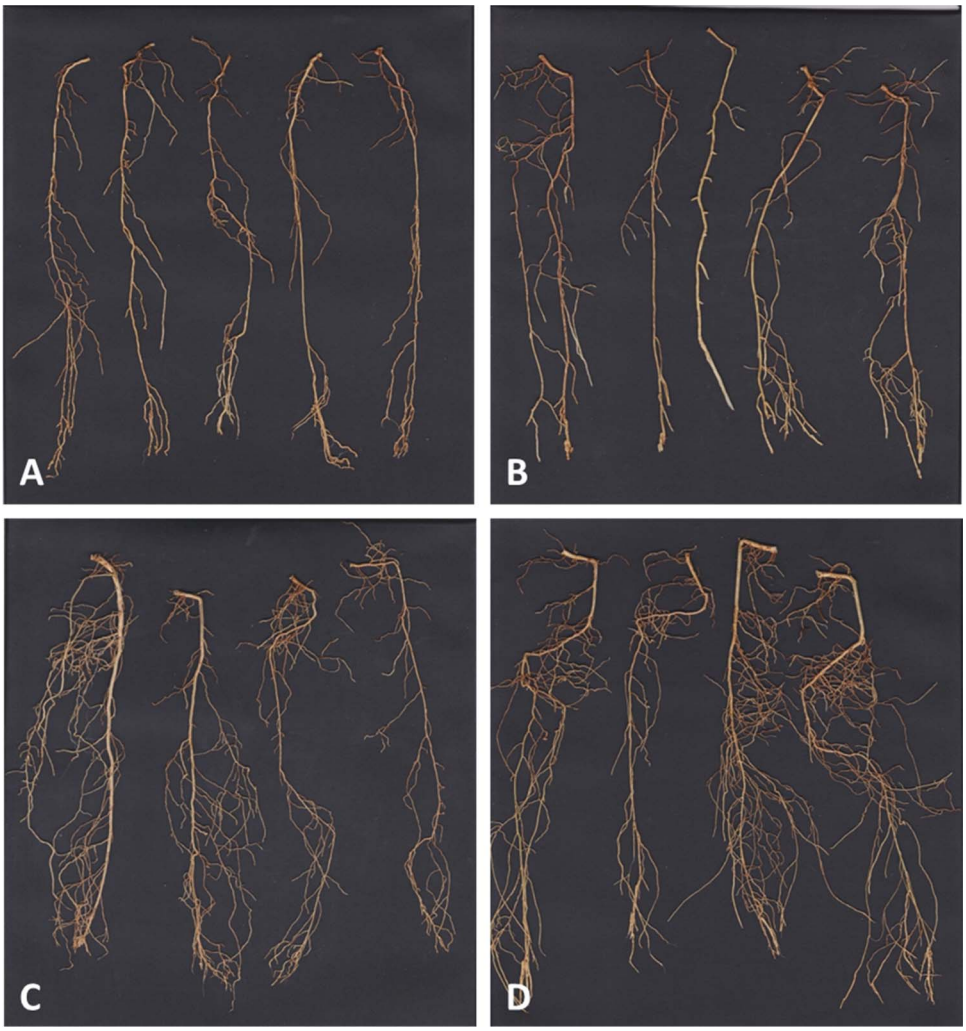

Fig. 6. Dissected adventitious roots from 20 week-old cuttings from four rootstocks with differences in the number of fibrous roots. A) Cleopatra, B) Swingle, C) US-897, D) US-942.

larger and justifies the additional time investment to develop the *in vitro* cultures.

Compared with other studies published on citrus cuttings, the methods described in this study appeared particularly useful for efficient citrus rootstock propagation, either for research materials or for commercial nurseries. The methods made use of one node per explant (maximizing the number of plants that can be propagated from limited source material), required only a simple mist bench, made use of an inexpensive commercial auxin powder and a standard soilless potting mix as used for normal citrus seedling growth, and rapidly resulted in healthy rooted growing plants. No modifications appeared necessary for the methods to work effectively with a wide range of genotypes. These described methods are proposed as an effective alternative for citrus rootstock propagation when adequate amounts of nucellar seed are not available, or when phytosanitary or other factors cause seed use to be undesirable.

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