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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.

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