In vitro propagation of citrus rootstocks and bioindexing indicators

Paulina Quijia-Lamiña, Vanessa Mendez, and Georgios Vidalakis

Citrus Clonal Protection Program, Microbiology and Plant Pathology, University of California Riverside

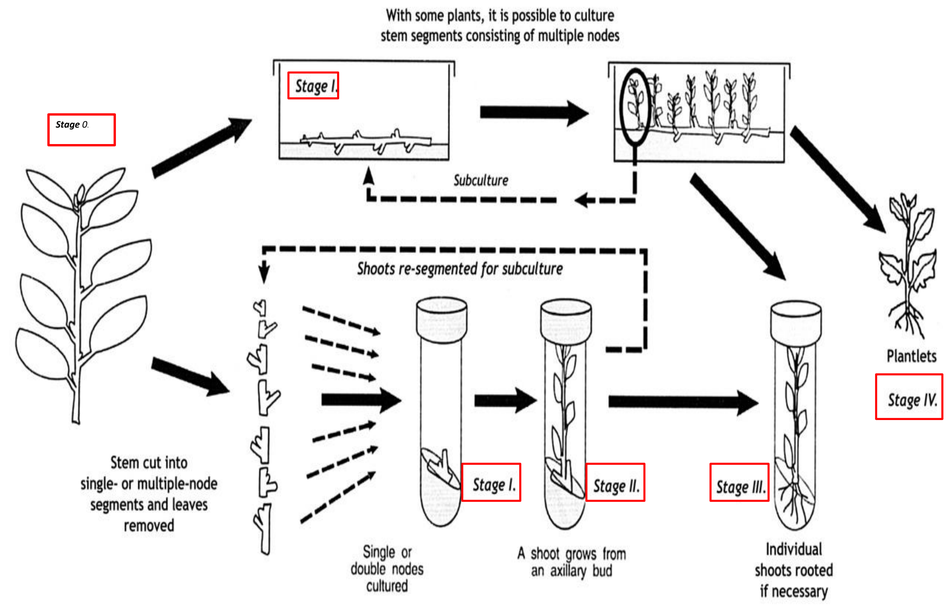
Introduction

The conventional method of rootstock propagation consists of the germination of open pollinated, highly apomictic and polyembryony seeds. Production rates are significantly reduced when genotypes exhibit low levels of nucellar embryony. Reduction in seed production of some valuable rootstock species (Fig. 1) have also limited the supply seeds to citrus nurseries. Introduction into California of new citrus varieties via the Citrus Clonal Protection Program (CCPP) also requires constant rootstock supply for therapy (shoot-tip grafting) as well as a high number of plant indicators for bio-indexing (VI index). Slow production of rootstocks and bioindicators plants have limited bio-indexing activities resulting in increased time for a variety to be released from quarantine. *In vitro* culture techniques such as micropropagation, can be used as viable strategy to alleviate these problems. These techniques allow the production of large number of propagules in relatively small space, reducing the necessity of a constant seed supply. Uniform, disease-free, and high-quality plant production can be obtained using this approach.

US942 donor plant maintained in the CCPP greenhouses.

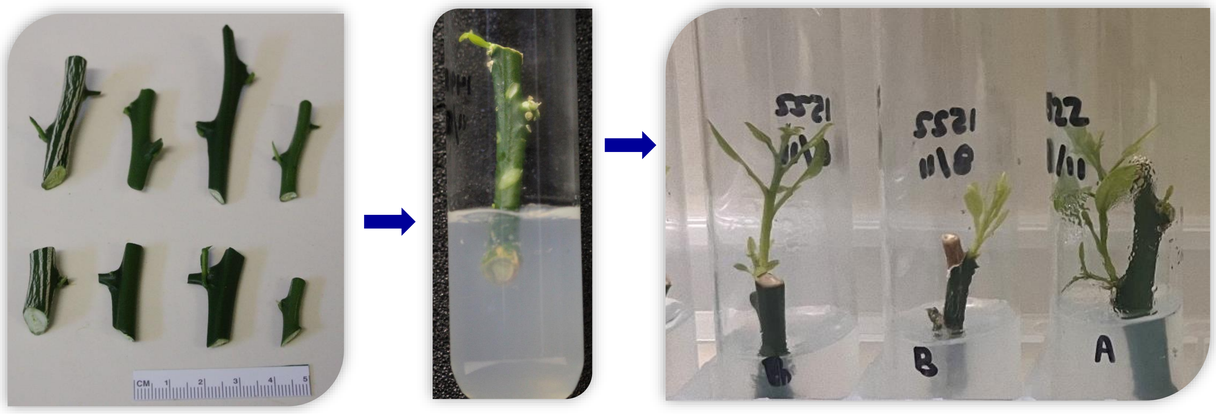
Aim

1. Standardize an optimal in vitro propagation protocol to produce citrus plant indicators and rootstocks species that are required for therapy and bioindexing procedures.
2. Standardize optimal in vitro propagation protocols to produce different rootstocks genotypes, that can be potentially implemented by the citrus nursery industry in commercial scale.
3. Upgrade and optimize the shoot-tip grafting (STG) tissue culture protocol used in the CCPP for therapy and pathogen elimination from citrus propagative materials.



Micropropagation stages: *https://users.ugent.be/~pdebergh/mic/mic2cd01.htm*

Methods



Surface sterilization: 70% ethanol for 1 min + 1.2% NaOCl for 12 minutes

Multiple culture cycles for shoot production

Media components	Concentration
Murashige and Skoog Salts	4.33 g/L
Thiamine	0.4 mg/L
Myo-inositol	100 mg/L
Nicotinic acid	0.5 mg/L
Pyridoxine-HCl	0.5 mg/L
Glycine	0.5 mg/L
Sucrose	30 g/L
Indole-3-acetic acid (IAA)	0.5-1 µM
Cytokines	1.2-10 µM

Results

- Differences in number of shoot produced in the same media between cultures cycles have been observed with both US492 and Rusk (Fig. 1)
- Optimized seed germination and seedling development protocol to obtain seedlings rootstocks used for shoot tip grafting
- Significantly higher germination rates, higher number and longer seedlings were observed with the media 1/4X MS. (Fig2 and 3)

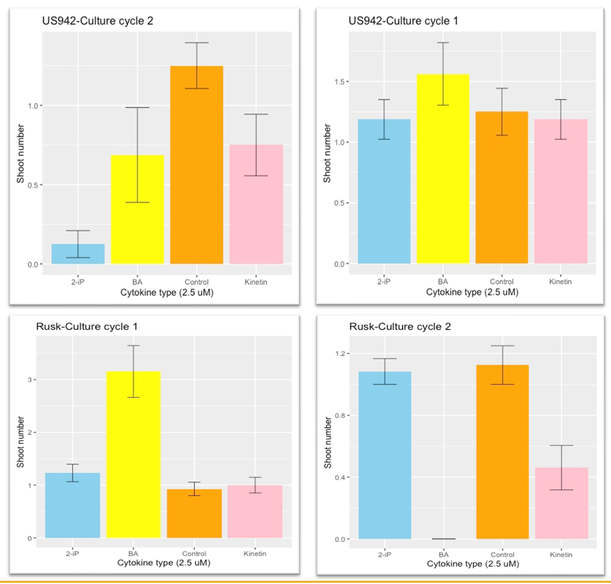


Figure 1. Number of shoots produced from US942 and Rusk budwood subcultured every 4 weeks in medium containing different cytokines types.

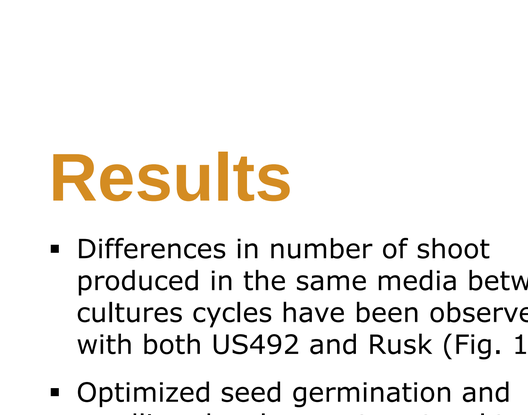
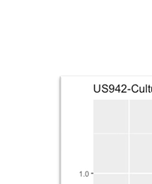


Figure 2. Germination survival curves for four different seed germination media



Media components	Concentration
Murashige and Skoog Salts	1.08 g/L
Thiamine	0.4 mg/L
Myo-inositol	100 mg/L
Nicotinic acid	0.5 mg/L
Pyridoxine-HCl	0.5 mg/L
Glycine	0.5 mg/L
Sucrose	10 g/L

Figure 3. Carrizo seedlings number and length after 21 days from sowing in different media