Combined Proceedings IPPS 73:89-95. 2023.

Propagating for Farms

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Keywords: tissue culture, micropropagation, biosecurity, quarantine, banana, avocado, papaya

Summary

Propagating for farms is to assist with improved crop production. Therefore, several factors ranging from the genetics to quality control have to be carefully considered while propagating for farms. As a veteran tissue culturist and from a decade of tissue culture production and management experiences as a commercial plant propagation Manger in Australia, I shall discuss some interesting experiences and lessons learned at the Yuruga Native Plants Nursery, Walkamin, Queensland (QLD); Fleming's Nursery, Australia and Skybury Farms, Paddy's Green, QLD for the benefit of the wider plant propagator community. I shall discuss some important aspects like the genetics, genetic improvement in vitro, fidelity, disease and pest control etc. in relation to commercial propagation of plants for farms with Banana, Avocado and Papaya as examples. Additionally, the usefulness of in vitro culture systems for rapid crop improvement, plant production, assisting with quarantine compliance etc. are discussed.

INTRODUCTION

Plants are propagated from seeds and cuttings for Millenia, ever since man ventured into agriculture/ horticulture. Techniques and tools were developed by ancient household cultivators, farmers, horticulturists and scientists over the years. Plant tissue culture is one of the revolutionary tools for propagation based on the totipotency of plant cells described by Haberlandt in 1902. The isolation and characterisation of auxins (Went and Thimann, 1937), gibberellins (Yabuta and Sumiki,1938) and cytokinins (Miller et al. 1955) assisted the rapid progress with plant propagation (Skoog and Miller, 1957). Murashige has comprehensively reviewed plant tissue culture and its applications to plant production (Murashige, 1974).

Discovery of somatic embryogenesis (Steward et al. 1958), the unique ability of plants to regenerate whole plants from a somatic cell, allows rapid, reliable cloning of some plants that are recalcitrant to other cloning methods as well as rapid crop improvement through selection and genetic engineering (Pierroz, 2022). Bergman (1977) reviewed cell cultures and its multiple applications like cell line selection for crop improvement and production of secondary metabolites.

Cocking (1960) reported enzymatic isolation of protoplast and discussed potential use of protoplasts. Takabe et al. (1971), for the first time demonstrated regeneration of whole tobacco plants from isolated protoplasts. Further rapid advancements in protoplast culture by Cocking's laboratory revolutionised this technique as detailed by Cocking et al. (1972). Further advances with protoplast culture and its applications came about in different parts of the world (Cocking, 2000). Protoplast culture technology was reviewed recently (Ranaware et al. 2023), due to its contemporary significance to the novel gene editing technology.

Although, plants can be rapidly cloned in large numbers in vitro using some or all of the above-mentioned techniques, propagating for farms requires additional considerations. Some of these considerations (e. g. genetic fidelity, uniformity, freedom from diseases etc) are highlighted in this article. Propagating for farms is also positively regulated in Australia for good reasons as I point out with specific examples in this paper.

CLONING STRATEGIES FOR FARMS

Of all the plant propagation methods, vegetative propagation ex vitro (using stem cuttings) is the most efficient method when the plant species concerned is amenable to this method of propagation. This is because such cuttings yield true to type plants (clones) demonstrating the characteristics of the mother plant (fidelity) if they are not affected by different growing environments, in other words not subjected to the impacts of G x E interactions. When the cutting production is efficient, it is also the most economical method of large-scale propagation as in the case of propagating ornamentals like *Begonia* sp., *Hibiscus* sp., *Hydrangea* sp. etc.

Banana Micropropagation

Micro-propagation (cloning of plants in vitro from shoot tip/ nodal segments) is a rapid, reliable method of cloning that is widely used in the farming sector because

of the efficiency, genetic fidelity, and freedom from diseases ensured by this process when the facility and operating procedures are properly managed. A good example is cloning of bananas (**Fig. 1** and **2**). Most of the banana farms in Australia use tissue cultured banana plants produced according to strict regulations of the quality approved banana nursery program (QBAN) - (https://abgc.org.au/qban/) to ensure quality standards, especially freedom from dreaded virus disease Bunchy top of banana caused by *Banana bunchy top virus* (BBTV) and the fungal disease (Panama wilt) caused by *Fusarium oxysporum* f. sp. *cubense* race 4. Main features of effectively managed QBAN includes NIASA accreditation (https://nurseryproductionfms.com.au/niasa-accreditation/), training, independent, disease indexing of the mother stock by Grow help Australia, as well as best practices in the grow on nursery, regularly monitored by the banana industry - the Australian Banana Growers Council (ABGC).

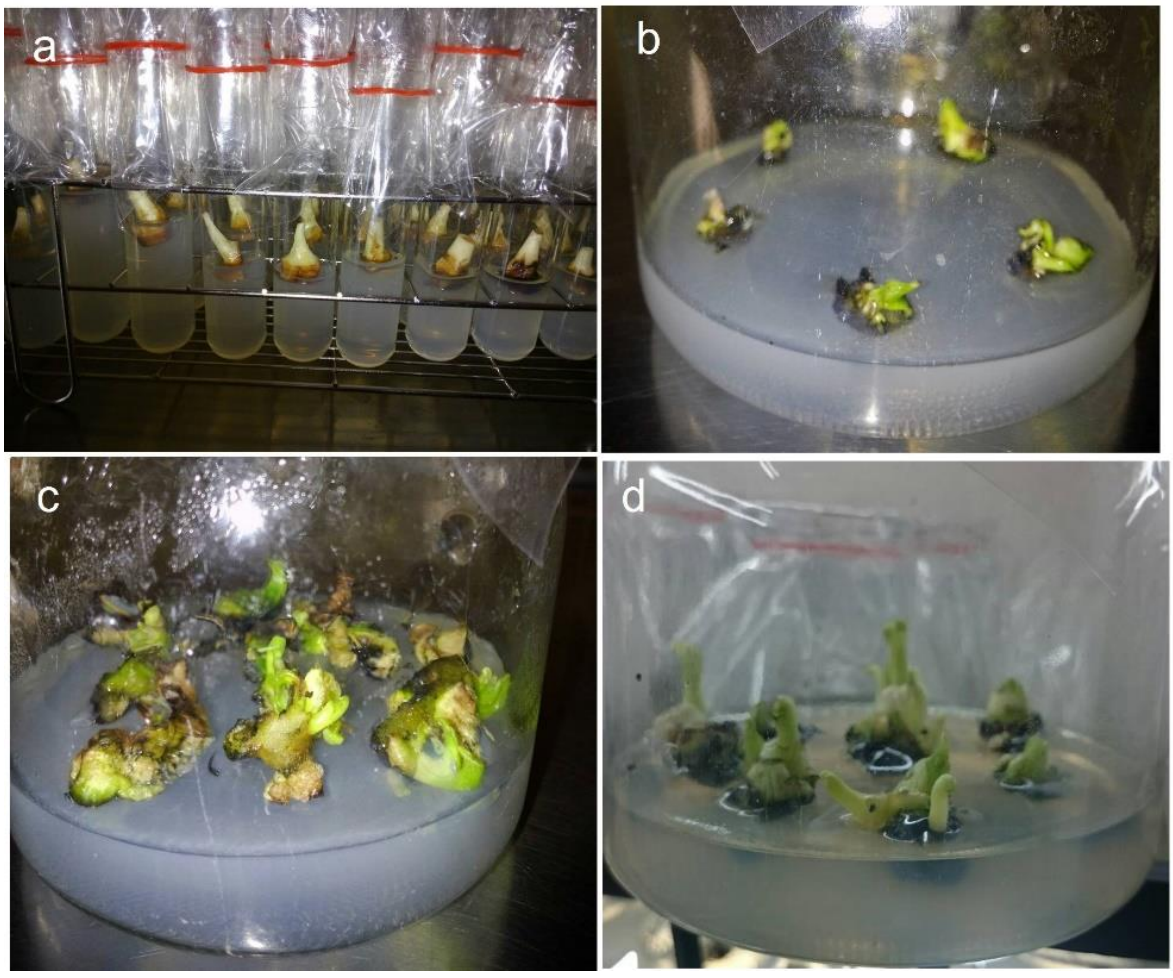

Figure 1. Stages in banana micropropagation. (a) Initiation from corm, after surface sterilisation and inoculation, (b) 10 days after transfer to proliferation medium, (c) One month after transfer to proliferation medium, (d) Shoot regeneration.

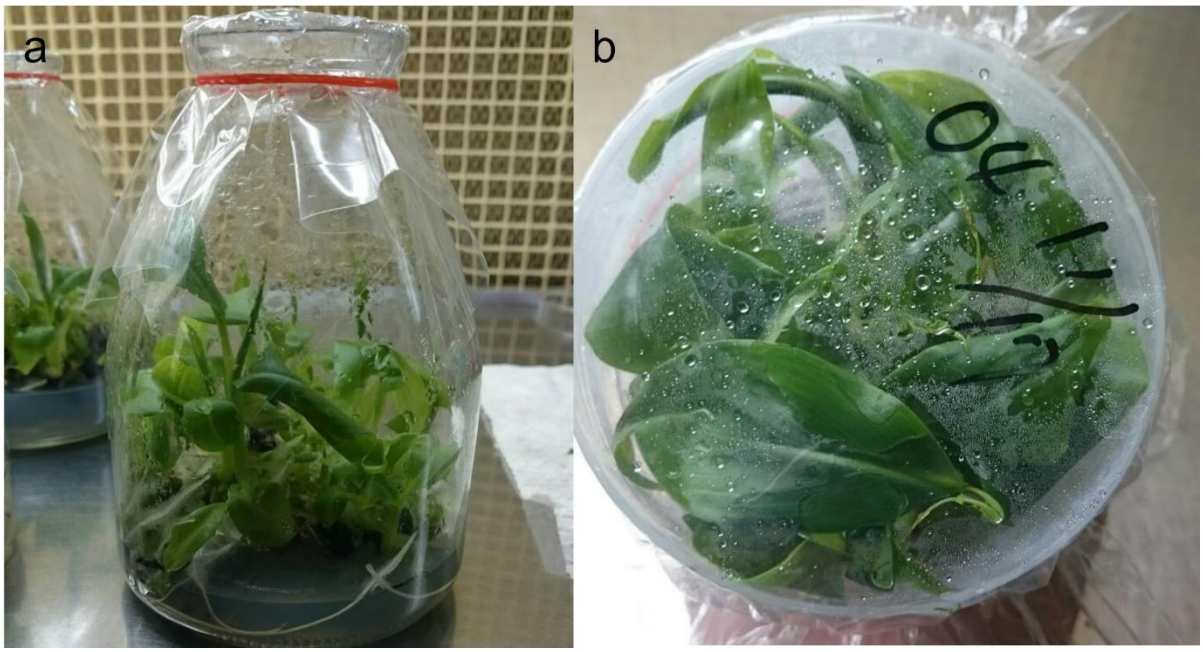

Figure 2. (a) In vitro rooted banana plants and (b) plants ready for acclimation in the greenhouse.

Avocado Micropropagation

Accreditation by the Avocado nursery voluntary accreditation scheme (ANVAS) – (https://avocado.org.au/wp-content/uploads/2019/01/ANVAS-Terms-and-Conditions-V3.pdf), is a scheme developed by Greenlife industry (https://www.greenlifeindustry.com.au/about/industry/green-life-industry), that regulates avocado nurseries and practices in Australia to ensure that the best possible avocado plants are supplied to the Industry. Although not a common practice like banana, recently developed avocado micropropagation methods (Josekutty, 2019) can revolutionise quality avocado plantlet supply to farms (**Fig. 3**). Additionally, the disease-free avocado plants micropropagated in vitro, could be a choice for exporting planting materials across the quarantine borders.

Cell Culture and Somatic Embryogenesis for rapid genetic improvement of Papaya

Another important aspect of propagating for farms is using appropriate propagation technology to generate genetically improved crops as Skybury farms did in the recent years. One school of thought is that somatic embryos are of single cell origin as demonstrated by (Nagamani et al., 1987). According to the principles of somatic cell genetics, it is possible to grow and screen millions of plant cells (potential plants as they are totipotent) in the tiny space of a 100 ml culture flask in the laboratory (Josekutty, 1998). A high-throughput somatic embryogenic system can then regenerate

thousands of variant plants from the micro-colonies originating from individual cells in vitro.

We used this power of plant cell culture to generate new lines of highly productive Skybury, Sweet, Red Papaya lines (Josekutty, 2022; Puthiyaparambil et al. 2023) (**Fig. 4**). Development of such advanced papaya lines enabled Skybury farms to rapidly enhance consumer preference and papaya market capture although papaya is a relatively less preferred fruit in Australia, largely due to the ignorance about its nutritional superiority and additional health benefits compared to other common sugary fruits like banana and grapes.

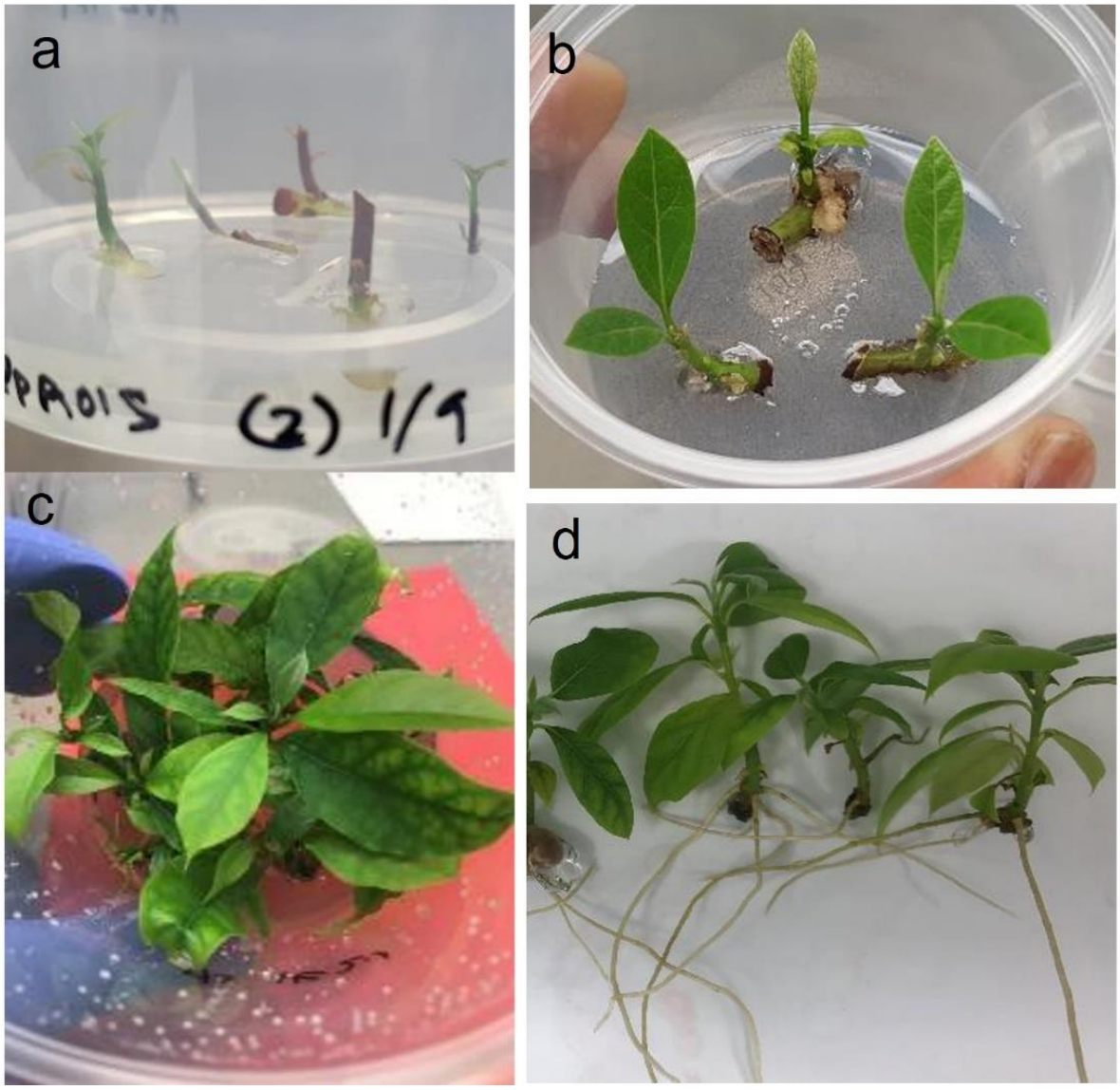

Figure 3. Avocado micropropagation for farms. (a) Initiation, (b) Shoot regeneration, (c) Shoot proliferation, (d) Rooted plants ready for acclimation.

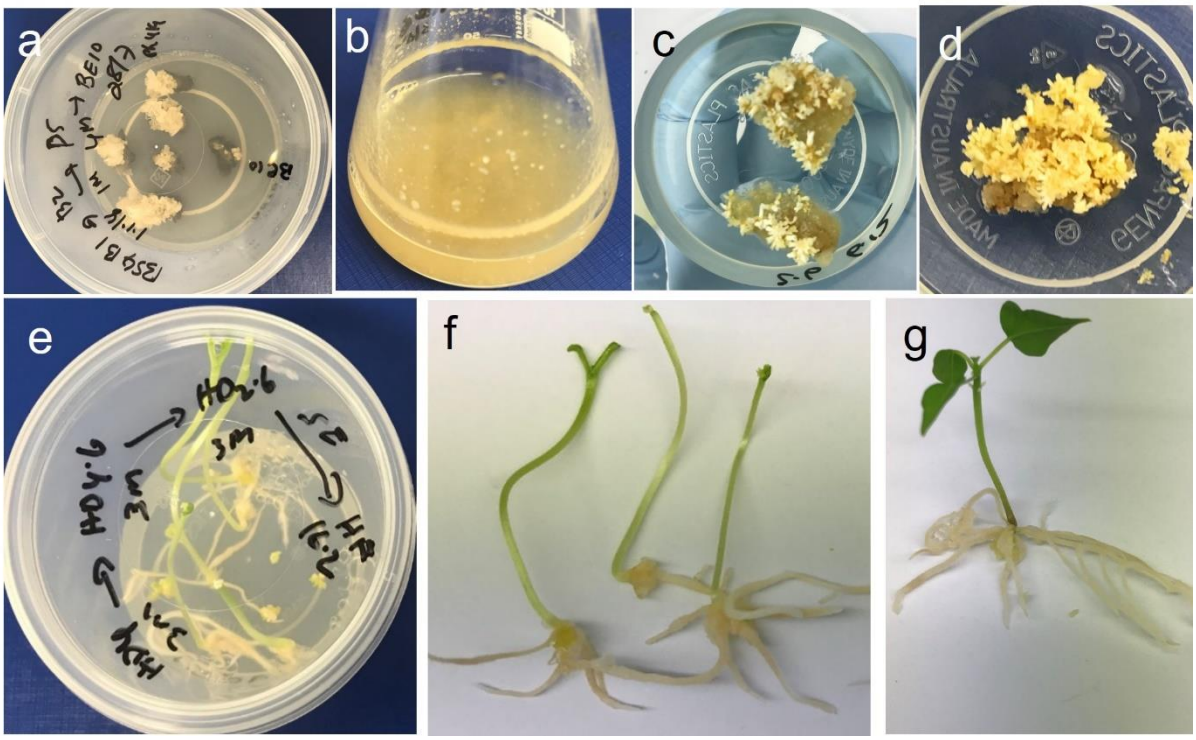

Figure 4. Papaya crop improvement through cell line selection. (a) Initiation of callus, (b) Cell cultures established from callus, treated with viricides, (c) Somatic embryo induction from callus, (d) Advanced somatic embryos ready for plant regeneration, (e-f) Different stages of plant regeneration from somatic embryos.

CONCLUSIONS

There are several methods for commercial propagation of plants for home gardens, conservation activities or commercial farming. Several methods for in vitro cloning of plants have been developed and micropropagation technique that revolutionised the propagation industry remains the preferred mode of propagation for many reasons such as:

(i) the ability to propagate in a mass scale
(ii) ability to produce plants throughout the year with no seasonal effects
(iii) production of high-health, disease free plants
(iv) ability to produce many plants within a limited time and space
(v) minimize requirements for quarantine compliance when sending plants across borders. Propagating for commercial farming requires several careful considerations and it is well regulated in Australia by the respective industries for valid reasons.

LITERATURE CITED

Cocking, E. C. (1960). A method for the isolation of plant protoplasts and vacuoles. Nature *187*: 962-963.

Cocking, E.C. (2000). Turning point article plant protoplasts. In Vitro Cell Dev Biol-Plant *36*, 77–82. https://doi.org/10.1007/s11627-000-0018-2

Bergmann, L. (1977). Plating of plant cells. In: Barz, W., Reinhard, E., Zenk, M.H. (eds) Plant Tissue Culture and Its Bio-technological Application. Proceedings in Life Sciences. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-66646-9_18

Haberlandt, G. (1902). Culturversuche mit isolierten Pflanzenzellen. Sitz-Ber. Mat.-Nat.KI. Kais. Akad. Wiss. Wien, *111*(1): 69–92.

Josekutty, P. C. (1998). Selection and characterisation of variant *Solanum xanthocarpum* that overproduce steroids. Phyton, 62 (1/2), 125-130.

Josekutty, P. (2019). Micropropagation of avocado (*Percea americana*). Proceedings of the IPPS – Australia conference 2019, Sunshine coast, Australia.

Josekutty, P. (2022). Accelerated In Vitro Breeding Creates Improved, Designer Papaya, Proceedings of the IPPS-Australia conference 2022, Blue mountains, NSW.

Puthiyaparambil, J. C. Pagie, M., Teressita, S., Jay, P.M., Bongani, N., Paul, F., Candy, M., Mark, M., Marion, M., Ian, M., Sanskruti, L. and Nitin, M. (2023). Rapid papaya crop improvement through accelerated in vitro breeding and molecular diagnostics. Acta Hortic. *1362*: 499-506. DOI 10.17660/ActaHortic.2023.1362.67

Miller, C.O. et al. (1955). Kinetin, a cell division factor from deoxyribonucleic acid. J. Am. Chem. Soc. 77**,** 1329–1334.

Nagata, T and Takabe, I. (1971). Planting of isolated tobacco mesophyll protoplasts on agar medium. Planta, 99: 12-20.

Nagmani et al. (1987). Single-cell origin and development of somatic embryos in *Picea abies* (L.) Karst. (Norway spruce) and *P. glauca* (Moench) Voss (white spruce). Plant Cell Rep., 6(2):157-159. doi: 10.1007/BF00276677.

Murashige, T. (1974). Plant propagation through tissue culture. Annual Review of Plant Physiology, 25:135-166.

Pierroz, G (2022). Making babies: how auxin regulates somatic embryogenesis in Arabidopsis tissue culture. The Plant Journal (2023) 113, 5–6. https://doi.org/10.1111/tpj.16024

Ranaware, A.S. et al. (2023). Protoplast technology and somatic hybridisation in the family Apiaceae. Plants, *12*: 1060. https://doi.org/10.3390/plants12051060

Skoog, F. and Miller, C.O. (1957) Chemical regulation of growth and organ formation in plant tissue cultured in vitro. Symposia of the Society for Experimental Biology, *11*: 118-131.

Takebe, I et al. (1971). Regeneration of whole plants from isolated mesophyll protoplasts of tobacco. Naturwissen. *58*:318-320.

Yabuta, T and Sumiki, T (1938). Communication to the editor. J. Agric. Chem. Soc. Japan. *14*:1526.