Using Tissue Culture to Help Develop New Crops©

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INTRODUCTION

Crops, new to New Zealand, are imported for a number of reasons. They might be considered to have market potential or the ability to grow in areas where other crops struggle. Importation might also expand the range of germplasm available for plant selection. Any crop imported into New Zealand is scrutinised and regulated by Biosecurity New Zealand since its health status may be unknown. It is held in a closed quarantine facility where it undergoes rigorous pathogen testing. Frequently, the plant material is found to be infected with one or many viruses that must be eliminated before the crop can be released and research and development studies commence. This paper describes an effective and efficient in vitro system that eliminates viruses in vegetatively propagated species. The system involves heat and chemical therapies. Five successfully treated species are described. Three of these are Andean root crops that are of culinary interest: oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*), and arracacha (*Arracacia xanthorrhiza*). Two other species, imported from Japan, are of both culinary and medicinal interest: mountain yam (*Dioscorea opposita*) and edible lily (*Lilium lancefolium*). In addition to applying tissue culture techniques to eliminating viruses, the processes of micropropagation and germplasm storage are also described.

METHOD

What Crops and Why Have They Been Imported?

Oca, grown and sold here as New Zealand yam, is an old and well established crop. In 1993, new accessions were imported by Alfredo Grau to expand this germplasm base (Grau and Halloy, 1994). It has high nutritional and culinary value (Martin et al., 2004). Oca is usually propagated by planting whole tubers (Popenoe et al., 1989).

Ulluco is a colourful and attractive new root crop that has been imported to extend the range of vegetables available to growers, producers, and consumers. It is usually propagated by planting small tubers (Popenoe et al., 1989).

Arracacha produces lateral roots, arising from the crown of the root, which resemble parsnip roots. They are tender and delicately flavoured and are another interesting choice of new crop. It is usually propagated with offsets or shoots that are produced on the crown of the main rootstock (Popenoe et al., 1989).

These three crops grow at high altitudes in the Andes in Argentina, Peru, and Bolivia. Along with potato, with which they are often interplanted, they were the main staple diet of the Incas and are an important part of the diet of Andean inhabitants today.

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Mountain yam is a culinary crop from Japan where it is peeled and cooked by any of the methods used for potatoes. In addition to its culinary value, it contains a range of phytochemicals and is documented to have medicinal attributes. Bensky and Gamble (1986) claim that it tonifies and augments the spleen and stomach; tonifies the lung qi and augments the lung yin; tonifies and stabilizes and binds the kidneys. Traditionally, mountain yam is propagated by cuttings or by planting small, whole, young tubers (Larkcom, 1991).

Edible lily has been grown as a food source in Japan and China since around the 17th century. The protein content of lily bulbs is twice that of potatoes. Medicinally, its active ingredient is lilioside, which is said to moisten the lungs, clear the heart, and calm the spirits (Bensky and Gamble, 1986). Edible lily bulbs are propagated using stem bulbils (Philips and Rix, 1993).

Virus Testing and Detection. Viruses were identified using electron microscopy, enzyme-linked immunosorbent assay (ELISA), herbaceous indicator hosts, and PCR analysis. Viruses detected in the Andean crops were: arracacha A virus, arracacha B virus, arracacha latent virus, papaya mosaic virus, ullucus virus C, ullucus mild mottle virus, ullucus mosaic virus (Fletcher and Fletcher, 2001). Viruses detected in mountain yam were cucumber mosaic virus and lily symptomless virus. Viruses detected in edible lily were dioscorea latent virus and an unidentified poty-virus.

Tissue Culture and Virus Elimination. The tubers of oca, ulluco, and mountain yam, the root of arracacha, and the bulb of edible lily were planted in sterile soil in the quarantine glasshouse. Plants were grown at 18 to 24 °C without supplementary lighting. The first step in establishing the plants in tissue culture was to select a suitable explant: stem nodal section (oca and ulluco); crown offshoot (arracacha); developing adventitious stem shoot (mountain yam); and bulb scale (edible lily). The explants were surface sterilized in a 1% (a.i.) sodium-hypochlorite solution for 20 min and then rinsed three times with sterile, distilled water.

Asceptically, the explants were placed in pottles on Murashige and Skoog (1962) tissue culture medium that had been modified by the addition of the anti-viral chemical ribavirin (a synthetic riboside,1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Virazole™. John Bell and Croyden, 51-54 Wigmore Street, London, United Kingdom) at a concentration of 50 mg·L^{-1}; providing the chemical component of the process. The pottles were placed in a growth cabinet set for alternating periods of 4 h light at 35 °C and 4 h dark at 31 °C; providing the heat treatment component of the process.

When the lateral or apical shoots (depending on the species) were approximately 1 cm long, they were excised and inoculated on to the same growth medium without ribavirin. They were grown for approximately 1 month in normal tissue culture conditions of 24 °C under fluorescent lights with a 16-h photoperiod and 8 h dark. The tissue-cultured plantlets were then tested for viruses. Those that tested virus-free were to be released from quarantine, ready for further research and development. The process would be repeated for plantlets that tested positive for virus.

The Micropropagation and Germplasm Storage Processes. These processes are applied to pathogen-free, tissue-cultured material where the plant is either micropropagated or is stored as in vitro germplasm that can be sourced at a future time. Micropropagation is the process used by tissue culturists to build up plant-

let numbers. Firstly, suitable growing points are excised. These may be shoot apical sectors, stem nodal sectors or, as with mountain yam, developing adventitious stem shoots. These are transferred to pottles containing appropriate tissue culture medium, which are then placed in a growth cabinet. After 4 to 6 weeks of growing under normal tissue culture conditions, healthy plantlets are ready for further micropropagation or for exflasking into a mist bed and then into the glasshouse. Germplasm storage retains the plantlets in tissue culture. Germplasm is maintained by subculturing and, for many species, the growing conditions are modified; e.g., cooler growing conditions, less light, and/or altered medium composition.

CONCLUSION

Tissue culture plays an important and useful role in new crop research and development by providing:

- An efficient and effective tool for virus elimination;
- A method for rapid multiplication of the new crop;
- In vitro, long-term storage of germplasm;
- A cost-efficient process for testing and establishing new crops in New Zealand agriculture.

LITERATURE CITED

Bensky, D. and **A. Gamble.** 1986. Chinese herbal medicine, Materia Merdica. Eastland Press, Seattle, Washington.

Fletcher, P.J., J.D. Fletcher, and **S.L. Lewthwaite.** 1998. In vitro elimination of onion yellow dwarf and shallot latent viruses in shallots (*Allium cepa* var. *ascalonium* L.). N.Z. J. Crop Hort. Sci. 26: 23–26.

Fletcher, P.J. and **J.D. Fletcher.** 2001. In vitro virus elimination in three Andean root crops: Oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*) and arracacha (*Arracacia xanthorrhiza*). N.Z. J. Crop Hort. Sci. 29:23–27.

Larkcom, J. 1991. Oriental Vegetables. Kodansha International, Tokyo.

Grau, A. and **S. Halloy.** 1994. Feast or famine. Growing Today, June 1994: 28–33.

Martin, R.J., G.P. Savage, B. Deo, S.R.P. Halloy, and **P.J. Fletcher.** 2005. Development of new oca lines in New Zealand. Proceedings of the first international symposium on root and tuber crops: Food down under. Acta hort. 670:87–92.

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 15:473–497.

Phillips, R. and **M. Rix.** 1993. Vegetables. Pan Books Ltd, London.

Popenoe, H., S.R. King, J. Leon, and **L.S. Kalinowski.** 1989. Lost crops of the Andes. National Academy Press, Washington D.C.