Grafting Tissue Cultures Directly onto Nursery Grown Stock©

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INTRODUCTION

Tissue culture is a useful tool to rapidly multiply material, rescue embryos from novel hybrids, generate haploid plants, or genetically engineer crops. In association with heat treatment and in vitro grafting it can be used to eliminate viruses and viroids from infected material. It provides a year-round supply of vegetative material that can be quickly multiplied to provide large numbers of propagules. In some cases it can be used to restore juvenility, often in turn increasing the propagation success of cuttings taken from tissue-culture-propagated motherstock.

One of its limitations is the relative difficulty in deflasking plantlets of many species. This can be due to poor rooting of plantlets or slow root growth of taxa in association with the very soft nature of tissue cultured plants.

Grafting tissue cultures in vitro or directly onto nursery grown stock are two methods to potentially overcome these problems. Providing a vigorous or well-developed rootstock allows for rapid development of the scion providing a much quicker growing plant than can be achieved through conventional deflasking.

IN VITRO GRAFTING

In vitro grafting has been widely used both in research laboratories and commercially. Three examples are summarised below:

- Navarro (1990) succinctly reviews the protocols and benefits of shoot tip in vitro grafting (STG) in the elimination of virus infection whilst maintaining the adult nature of scion material, a procedure adopted worldwide.
- Espen et al. (2002) showed in vitro grafting of internodes allows a rapid evaluation of the "localised type" of graft incompatibility, as exhibited by some pear and quince combinations in as little as 30 days. Using this technique for other woody perennials has the potential of elucidating the causes of incompatibility, as well as significantly reducing the time to confirm incompatibility of new combinations, saving valuable time in the search for appropriate combinations.
- Senthil et al. (2004) used in vitro grafting onto seedlings germinated in culture as a means of ensuring rooting and survival of genetically modified chickpea after deflasking.

Of these examples, ensuring successful deflasking is where direct grafting onto nursery stock has the most likely potential benefit over in vitro grafting. Reducing the number of steps requiring a completely aseptic environment potentially saves laboratory space, time, and money.

PROPAGATING DIFFICULT-TO-ROOT TISSUE CULTURES

Grafting tissue cultures directly onto nursery-grown rootstocks offers a very simple solution to deflasking difficult-to-root tissue cultures. Often in the development of new taxa through embryo rescue, mutagenic treatment, or genetic engineering as little as a single plant is needed to be deflasked to allow further propagation or breeding.

CASE STUDY — *ERIOSTEMON AUSTRALASIUS*

We first tried this technique with *Eriostemon australasius*. Conventionally, this species is regarded as both hard and slow to strike by cuttings with only a few clones propagated in the nursery trade. As part of research introducing new clones to cultivation we selected new adult forms based on flower and habit characteristics and attempted to propagate them by cutting and tissue culture. The percentage rooting of cuttings was very low and very slow. In contrast the introduction to tissue culture was more successful. Bushfires destroyed a number of the original plants and all that remained of the clones was material established in culture. Very low root strike in vitro hampered deflasking and an alternative method had to be developed.

Using simple leafy-scion grafting techniques and allowing for the softness of tissue culture material a method was developed to graft tissue cultures directly onto nursery-grown seedlings. As tissue cultured material is often only 0.5–2 mm in diameter, scalpels or preferably razor blades were used to prepare the rootstock and scion.

A simple top wedge graft was used and the union wrapped in a fine piece of Gladwrap® or Nescofilm®. Young vigorously growing rootstocks were selected and the wedge was made in the transition zone between soft tip and semihardwood and any branches or axillary buds were removed. Scions were kept in their jars until required then selected for stem thickness and trimmed to reduce the level of very soft tissue usually to 1–4 cm long. Grafted plants were misted with distilled water or 0.2% Amistar® solution to maintain turgor and reduce the risk of fungal infection, then sealed in a polyethylene bag, and placed in a lit growth room at 25 °C yielding 60 μmol $m^{-2} \cdot s^{-1}$. After 2 weeks the light level was increased to 90 μmol $m^{-2} \cdot s^{-1}$. After 3 weeks the tie was removed if necessary and new axillary buds on the rootstock were removed. Grafted plants were transferred to a shaded mist bench and progressively hardened off.

Success rate was variable depending on the scion and rootstock quality and seasonal climactic conditions. Invariably it was harder to harden off plants during midsummer. In all 60/129 (47%) grafts were successful including a number of plants rescued from contaminated jars. In some batches success reached 100% (Lidbetter et al., 2002).

Plants originating from tissue cultures of adult origin flowered within 9–15 months of grafting effectively maintaining the same ontogenic age as the source plant similar to that observed in citrus (Navarro, 1990). Tissue cultures of seedling origin still took just over 2 years to reach flowering as per seedlings germinated in the nursery.

OTHER SPECIES

At Gosford Horticultural Institute we have also used this technique to rapidly deflask new *Boronia* hybrids germinated in vitro following embryo rescue. Grafted plants flowered within 20 months.

Alexander and Lewis (1998) reported the use of this technique to graft new avocado hybrids germinated in vitro following embryo rescue. Vic Hartney (pers. comm.) has also reported using this technique in the development of cocoa in Malaysia.

Pniewski et al. (2002) have also used this technique to deflask lupins after noticing a severe decline in rooting success after only four to five subcultures in vitro.

The only obvious reason limiting the potential species with which this technique can be used is the issue of large rootstock stem diameter. However, other grafting techniques can be used to overcome this problem.

REDUCING DELAYS IN QUARANTINE

One of the major delays to the introduction of new plants into Australia from overseas is the period that they spend in quarantine. Significant time and hence cost savings can be achieved using this technique, particularly for ornamental plants and potentially for field fruit crops. For instance, in the case of most ornamentals, once an import permit has been acquired, the import fees paid, satisfactory documentation presented, simple logical procedures followed, and an inspection completed showing the tissue cultures are free from bacteria, fungi, insects, and disease symptoms, the material may be released with no further impediment. In contrast, all propagation material other than tissue cultures face obligatory methyl bromide fumigation prior to propagation and 3 months in an approved post entry quarantine facility with the attendant costs of AQIS inspection (AQIS-ICON 2005).

For fruit crops the only difference between tissue cultures and all other propagation material is the requirement for methyl bromide fumigation of nontissue cultures. Both tissue cultures and all other propagation material require the same prolonged period of growth and testing in post-entry quarantine. However with greater acceptance of overseas virus test results and accreditation of overseas tissue culture laboratories potential exists for a reduction in the testing needed in Australia. Similarly, the requirements for interstate transfer of tissue cultures are lower than for other material and tube stock.

OVERVIEW

The procedure of grafting tissue cultures onto nursery grown stock opens up possibilities for plants that are difficult to root in vitro, from breeding programs and genetic engineering research, to crops that don't currently have a satisfactory root initiation or deflasking protocol developed. This technique has already been utilised for crops from herbaceous legumes to small trees from a wide range of families (Rutaceae, Lauraceae, Fabaceae, Sterculiaceae). Furthermore if a crop is routinely grafted to confer disease resistance or vigour, both grafting and deflasking can be combined in one simple operation. The minimisation of time delays and costs associated with quarantine is another potentially significant benefit for the nursery industry.

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"Long John" Grafts©

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INTRODUCTION

Usually when short scions (graft pieces) are grafted to any fruit tree or rootstock the scions used have only leaf buds evident. When the graft "takes" it will only produce leafy shoots, which form branches. It can then take from 3 to 5 years or more before that branch will be large enough or mature enough to produce significant amounts of fruit. This is especially so if spur pruning is the method of pruning used.

I have found that by using a different technique, selected scions with flower buds already formed on them can be encouraged to produce lots of fruit in the same season that they are grafted to the tree. The new approach I will describe developed from the success I achieved by using short scion pieces with flower buds attached, and grafting them onto apple trees. These produced some fruit on those graft pieces in the same season that the grafts were done, but I found that the longer pieces resulted in a heavier crop in the first season.

THE "LONG JOHN" TECHNIQUE

The technique of using long scions (20–100+ cm) together with a plastic sleeve has developed as a result of experiments over a period of 15 years. It was originally used for grafting a 'Granny Smith' apple scion (*Malus domestica* 'Granny Smith') onto an established green/purple fruited 'Northern Spy' (*M. domestica* 'Northern Spy') apple tree in July 1998. In this first attempt, I used an unpruned single 1-year growth scion (lateral) over 1 m long that contained leaf buds but no flower buds or spurs. The graft piece was positioned almost horizontally on the tree and was covered with a plastic sleeve, moisturised inside, sealed at the top end, and open at the base to allow air circulation. The warming aspect of the sleeve and the length of scion used is the reason I started calling these longer scions "Long Johns."

The attached scion formed spurs with flower buds along its entire length in its first season of growth (1998–1999). During the 1999–2000 season it produced many green apples that had developed from these formed flower buds. The weight of the apples bent the branch downward and it was easy to pick from the ground and the grafted branch stayed bent in the same position even after the fruit was picked adding to the low-profile shape of the tree. This low-profile shape is also characteristic of the little or no pruning approach I have been developing (Gilbert, 2001).

I have also grafted lengthy unpruned single scions on plums, prunes, cherries and pears with great success. It is worth noting, however, that if the attached scion is pruned at the tip before or after grafting occurs, flower bud and spur formation