Successful Exflasking©

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INTRODUCTION

What I would like to discuss with you today is how the tissue culture laboratory and the nursery can work together more effectively to produce more successful exflasking.

To get the very best results at exflasking, it is necessary to have a greater understanding of the plant and its needs both while it is in the laboratory as well as when it leaves. It is important to have input from both sides. This will greatly improve the chances of a successful result. Some of you may recall a very good paper presented at the 2003 I.P.P.S. New Zealand Conference in Palmerston North entitled: "Exflasking, A Shocking Experience" (Seelye, 2003). To refresh your memory, I will return to this paper and will go over some of the points raised, looking again at these issues from perhaps a practical viewpoint. I hope in this way to foster a better understanding of the roles the plant nursery and the tissue culture laboratory have and how collectively they may improve their chances of successful exflasking.

USING A LABORATORY

Why plants are introduced to a laboratory can usually be broken down to three main reasons:

- **Maintaining true-to-type material** where a homogenous (uniform) crop of plants is required, which cannot be achieved with more conventional means such as seed or vegetative cutting production, as used in the cut flower, forestry, and nursery industries.
- **Speed to market** is becoming more and more important as an industry objective in ornamental types. The potential of rapid multiplication of plant tissue is achievable and can be used when the introduced plant may only have a window of opportunity of 3 years, before it is seen as being behind current trends, e.g., massed flowering perennial garden-type plants to now more formal, structural, singular plantings.
- **The introduction of clean stock** into the nursery is also a service the lab can provide when virus-indexed plant material is required (such as for the cut flower industry) or when stock plants in the nursery have succumbed to disease and need replacing. Many times cuttings can be taken off young plants coming from tissue culture, because these plants have more vigour, e.g., *Lavandula* species. While this has no real bearing on how successful exflasking will be, it may prompt the question; do you need to place your plant in to a laboratory in the first place?

Plant Selection. During the time of plant selection, several processes of elimination are underway, and these do vary from plant breeder to plant breeder, from sales marketer to sales marketer. This process can be highly subjective, but it is of great importance because it is here at this crucial moment that successful exflasking actually begins.

Plant breeders love their plants; if it were left up to them they would select all their plants, and sales and marketing people would have them sent to all four corners of the earth. Luckily there is a more sensible approach where form and vigour may or may not be foremost in the selection criteria. The latter is very important regarding how that plant will respond during the laboratory and nursery production cycle. If, on the other hand, a plant is chosen on form only and not much importance is given to vigour or perhaps disease resistance, then success can be severely checked at exflasking or soon after. Market trends and economics have major influence when selecting new plants; however these has very little or no relevance regarding successful exflasking.

THE ENVIRONMENT

When we look at humidity, CO_2, and temperature levels during the growth period while in a laboratory environment, these are relatively much higher than what is experienced in a nursery environment. The plants, while growing in the laboratory, are housed inside a culture vessel with a sealed lid that allows for minimal gas exchange. The level of CO_2 at this stage is greater than that which is experienced outside, and the temperature range of 20–24 °C on average is both higher and more consistent in comparison. At the other end of the scale, photosynthesis, root development, and light levels are relatively much lower in the laboratory environment when compared to plants growing outside (Seelye, 2003).

You may be wondering how is it that plants can grow so quickly in a laboratory environment while being less photosynthetically active. In the laboratory the tissue culture media the plants are growing in plays an important role. The components of the media are gelled agar containing nutrients, growth promoters, and sucrose, the latter being the plants carbohydrate source. This allows the plant to grow quickly while in an artificial environment or fluorescent lighting that is very low on the light spectrum scale.

Because of the environment provided by the media, plants do not need to develop as many roots. Those roots that are present are likely to fall away once planted out into the greenhouse. However the existence of roots at the tissue culture stage usually enhances transplanting success because functioning roots can create a favourable plant water balance (Diaz-Perez et al., 1995). New roots appear during the hardening off phase when the plant becomes more photosynthetically active. The leaves of the young plant gain more stomatal control, which regulates water loss through transpiration, and the cuticle or waxy outside layer of the leaf develops. The process of respiration also begins when the plant starts to become self-sufficient.

Recognising the marked differences between the two environments can give the nursery a better understanding of what to provide in the initial assimilation period.

PLANT INTEGRITY

The quality of the parent plant introduced to a laboratory is very important. The nursery/plant breeder needs to ensure that the plant does represent the best example of its type and that an evaluation has been done to that effect. Several stock

plants should be kept that are true to type. This gives an insurance cover in the event of an initiation not being successful and these plants may be used in subsequent introductions to the laboratory. The plant should be in a healthy state. Application of a spray programme for bacteria and fungal disease at least 1 month prior to introduction to the laboratory will greatly improve the initiation results and in addition improve plant health in the laboratory. For plants that have a growing tip near or at the base, the use of a soilless medium such as perlite is preferable. A plant's history from the breeder is really helpful especially if the nursery is acting as a contract grower and providing a service like the laboratory.

Factors that are important:

- Was the plant originally in acid and dry environment, or high humidity?
- Low or high light?
- Low or high temperature?
- Understanding a plant's natural environment will determine how the plant is manipulated in the laboratory and greater knowledge in this area will be beneficial to a better understanding of how to handle a plant at pre- and post-exflasking.

Trial Plants. This area cannot be emphasised enough. A laboratory will have been pursuing the best procedures in formulating a protocol that works in a laboratory environment several months prior to any plants being produced. The opportunity for research and development between the laboratory and the nursery can be realised when trial plants are sent out. At this stage it is important to monitor the plants performance, take accurate records of results, relate these to expectations, and then report these back to the laboratory. This feedback can lead to a more desirable plant and better success at exflasking. Optimal planting seasons will be realised over the course of several trial deliveries within a 12-month period. However, this may cause some conflict with speed to market.

REDUCING STRESS

In these days of time constraints, tight schedules, and fixed deadlines there is less flexibility now than ever before in plant production, but we are still dealing with the same living plant. It is not like producing nuts and bolts that can sit on a shelf until required. A laboratory's responsibility is more acute now, and timing and communications prior to shipping are very important to ensure the nursery is ready and equipped to handle tissue culture plants.

Undue delays around this time may mean the tissue culture plant has to sit around twiddling its leaves. When tissue culture plants are dispatched from the laboratory they are ready to be dealt with straight away — any delays may result in a plant not being exflasked at an ideal period when the in-vitro plant is at its optimum health and vigour. Maintaining the plant's growth and quality around this period will improve success at exflasking.

Acclimatisation. Recognising and understanding the differences between the two environments will help you create exflasking conditions suitable for root and leaf developments (Seelye, 2003).

The main points to recall are:

- Relative humidity.
- Light levels.
- Temperature.
- Media.
- Perhaps pH levels now need to be looked at more closely.

LITERATURE CITED

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