Micropropagation and Plant Health

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INTRODUCTION

When plants are asexually propagated, there is always a risk of diseases being transferred. This is especially a problem when the disease is caused by a virus. In the past the only way to avoid this problem was to select stock plants that were not infected. Since the beginning of the 1950s, research has shown that it is possible to inactivate viruses in plants with heat.

TEMPERATURE EFFECTS

Increasing temperature increases the probability of infection. Not only do plants grown at high temperature have a higher risk of infection, but the subsequent multiplication of the virus is also temperature sensitive. Some viruses grow best at a high temperature, whereas others prefer a low temperature. High temperature can also often cause the symptoms of an infection to disappear, but as soon as the temperature decreases, the infection symptoms become visable again. This temperature effect is important because one may assume the infection occurred simultaneously with the appearance of the symptoms.

When it was shown that high temperature can inactivated viruses, a tool became available to treat infected plants. The temperature tolerance of plants is very unpredictable. Carnations will withstand 37°C for several month, whereas *Pelargonium* exhibits severe disorders such as etiolated shoots after only 3 weeks.

TEMPERATURE TREATMENT PROTOCOL

Plants that are in active growth are placed in incubators maintained at a constant temperature. The temperature selected may vary between 34 and 37°C, most often 34°C is used. It is important to keep the plants well watered, fertilized, and at a sufficient level of irradiance during the temperature incubation period. A 16-h photoperiod is used for most plants. The duration of the treatment will depend of several conditions. For most plants, 3 to 4 weeks is sufficient but as long as several month may be needed. It is unusual that a virus following treatment is absent from the whole plant, but it seems to be absent from the young growing shoots. If a plant is propagated from the apical meristem of a shoot, it will most likely be free of virus. Table 1 shows the effect of treating apple trees at 37°C for various lengths of time.

Stockplants were heat treated for various lengths of time. Shoot tips from heat treated plant material were used for further propagation. Out of 96 heat treated plants, infection was only observed in six shoot tips.

MICROPROPAGATION

In micropropagation only the shoot meristem is used. It is possible to use a growth medium where internal fungal and/or bacterial infections can be detected. It is thereby possible to discard such infected material at an early stage. If the stockplants have been infected with a virus, the plants can be heat treated before the meristems are used for propagation.

Table 1. Heat treatment of mosaic virus infected apples (*Malus domestic* 'Ingrid Marie').

Treatment	Infected plants (%)
Control, untreated stockplants	100
Heat treated stockplants (37°C, whole plant)	100
Shoot tips from stockplants at 37°C for 20 days	17
Shoot tips from stockplants at 37°C for 30 days	0
Shoot tips from stockplants at 37°C for 40 days	3

Stock Plant Material. It is important that the stock-plant material has been selected for genetic uniformity and that the health status in known. If the plants have a virus infection, one must know which virus is present. When plants are grown in a greenhouse, shoot tips from growing plants are used for micropropagation. If the stock plants are field grown, dormant buds are used.

Micropropagation as a Method for Propagating Virus-Free Plants. Micropropagation can be used, not only for making plants free of known diseases, but for the mass propagation of plants. It is possible to produce 1/2 to 1 million plantlets from a single cutting. However, some risks are involved and the method may only be used on genetically stable plants. Examples of genetically stable plants are fruit trees and strawberries. At the other end of the scale is *Pelargonium* which is very unstable. If *Pelargonium* shoot material is grown on a medium with a high cytokinin level, callus and adventitious shoots will develop. Such shoots may have mutated, and those mutated shoots could produce abnormal plants.

Testing Micropropagated Plants for the Presence of Virus. The newly propagated plants must first be declared free of the particular virus(es). The virus may be present at a low concentration after heat treatment, and testing is very important. In addition, the plants must be kept isolated from the surroundings and tested regularly. If the disease has not been found within 1/2 to 1 year, the plants may be declared free of those diseases they have been tested for. For field-grown plants, at least two growing seasons are needed before they may be declared free.

Table 2. Examples of important crop plants which have been made disease free by micropropagation.

Plant	Virus eliminated
Euphorbia pulcherrima	Poinsettia mosaic virus
	Poinsettia cryptic virus
Solanum tuberosum (potato)	Potato virus Y, A, X, S, and M
Kalanchoë blossfeldiana	Kalanchoe latent virus
Populus blasamifera	Populus mosaic virus
Ribes red current group	Raspberry ringspot virus
Malus pumila, M9	Chlorotic leafspot virus
	Epinasty virus