D-30: Thermal inactivation of strawberry latent ringspot virus (SLRV) from seeds of parsnip (*Pastinaca sativa* L)

N Salim
(*Dept. of Biological Science, Univ. of Bath, UK*)
(present address: *Dept. of Botany, Univ. of Sri Jayewardenepura, Nugegoda*)

Virus-infected plants exposed to high temperatures have been reported to produce virus free shoot tips which can be propagated directly as cuttings or may be followed by tissue culture of the vegetative meristems to produce virus-free plants. In contrast, attempts made to eradicate virus in embryo by heat treatment of seeds have been relatively unsuccessful.

The strawberry latent ringspot virus (SLRV) is transmitted in 80-90% of the parsnip (*Pastinaca sativa* L.) seeds, thus, the seeds were found to be the most important source of infection. In the present study attempts were made to eliminate or eradicate SLRV by thermotherapy, from germinating seeds of parsnip grown in tissue culture.

Parsnip plants infected with SLRV were raised in the greenhouse at a temperature range 18-30°C for seed production. The presence of SLRV in mother plants and harvested seeds were confirmed by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) test using an antiserum produced to an isolate of SLRV from parsnip.

Seeds were grown in static cultures, in a medium as that used by Cooper and Walkey, but a solid medium was used instead of a liquid medium.

Infected seeds of parsnip were surface sterilised in 5% sodium hypochlorite containing 1% Tween 20, for 5-10 min, and thoroughly rinsed in 3 changes of sterile distilled water. Then the seeds were dehusked and introduced aseptically to the medium. Seed cultures were transferred to fresh medium at 3 weeks intervals when necessary.

The cultures were held under controlled conditions at 28 and 33°C in growth chambers illuminated 16/day. Seed cultures of control tubes were maintained at 20±1°C. Initially the heat treatments were given for 2, weeks before assaying for the virus. In the subsequent experiments, seed cultures were assayed for virus at 4 day intervals over a period of 16 days.

Seedlings of heat treated and control experiments were assayed for virus by DAS-ELISA test.

The results showed that SLRV could be inactivated in seed cultures grown *in vitro* at 33°C for 2 weeks but not at 20°C or 28°C.

In the subsequent experiments to determine the minimum time required for thermal inactivation of the virus at 33°C, SLRV was detected in 40% of the cultures after 8 days treatment, but virus was not detected in 100% of the cultures after 12 days treatment. In comparison, 80-90% of the cultures grown at 20°C remained infected throughout the experiment.