# Producing Crown gall Resistant Rootstocks for Almond Trees by Genetic Modification

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Regeneration of complete plants from peach tissues, a necessary prerequisite for genetic transformation, has been unusually difficult to accomplish. Two discrete methods may be used, organogenesis and somatic embryogenesis. In organogenesis shoots or roots are induced to form from a specific part of the plant such as leaves and stems. Somatic embryogenesis is the process of embryo formation from any non-gametic source. Both systems require that initial tissues be induced to form new kinds of cells and, subsequently, an entire plant. The most important factors in both organogenesis and somatic embryogenesis are plant growth regulators. When added to an artificial medium containing salts, vitamins, and sucrose, cells of organs such as leaves, stems roots and floral parts have the potential to form complete plants.

In our initial studies we focused on regeneration from leaf sections. We selected this approach because of the well-developed methods of genetic transformation that have been developed using leaf discs and *Agrobacterium tumefaciens*.

## Culture initiation

Upon the advice of Dr. Tom Gradziel we selected a dihaploid Lovell genotype. Seedlings collected from these trees were surface-sterilized, with laundry bleach, the embryos excised and placed in vitro. The resultant shoots were then multiplied in vitro on one of two nutrient media, one based on Murashige – Skoog salts and vitamins and one based on Almehdi and Parfitt salts and vitamins, to provide leaves for subsequent regeneration. These are referred to as juvenile cultures. Cultures were also started from shoots of adult trees in the field. Surface sterilization techniques were more rigorous consisting of treatment with ethanol and laundry bleach. Stem sections containing 2 nodes were placed in vitro on half strength Murashige -Skoog medium containing 2 mg/l benzyladenine (BA).

## Regeneration studies

Leaves were taken from both adult and juvenile cultures, cut in half transversely and placed on test media. Over 20 different media formulations were used with combination of the cytokinins thidiazuron (TDZ), BA, 6 γγ dimethallylaminopurine (2 ip) and the auxins naphthaleneacetic acid (NAA), indolebutyric acid (IBA) and 2,4 dichlorophenoxyacetic acid (2,4-D). Each experiment was conducted twice. Cultures were placed either in low light (40 Wm-2s-1) or in the dark. Cultures in the light produced light green or tan callus whereas cultures placed in the dark produced pale white to yellow callus. Callus was produced on 98% of the leaf sections. Additional treatments were established by moving callus from one hormone combination to another. In spite of these many different treatments, shoots were formed only on two explants. These had been initiated on Almehdi-Parfitt medium containing 6 mg/l BA and glucose rather than sucrose, and had been transferred to half strength Murashige-Skoog medium containing 2 mg/l BA and sucrose. We were not able to repeat this result.

Future studies

It is clear that the reputation of Prunus persica being difficult to regenerate is well-earned. We have chosen to continue the studies by using 2,4-D in order to induce somatic embryogeneisis. In addition, since it is well known that regeneration is genotype-dependent, we have placed two additional rootstock genotypes in culture, 'Nemared' and 'Nemaguard'. Both were started from juvenile material. 'Nemared' cultures originated from greenhouse grown seedlings. 'Nemaguard' cultures originated from excised embryos.