Production of a New Generation of Avocado Rootstocks by Somatic Hybridization and Genetic Engineering

Richard E. Litz
Tropical Research & Education Center
University of Florida
18905 SW 280 St
Homestead FL 33031-3314

Cryopreservation:

Embryogenic avocado culturesthat are being maintained as suspension cultures are briefly exposed to a cryoprotectant solution consisting of 5 % DMSO (dimethylsulfoxide) and 5% glycerol before cooling to -196^{o}C. The cultures are cooled from room temperature (25^{o}C) at a rate of 1^{o} min^{-1} to -75 to -80^{o}C and then rapidly cooled by direct immersion into liquid nitrogen (-196^{o}C). This protocol for cryopreservation of embryogenic avocado cultures has been highly repeatable with several embryogenic culture lines. Following their removal from liquid nitrogen storage, the embryogenic cultures are washed thoroughly with liquid maintenance medium before plating onto standard semi solid avocado maintenance medium containing picloram. Mature somatic embryos derived from cryopreserved cultures have been recovered ('Fuerte', 'Gwen', 'Hass', 'Reed', etc.), and these have germinated

We are using this procedure for storing embryogenic avocado cultures (**Figure 1**). This procedure has demonstrated great utility, and could revolutionize the long-term storage of avocado genetic resources.

Genetic transformation

Embryogenic cultures have been transformed with several gene constructs using *Agrobacterium tumefaciens* as a vector. We are using *A. tumefaciens* strains EHA101 and hypervirulent EHA105.

Genes implicated in the host defense response

1. Antifungal protein gene (AFP)

Transgenic embryogenic 'Hass' cultures were produced through April 30, 2000 after co-culture of embryogenic cultures with *A. tumefaciens* containing the pGPTV binary vector that contained the antifungal protein gene (*AFP*), the *BAR* (basta resistance) selective marker gene, *GUS* and the constitutive 35S promoter. Cultures with resistance to hygromycin were selected, and embryogenic cultures (**Figure 2**) and mature somatic embryos (**Figure 3**) were recovered that strongly expressed the *GUS* scorable marker gene. Mature somatic embryos containing the *AFP* gene began to germinate unevenly, with more synchronized germination in December-January, 2001. Germinating somatic embryos were grown under light conditions (16h photoperiod with 60 μol m^{-2} s^{-1}). Because so many of the somatic embryos were forming shoots without roots (**Figure 4**), we initiated *in vitro* micrografting in order to rescue these regenerants. Somatic embryo microshoots are being grafted onto *in vitro*-germinated avocado seedlings (**Figure 5**). Shoots are now being bulked for evaluation (**Figure 6**).

2. Chitinase and Glucanase genes

Transgenic embryogenic 'Fuerte' and 'Hass' cultures were produced in the year through April 30, 2000 after co-culture of embryogenic cultures with *A. tumefaciens* containing the pGPTV binary vector that contained the chitinase and glucanase genes, the *BAR* (basta resistance) selective marker gene, *GUS* and the constitutive 35S promoter. The development of somatic embryos from transformed cultures has been sporadic, and the transformation was carried out a second time. It is suspected that the developmental pattern of somatic embryo development has been affected by this gene construct. Micrografting (see above) has been attempted with these shoot regenerants; however, their numbers are very low.

3. Chalcone synthase gene

Transgenic embryogenic cultures of 'Gwen' were produced in the previous year (through April 30, 2000) and cultures have been selected that strongly express the *GUS* scorable marker gene. The cultures had been transformed with the pBI121 binary vector that contained the chalcone synthase gene, the *NPTII* (kanamycin resistant) selective marker gene, *GUS* and the constitutive 35S promoter. Somatic embryos have been recovered at low frequency and these are beginning to germinate. We will be micrografting these shoots onto *in vitro*-grown seedlings and plan to micropropagate the established grafted shoots.

Genes implicated in control of fruit ripening

'Booth 7', 'Hass', 'Lula', 'Reed' and 'T362' embryogenic cultures were transformed with the two gene constructs: 1) pAG-4092 containing the SAMase gene, *NPTII* (kanamycin resistant) selective marker gene and avocado fruit cellulase promoter; 2) pMON10117 containing the ACC deaminase gene, *NPTII* selective marker gene and the constitutive 35S promoter. The cultures that were transformed with the ACC deaminase gene appear to have los their morphogenic potential. 'Booth 7', 'Hass', 'Lula', Reed' and 'T362' somatic embryos transformed with SAMase have developed to maturity, and germination is occurring. We are ready to begin ethylene measurements in order to verify that ethylene biosynthesis has been blocked in these transformants.

Somatic hybridization

We initiated a selection protocol for fusing avocado + *P. pachypoda* and avocado + *P. cinerascens* cells. *Persea pachypoda* and *P. cinerascens* nonembryogenic cultures were genetically transformed with pGPTV binary vector that contained the *AFP* gene, the *BAR* (basta resistance) selective marker gene, *GUS* and the constitutive 35S promoter. Callus has been recovered from the protoplasts. Controlled fusions have been made between *Persea* spp. protoplasts and protoplasts from embryogenic avocado cultures that were transformed with the pBI121 binary vector that contained the chalcone synthase gene, the *NPTII* (kanamycin resistance) selective marker gene, *GUS* and the constitutive 35S promoter. Cultures have been recovered on plating medium containing BASTA and kanamycin following fusion, and are growing on maintenance medium.

Figures: *(see next page)*

Figure 1. Procedure for cryoconservation of embryogenic avocado cultures.

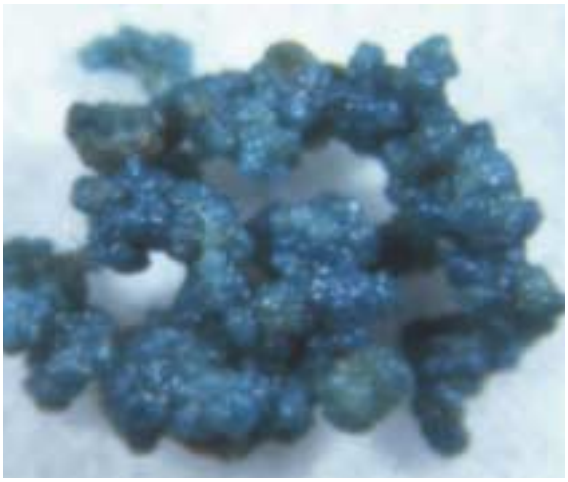

Figure 2. Embryogenic culture demonstrating the GUS reaction for positive genetic transformation.

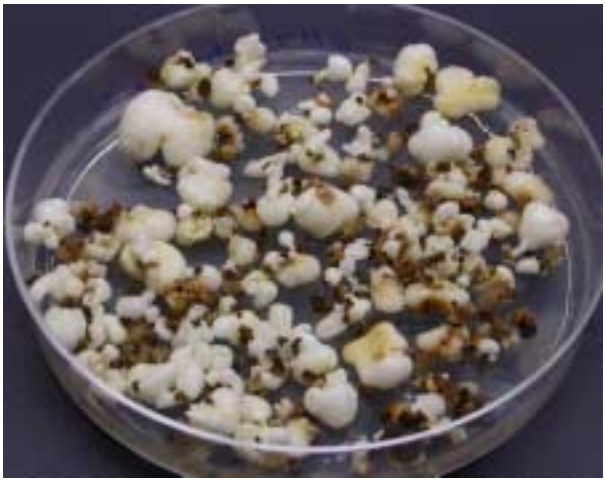

Figure 3. Genetically transformed avocado somatic embryos.

Figure 4. Transformed avocado shoot developing from a somatic embryo.

Figure 5. Micrografting of a transformed avocado shoot onto a seedling rootstock in vitro.

Figure 6. Transformed avocado plantlets that have been rescued following micrografting.