Development of Disease Resistant Hybrid Rootstocks through Cell Culture

Sriema L. Walawage[a], Paulo A. Zaini[a] , Abhaya M. Dandekar[a]
Cooperators: Thomas Gradziel[a],Charles Leslie[a], David Tricoli[a], Takao Kasuga[b,c], Greg Browne[b,a]

[a] Department of Plant Sciences, UC Davis, [b] USDA-Agricultural Research Service, [c] Department of Plant Pathology, UC Davis
✉ *amdandekar@ucdavis.edu*

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Abstract

Almonds (*Prunus dulcis*) are California's #2 agricultural commodity, grown on ~870,000 acres with an annual production of two billion lbs valued at $5.9 billion (farm gate value). In many production areas, soil borne disease, pests and environmental problems besiege almond productivity and reduce profitability. Many of these problems can be addressed by grafting almond scion varieties to resistant rootstocks. Currently, growers use a peach-almond hybrid rootstock that has significantly improved almond productivity for the industry, but which also increases susceptibility to certain disease, pests and environmental stressors. Creating new hybrid rootstock varieties resistant to these ailments is a path forward and the focus of this investigation. *Prunus*-almond hybrid seeds were obtained using parental materials that contain resistance traits of interest. A key objective of our study is to isolate and culture plant stem cells from these hybrid tissues and to develop cell and tissue culture systems to propagate individual hybrid seed sources, leading to the development of novel hybrid rootstocks for almond orchards in California. Somatic embryogenesis system was developed using immature nuts of almond-peach crosses. Plantlets obtained are currently being tested for disease and pest resistance.

Goal: Develop cultures of peach-almond hybrid rootstocks that capture disease resistance present in one of the parental genotypes.

Objectives.

1) Develop embryogenic cultures of peach-almond hybrid rootstocks that capture existing disease/pest resistance traits.

Activity 1: Culture immature embryos from hybrid almond seed.

Stem cells have the potential to generate a whole organism. In plants, they occur in the embryo within seeds, and also in meristematic (growth) tissues in developing and adult plants. When mature flowers receive compatible pollen, fertilization occurs and seed formation begins. As the initial stem cell grows and multiplies, embryogenesis progresses defining the meristems on the root and apical tips (Figure 1 and 2).

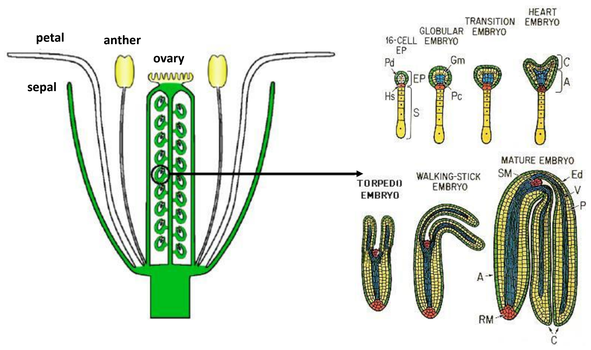

Figure 1. Plant embryogenesis. Image modified from http://www.bio.miami.edu/dana/226/226F09_4print.html

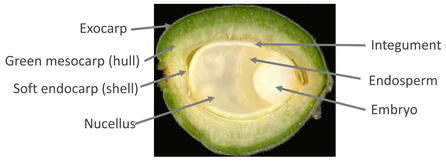

Figure 2. Longitudinal section of almond fruit.

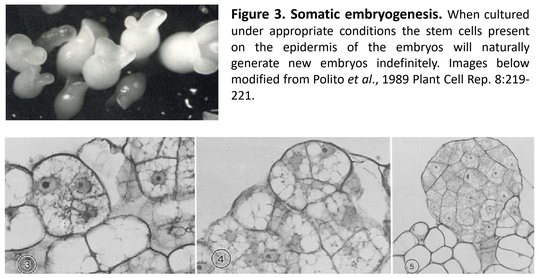

Figure 3. Somatic embryogenesis. When cultured under appropriate conditions the stem cells present on the epidermis of the embryos will naturally generate new embryos indefinitely. Images below modified from Polito *et al.*, 1989 Plant Cell Rep. 8:219-221.

Our laboratory has extensive experience in somatic embryogenesis of walnut and grape. We adopted the procedures to almond, and this year for the first time we successfully established somatic embryogenesis of a almond-peach hybrid intended for rootstocks (Figure 3 and Figure 4). Those somatic embryos were proliferated in no hormone media to obtained embryos for germination (Figure 5). The well developed, healthy, white embryos were dried in a desiccator (Figure 6) for a few days until they were ready to germinate and then cultured them on shoot induction media to obtain hybrid plantlets (Figure 7).

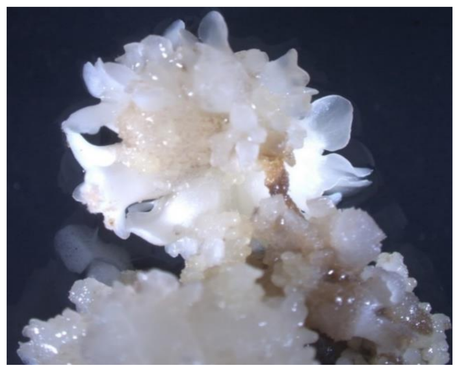

Figure 4. Somatic embryos generated from almond and peach crosses

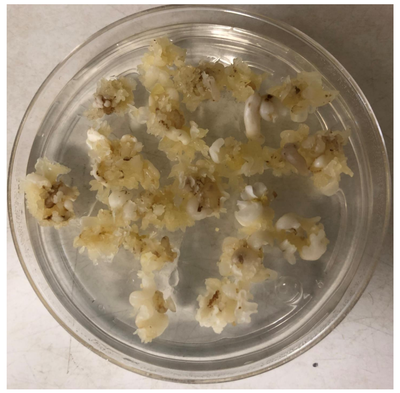

Figure 5. Proliferating embryos

Figure 6. Drying well developed embryos in a desiccator

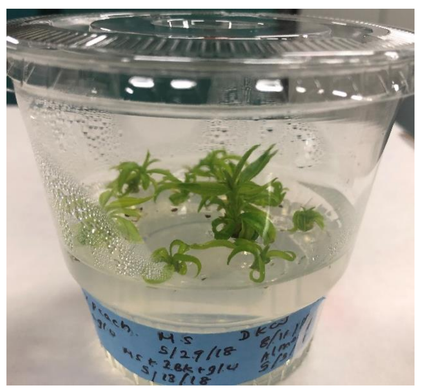

Figure 7. Plantlets obtained by germinating hybrid somatic embryos

2) Micropropagation and validating disease/pest resistance.

Activity 2: Develop micropropagation system for new genotypes.

Media and culture conditions were optimized for shoots and root proliferation to use for validating disease resistance assays in the lab and greenhouse. These proliferated shoots (Figure 8) with well developed root system (Figure 9) can be used for different assays under *in vitro* conditions.

Figure 8. Shoot proliferation in shoot induction media

Figure 9. Root proliferation in root induction media

Activity 3: Validating disease resistance in the lab and greenhouse.

Propagated plants or shoots can be exposed to disease causing organisms and pests in culture and efficacy documented by a disease phenotype assay. Disease resistance will be evaluated in the lab and greenhouse under controlled conditions. Elite lines will be selected and further propagated.

Deliverables

Development of hybrid rootstock cell/tissue cultures to improve the efficiency of propagation of rootstock genotypes available for almond production.

Development of disease and/or pest resistance in rootstock genotypes to decrease the input of chemicals and to make almond production more sustainable.

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