THE HUNTINGTON
Library, Art Museum, and Botanical Gardens

OPENING ROADS FOR MICROPROPAGATION OF AVOCADO IN CALIFORNIA

Final Report to the California Avocado Commission

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Background

The popularity and demand for avocado (*Persea americana*) have increased tremendously. Like many other clonal crops, a few varieties and rootstocks dominate the avocado market. Nowadays, avocado trees are conquering new farming areas. However, at the same time, they are facing new threats, such as new pests and changing environmental conditions. The search for clonal rootstocks that can better adapt to the biotic and abiotic stress conditions is one of the challenges. That could be solved using the large reserve of genes present in the avocado germplasm because there is a diversity in the native areas where the avocado originated. Thus, the creation of Shepherd-Brokaw Orchard at The Huntington as a field avocado collection was our first attempt to preserve avocado diversity. Thirty-three different avocado cultivars, clones with importance in the history of the avocado industry in California, were selected for this purpose. However, ideally, we should be able to secure the avocado crop and its wild relatives with minimal cost, maximum diversity, and no loss through time.

Avocado propagation is very slow and labor-intensive, limiting plants' availability for growers and increasing their final cost. Although various protocols reported micropropagation of some avocado clones of interest, the mass propagation of avocado for most commercial rootstocks and cultivars needs optimization. It is broadly accepted that shorting the propagation time in the nurseries will increase the plants' availability for growers and decrease their final cost. Our approach to clonal micropropagation of avocado primarily focused on rootstocks of interest for the California industry and The Huntington field collection.

Figure 1. Avocado (Persea americana) trees at The Huntington: the Shepherd-Brokaw Orchard

Initiation and establishment of avocado in tissue culture

From September 2018, before the CAC project officially started, selected avocado rootstocks and cultivars have been initiated in tissue culture. Additionally, we collected and introduced juvenile materials to obtain explants for the establishment experiments.

The first step that needed optimization was the sterilization protocol, and contamination levels were drastically reduced in all varieties. Although cleanness and oxidation of initiated buds were dependent on the cultivar and the state of the donor tree, we obtained an optimized surface decontamination method for juvenile or adult avocados. The improvement of the technology incorporated steps like antioxidant incubation and other chemicals to minimize late endogenous contamination. We have collected materials from the selected rootstocks planted at Riverside and Irvine and the cultivars from The Huntington and the Brokaw Nursery avocado field collections. A total of eighty clones are in tissue culture at the reported time. We will continue to back up the field collections to ensure they are safe.

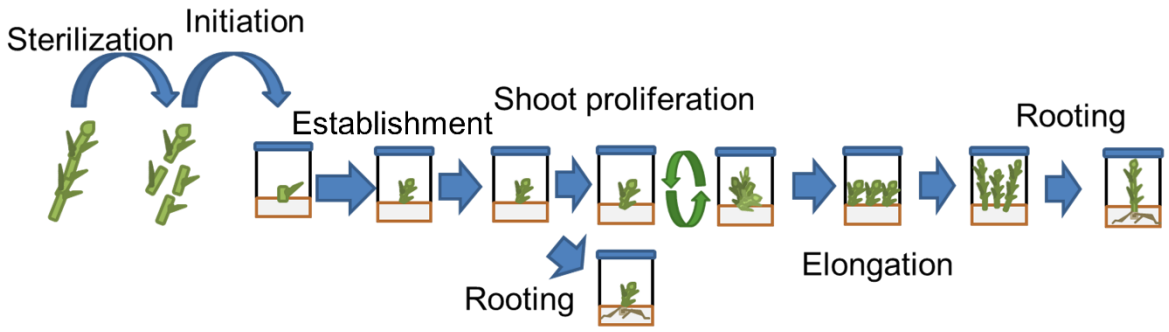

Figure 2. The avocado micropropagation system.

After obtaining clean explants in tissue culture conditions, we could aim for the in vitro multiplication of avocado rootstocks. Before the propagation, a previous stage that we named establishment showed to be critical for avocado. The micropropagation of avocado depends on de cultivar, making the introduction in tissue culture more challenging, especially if adult plants are the donors. The experiments focused on exploring different media containing changes in the concentration of various minerals. The trials allowed the identification of critical components in the medium that we continue to test. The acquired knowledge has led us to media where the avocado shoots initiate and establish in tissue culture. Up to date, more than fifty cultivars and rootstocks have been successfully established.

It is worth noting that the preliminary experiments were made using magnolia tissue culture due to a lack of avocado shoots. We could observe similar symptoms between the two plant groups. The magnolias and the avocados belong to related families, so we expected to apply results from magnolia trials to avocados. Later, we experimented using avocado shoots when we obtained enough explants. Last year, we focused on the response of the avocado cultivars to the culture conditions during the introduction and establishment phases of the in vitro procedure.

Figure 3 *In vitro* avocado shoot at the initiation and establishment phases.

Multiplication

The multiplication phase depends on the cultivar and the state of the explant. The juvenile materials seem easier to establish in culture and reach the multiplication phase earlier. In the third year, some of the experiments aimed to improve the multiplication rate of the previously initiated cultivars and obtain enough explants to study the rooting process better.

We have started an avocado in vitro repository at The Huntington, and more than fifteen genotypes are in active multiplication thanks to the knowledge we acquired. Further experiments are focusing on the multiplication of the rootstocks that are established. This will allow the mass propagation of commercial avocados which is the previous phase to obtain rooted plants that can be acclimated.

Figure 4 *In vitro* avocado shoot at the propagation stage.

Rooting

The lack of explants during part of the project and the difficulty of adult materials to root have delayed rooted plants' obtention. As for the mineral studies, we performed additional rooting experiments with magnolia explants, which could not respond to rooting media in previous tests. We have been able to root magnolias in the avocado salts. Besides, we identified similar symptoms between avocado and magnolia, allowing new experiments with the selected rootstocks.

We performed several rooting experiments and obtained some rooted plants during the third year. However, the work continues to better understand the in vitro rooting process of the avocado. We have shared some rooted plants with our partner at the UCR to further studies of the avocado roots.

Figure 5 Rooting experiments with avocado in vitro.

Micrografting and cryopreservation

Micrografting trials were performed during the last year to test the possibility of using this tool to better regenerate avocado microplants. There are cultivars more sensitive to the tissue culture conditions and the cryopreservation. The preliminary results were very positive. We consider micrografting an alternative technique to back up the avocado field collections.

Figure 6. Avocado micrografting. Shoot tips are dissected and grafted on tissue-cultured rootstock.

On the other hand, experiments in partnership with the University of Queensland resulted in the first cryopreservation protocol for avocado clonal materials. We intend to continue the studies that will lead to the first cryobank for this crop.

Conclusion

During the last three years, this project developed at The Huntington has focused on the micropropagation of avocado, including clonal rootstocks, cultivars, and wild relatives. Since the avocado is very sensitive to the tissue culture conditions, protocols are needed, from the initiation of bud woods to the rooting of microplants. The development of tissue culture protocols for different avocado varieties and rootstocks allows the shortage of avocado propagation cycle, which might positively impact the California avocado industry. Support from CAC also helped create the first in vitro repository of avocados in the USA, complementing the field collections. The tissue culture collection holds more than eighty specimens (more than seventy accessions were collected from adult trees). We expect to secure the avocado genetic resources, which will be used as a source of avocado plants by the California avocado industry, including growers and breeders. Besides, the tissue-cultured avocados at The Huntington are a source for cryopreservation studies, which aims for the long-term conservation of avocado genetic resources.

Figure 7. The Huntington avocado in vitro repository.

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