The Development of a Gene-Editing System in Olive for Florida

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There has been substantial interest in olive cultivation in Florida. It has been suggested as a potential high-value replacement for a portion of lost citrus acreage, and value is expected to increase as disease and climate affect foreign production. Farmers across the state seek new high-value options to diversify their markets and limit exposure to specific losses. Existing olive varieties have been trialed throughout the state with mixed success, owing in part to Florida's diverse soils and weather patterns.

The central drawback to profitable olive production is misplaced genetics. Olive trees were bred primarily for a Mediterranean climate, so trees perform well when those varieties are grown in similar climates. Areas of California and Australia match well with the temperature extremes, chilling hour requirement and photoperiod (length of day), facilitating production in these regions. However, while many of these factors are paralleled in Florida, several issues (like photoperiod, chilling hours, and disease pressure) remain formidable barriers to realistic production.

The traditional solution would be to breed new trees particularly suited for Florida. However, the seed-to-seed cycle for olives is approximately 10 years, so new elite varieties take decades to produce. Making matters worse, the specific traits desired may not even exist in trees relevant to Florida production, making traditional genetic approaches impossible in some cases.

The solution is in gene editing, using modern molecular biology techniques like CRISPR/Cas9. These tools allow precise and custom changes in DNA. The resulting trees contain no "hardware" associated with transgenic ('GMO') technologies, so they are not regulated as genetically engineered plants in the USA. While traditional breeding and trials of existing materials need to continue, there needs to be a parallel effort to create new trees with gene editing.

The goal of this proposal is to initiate a development of low-chill/no-chill olive trees through gene editing. The gene editing process can take cultivars that are nearly fit for the Florida environment and make pinpoint genetic adjustments, making new trees genetically appropriate for Florida in years rather than decades.

Objective 1. Engineering Elite Olive Trees. A gene editing approach would target the vernalization machinery, allowing plants to flower year-'round, where flowering is not gated by chilling. Other edits could remove floral suppression genes, leading to precocious flowering. The genes controlling these processes have been well described and are logical targets for inactivation. Protocols will be devised and specialized for future introduction of disease-resistance genes.

Objective 2. Engineering a Florida Rootstock. It may be possible to produce a rootstock that also break the chilling requirements of the attached scion. Such a rootstock would be tested against multiple scion combinations to identify combinations that best match Florida conditions and production demands. Because the rootstock was genetically altered but the scion was not, the products from the tree would come from unchanged scions and would be acceptable to export markets that do not accept gene editing at this time.

General Experimental Plan.

1. **Optimize tissue culture protocols for at least three olive varieties.** We will adapt existing protocols to Arbequina (for its reports of production in Florida), Empeltre (recognized for its ability to grow in challenging soils) and which has potential as a low-chill variety that may be readily improved for lower requirements.
2. **Introduce edits using the Cas9 system.** Using the well-established gene editing system we will introduce edits into genes associated with repression of flowering and vernalization. We will target at least 4 genes.

The current literature indicates that it takes at least nine months to be able to create genetic changes in olive materials and produce young trees in soil. We will produce as many trees as possible from as many specific editing events. Trees will be examined for the presence of specific edits and those where floral repressors have been abolished will be replicated in culture early in Year 2. Trees will transition to multi-site field trails and monitored for flowering.

What will be gained

1. Adaptation of known techniques to these olive varieties.
2. Development of trees with edits in known floral repression pathways.
3. We will establish working protocols that may be later extended to disease resistance genes.
4. Trees developed can be used both as scions and rootstocks in subsequent mix-and-match experiments.

Pitfalls.

1. While tissue culture is routine in our hands, some crops can be challenging and may require more time to produce successful shoot material.
2. Current guidelines on gene edited material allow for their propagation and growth without special permitting. It is possible that the guidelines could change and block the use of these (and all) gene edited materials in production.
3. Removal of floral repressors may generate trees that are ever-bearing and not conducive to practical production because of the lack of a defined harvest window.

Budget.
The cost of a two-year proposal is $240,000 This breaks down as (subject to change slightly upon discussion with UF financial offices and specific current rates).

Postdoctoral researcher (Ph.D. level) $47,484 per year (federal minimum) + UF mandated fringe at 12. 1%. Total cost is approximately $54,000/ year, or $108,000.

Tissue Culture Technician – $30,000 per year for two years, no fringe, hourly part-time position. This person would assist in the preparation of media and transfer of materials, a process that can be extremely time consuming and best performed by a technician than by a postdoc at higher cost. $60,000 total.

Material and Supplies - We will require the use of a large amount of plastic ware, tissue culture media, hormones, nutrient solutions, pots, soilless mix, and other lab supplies. We also need to rent chamber space. The cost is approximately $20,000 per year for work of this scale. Total cost $40,000

Travel costs – We request $1000 per year to defray the cost of in-state travel. The budget will be used for vehicle mileage costs and lodging when traveling to monitor trees or obtain materials. $2000

Indirect Costs.   The University of Florida mandates an overhead cost of 12% be charged to every award for facilities and administrative costs. ~$30,000 over two years.

The work would be performed from 9/1/19 to 8/31/2021