

Germinating Coffea liberica Rootstock Seedlings for Grafting and Coffee Root-knot Nematode Tolerance

Introduction

In Hawai'i, *Coffea liberica* 'Fukunaga' and 'Arnoldiana' seedlings are the recommended rootstock when grafting coffee for tolerance against coffee root-knot nematode (*Meloidogyne konaensis*) [1,7]. *C. liberica* seeds can be more difficult to germinate and require an extended germination period than *C. arabica* (e.g., 'Typica', 'Caturra') seeds, so the purpose of this document is to help growers obtain good germination of *C. liberica* seeds and establish healthy rootstock plants for grafting purposes.

For additional and more detailed information about coffee root-knot nematode (CRKN), cultural management of nematodes, sampling procedures [3], replanting and more, visit <u>https://www.hawaiicoffeeed.com/coffee-root-knot-nema-tode.html</u>.

Why it's important to graft coffee onto nematode-tolerant rootstock

There currently are no chemicals or nematicides that can legally be used to treat for CRKN in Hawaii's coffee orchards. As such, grafting susceptible scions onto 'Fukunaga' and 'Arnoldiana' rootstocks is the only effective means for managing this root pest [2,5,7]. Planting or replanting with grafted trees (Fig. 1) allows coffee to thrive in soils infested with CRKN. Without tolerance, these nematodes cause damage to the coffee root system, resulting in reduced vigor, yield, and overall decline. Susceptible coffee trees struggle to survive, even with adequate rainfall, irrigation and fertilization, and often are killed as a result of over-bearing dieback during years of high production due to the extensive root damage caused by CRKN [4,6].

Supplies and tools for germination and transplanting

• C. liberica 'Fukunaga' and/or 'Arnoldiana' rootstock cherries or seeds gathered from known mother plants (Fig. 2). Obtaining seeds from clones of mother trees from UH CTAHR's Kona Research Station is highly recommended. Research [7] shows variation in rootstock seedlings and the potential loss of tolerance to CRKN when seeds are taken from seedlings (vs clones) of mother plants. That is, C. liberica does not seem to breed true, at least for CRKN resistance.



Figure 1. A 6-month-old, grafted tree with 'Kona Typica' scion on 'Arnoldiana' rootstock ready to be field planted. Planting holes should be dug as deep and wide as possible to aid root development.

December 2022 Subject Category: Fruit, Nut, and Beverage Crops, FN-65

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- Sterile, soilless media.
 - o Peat mix (Pro-Mix, Sunshine, etc.)
 - o Vermiculite
 - o Perlite
- Clean planters (plastic tray, box, container, pot, etc.) with good drainage for sowing seeds. Planters should allow media depth of at least 2½ to 3 inches so the roots do not bend.
- Clean forestry tubes, tall pots, bags, etc. with good drainage for transplanting seedlings.
- Dishwashing detergent, a freshly made 10% chlorine bleach and water solution, and/or at least 70% ethyl, ethanol, rubbing, or isopropyl alcohol for cleaning, decontamination and sanitation of tools, planters, pots, etc. as needed.



Figure 2. Large, vigorous, full-grown 'Fukunaga' and 'Arnoldiana' trees planted in coffee root-knot nematode infested soil in Kona. This 50+ year-old tree shown is about 20 feet tall and 20 feet wide.

Preparing the rootstock seed materials

Obtaining seeds of *C. liberica* 'Fukunaga' and 'Arnoldiana' can be challenging. Some coffee producers have their own 'Fukunaga' or 'Arnoldiana' trees and may be willing to sell or share seeds. Farmers can also reach out to their local CTAHR Cooperative Extension agent for assistance in locating and obtaining rootstock seeds or mother plants. Seeds may be received as cherry or dried parchment (Figure 3). Since *C. liberica* seeds take longer to germinate than *C. arabica* scion material, be sure to establish rootstock seedlings at least 2 to 3 months in advance to accommodate time needed to germinate, transplant, and grow the rootstock prior to grafting. The following are steps for preparing suitable rootstock plants.



Figure 3. *Coffea liberica* 'Arnoldiana' cherries (left) and dried seeds in the parchment form (right). After fermentation and during drying, caramelization of remaining mucilage on coffee parchment can cause seeds to be darker in color than those completely free of mucilage.

If receiving fresh, unprocessed cherry:

- 1. Harvest and pulp the ripe *C. liberica* cherries. Do not refrigerate or freeze the cherries or seeds.
- 2. Soak the seeds (parchment) in water to begin the fermentation process to remove the thick, sticky mucilage around the hull or wet parchment. Discard any seeds that rise to the surface and float. Seeds that float indicate hollowness and poor development. Do not change the water until fermentation is complete.
- 3. Ferment the seeds for approximately 24 to 48 hours at room temperature. Do not boil, chill, refrigerate, or freeze the seeds during fermentation. A thorough fermentation is important to remove the pectin and mucilage around seeds, and reduce mold and rot issues during germination, drying, and storage.

Small seed batches may take longer to ferment because there are fewer natural coffee yeasts, enzymes, and microbes compared to large seed batches. To improve and/ or hasten the fermentation or demucilation process, you could 1) physically remove the mucilage around the seeds, 2) add a pectinase or yeast to the seeds and water in the fermentation tank, or 3) add warm water (86-95° F; 30-35°C) to the fermentation tank to activate natural coffee yeasts, enzymes and microbes present on the seeds [10,8]. If adding warm water, use a thermometer to determine water



temperature and then add the warm water to the coffee seeds to start the fermentation process. Do not heat the water with seeds in it.

Sow the seeds immediately or dry them to store. Freshly harvested, pulped, fermented, and then rinsed 'Fukunaga' and 'Arnoldiana' seeds may have improved viability and germination over dried seeds [1]. Scarification of the seed coat (parchment) using a file or sandpaper will allow moisture and air to the seed and embryo, which could enhance germination.

When storing coffee seeds for germination, store at the highest moisture content possible without causing mold or rot of the seeds during storage.

Sowing fresh or dried seeds for rootstock plants

1. Prepare a mixture of 2:1 perlite to peat mix, or a 3:2 perlite to vermiculite mix, or a combination of soilless media that provides good moisture and airflow during seedling germination.

Note: Your optimal media mix and ratio may vary from our suggestions. More frequent watering or irrigation may require a higher ratio of perlite for media aeration. Less frequent watering may require a higher ratio of vermiculite, or a peat mix for better water-holding capacity.

- 2. Mix and dampen the media with water, but not to sopping wet. Break-up any compressed, dry portions of peat for thorough media wetting.
- 3. Fill a clean planter (tray, box, container, pot, etc.) with the moist media and sow the seeds about ¼- to ½-inch apart and then cover the seeds with about ½-inch of media. Do not compact the media over the seeds. Your planter should provide good drainage.
- 4. Place the planter at least 18 inches off the ground and away from soil, splashing water, weeds, and other plants that could contaminate the planter with unwanted pests and diseases.

Note: Bottom heat has been shown to expedite the germination of seeds [9].

- 5. Keep the media moist but not waterlogged, and do not let the media dry out or seedlings wilt.
- 6. Protect the seeds and seedlings from birds, snails and slugs, and rodents.

Note: If media moisture and humidity is not an issue, seedling wilt or death may be the result of a bacterial, fungal, or insect problem. Identify and treat as necessary. 7. Be patient. Depending on factors such as seed viability, seed freshness, media moisture, temperature, and depth of sowing, germination can take between 2 and 4 months. Check weekly to every fortnight for sprouting seedlings.

Note: If germination does not occur within 4 months, uncover and inspect some of the seeds. Look for roots or root tips arising from the seeds as an indicator that the seeds are still viable but taking longer to germinate than expected.

If the seeds are no longer alive, try to determine the reason(s) for poor germination. Was the media too compact? Too wet? Too dry? Did you forget to water the planter regularly and the media dried out? Is the irrigation functioning properly? Were the seeds too old? Dried too long? Is there a bacterial, fungal or insect problem? Could slugs or snails have eaten the seedlings? Did the seeds rot? Are they still there or are the seeds missing? Once the problem is identified, treat for the issue and make modifications as necessary for better germination in the future.

8. Transplant individual seedlings after they have germinated and are fully upright. The seedlings can be transplanted when the parchment still covers the cotyledons (Fig. 4A) or when the cotyledons have opened. However, waiting too long to transplant can result in seedlings with deformed taproots (e.g. J-rooting, bent), especially if seeds are sown in shallow planters.

Note: Cull seedlings with deformed or poorly developed roots (Fig. 4B), lack of vigor, disease, insect, vertebrate or mechanical damage prior to transplanting. Only the healthiest seedlings should be used for rootstock.

9. Transplant the seedlings into clean forestry tubes or tall pots or bags that allow the taproot to grow without twisting, bending, or growing upward. Use a sterile media mixture similar to Step 4. When transplanting, be careful to not break or bend roots and do not compact the media, which could cause soil moisture and root growth issues. Water after transplanting.

Note: When transplanting, a general slow-release or controlled fertilizer (Nutricote 13-13-13, Osmocote 14-14-14, etc.) can either be top-dressed (1 tsp. per plant) on the media or incorporated (mixed well) into the media at a rate of 1 tbsp. of fertilizer per 1 gallon of media. Do not top dress or mix granular and non-coated fertilizers into or on the media, as doing so can cause damage to the seedling roots and leaves, or plant death.

Consider also adding calcium fertilizer if needed.

- 10. Place the repotted seedlings at least 18 inches off the ground and away from soil, splashing water, weeds, and other plants that could contaminate the planter with unwanted pests and diseases.
- 11. Keep the seedlings well-watered. Maintain moist but not waterlogged media for good seedling growth. Excessive drying of the media can cause plant stunting and death. Note any leaf flagging, then water immediately and thoroughly. Check, modify, and increase irrigation or hand-watering as necessary for proper soil moisture and rootstock growth.
- 12. Rootstock seedlings are ready for grafting when at least three pairs of true leaves emerge (Fig. 5). However, they can also be grafted following complete expansion of the cotyledons as long as the root system is vigorous enough to support the graft.
- 13. Monitor and treat for slugs and snails, insect pests, and fungal or bacterial diseases as needed.
- 14. Follow instructions for coffee grafting provided by the Extension publication "A Pictorial Guide to Coffee Grafting." <u>https://www.ctahr.hawaii.edu/oc/freepubs/</u> <u>pdf/F_N-54.pdf</u>



Figure 4. (left) Seedlings that are fully upright and ready to be transplanted as in Step 8. (right) Rootstock seedlings showing good and bad root development. Those in the bottom row should be discarded due to poor rooting.



Figure 5. Healthy *C. liberica* 'Fukunaga' seedlings that were transplanted into dibble tubes and are now ready for grafting



Acknowledgements

This work was funded by the County of Hawai'i's Department of Research and Development, Hawai'i Department of Agriculture, USDA National Institute of Food and Agriculture Hatch and Smith-Lever project 20-013 and integrated project HAW02033-H funds managed by the University of Hawai'i at Mānoa's College of Tropical Agriculture and Human Resources, and was part of a collaborative project between UH-CTAHR and USDA-ARS DKI PBARC titled "The Long-term Responses of Coffee Rootstocks to Root-knot Nematode in Kona."

The authors thank Dr. Roshan Manandhar of UH-CTAHR and Dr. Roxana Myers and Dr. Tracie Matsumoto of the USDA-ARS Daniel K. Inouye Pacific Basin Agricultural Research Center for their valuable time and review of this publication.

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