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A Beginner's Guide to Begonias: Micropropagation and Tissue Culture[1]

Julian Ginori, Sandra Wilson, Wisnu Handoyo, and Heqiang Huo[2]

Introduction

Due to genetic variability found throughout the genus, many species and varieties of *Begonia* are primarily propagated via asexual propagation. For mass clonal propagation to maintain select traits or for controlled genetic studies, micropropagation and tissue culture serve as alternative means of propagation. This publication explains the differences between micropropagation and tissue culture, providing a breakdown of the stages involved in each process. It was developed to educate students and growers on the uses and benefits of tissue culture and micropropagation. While these terms are sometimes used interchangeably due to similar procedural stages, they differ in their source of explant materials and primary objectives. Micropropagation serves as a tool, in addition to propagation via cuttings (Ginori et al. 2022a) or seeds (Ginori et al. 2022b), with the goal of multiplying plants of interest.

Plant tissue culture is a biotechnological technique that involves the *in vitro* (in a controlled laboratory setting) cultivation of plant cells, tissues, or organs on a nutrient-rich growth medium under sterile conditions. It is used for a variety of applications, including plant breeding, genetic modification, conservation, and mass propagation.

Micropropagation, a specialized subset of tissue culture, is primarily used for rapid mass production of genetically identical plantlets (clones) from shoot tips, nodal segments, or axillary buds. It is widely used in commercial horticulture, agriculture, and forestry to propagate elite plant varieties with desirable traits. During micropropagation, shoots develop directly from meristems, shoots, nodes, and other actively dividing tissue, a process known as direct organogenesis. While tissue culture follows a similar framework, it involves additional plant regeneration pathways beyond direct organogenesis. These pathways include somatic embryogenesis, where somatic (non-reproductive) embryos form first and develop into shoots later, and indirect organogenesis, where a callus (a cluster of undifferentiated plant cells with no organized structure) forms first, followed by the development of shoots. It is worth noting that somatic embryogenesis can occur either directly or indirectly.

Micropropagation includes five key stages: Stage 0, donor plant selection; Stage I, establishment and stabilization; Stage II, shoot multiplication; Stage III, Root formation; and Stage IV, Acclimatization (Figure 1; Davies et al. 2018). See Figure 1 for a summary of these stages.

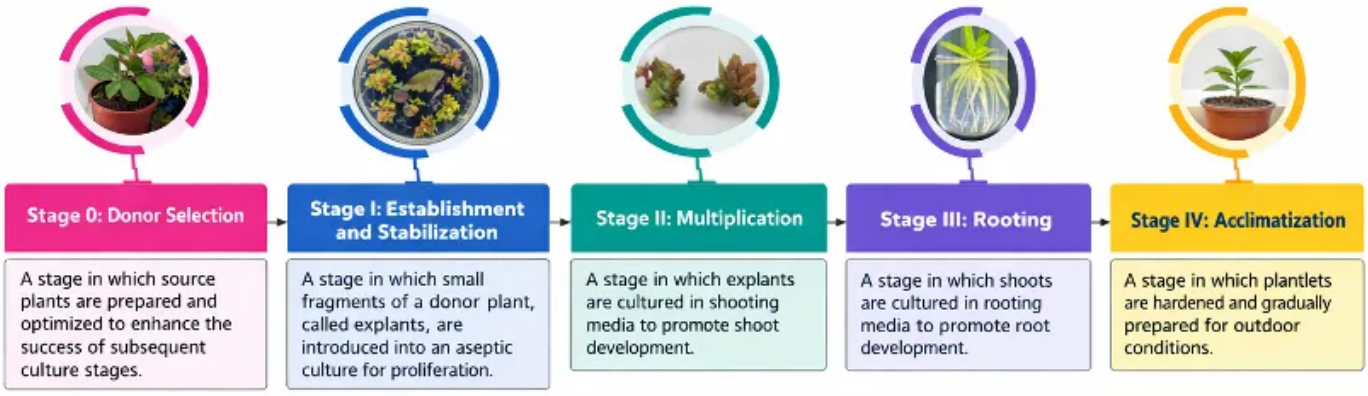

Figure 1. Overview of the key stages to tissue culture and micropropagation.
Credit: Heqiang Huo and Julian Ginori, UF/IFAS

Stage 0: Donor Plant Selection

Stage 0, the donor plant selection stage, is arguably the most critical step in the tissue culture process, as it sets the foundation for success in later stages. This step defines goals, determines what plant material and media formulas to use, and prepares the work area to reduce the possibility of contamination in samples. Good planning in Stage 0 leads to higher success in future stages. Prior to introducing any plant to tissue culture, a review of literature pertaining to the species of interest is essential. This preliminary step can save weeks in trial and error as well as significant costs in chemicals and other supplies. Tissue culture is all about making minor adjustments to media formulas and plant materials. Therefore, finding peer-reviewed, published research on the plant material of interest could provide an advanced starting point to better refine media formulas before introducing any tissue cultures.

Stage I: Establishment and Stabilization

The goal of this stage is to establish a small fragment of a plant, called an explant, into aseptic (contamination free) culture and achieve stable shoot production. This relies on using an artificial medium that contains nutrients needed for explant growth. Possible explants include seeds, embryos, organs, and more; however, the most common explants include leaves, shoots, and roots from which a callus may be induced (Figure 2). Determining which plant material has the highest success rate for the species of interest is key to an efficient tissue culture pipeline.

Figure 2. Examples of potential plant material from begonia for use in micropropagation and tissue culture protocols. Credit: Wisnu Handoyo, UF/IFAS

After collecting the plant material, the next step is to sterilize its surface. To accomplish this, run the plant material under water to remove any loose debris, then wash the material using 70% ethanol. Follow this with a rinse of sterile autoclaved water and then wash with bleach (Figure 3). Depending on the explant and the species of interest, the concentration of bleach may vary. A good starting point is 10%, gradually increasing the concentration of bleach until the most suitable concentration is discovered. The active ingredient in bleach is sodium hypochlorite. Standard household bleaches contain 5%–6% sodium hypochlorite. A 10% bleach solution contains between 0.5% and 0.6% sodium hypochlorite. It is important to acknowledge the concentration of sodium hypochlorite in the bleach before diluting it in water to make bleach solutions. For *Begonia semperflorens-cultorum*, this concentration is sufficient, but higher concentrations can be utilized for species with thicker stems or leaves. Follow the bleach wash with multiple repetitions of sterile water rinses. There is significant variation in the sterilization process. Some plant species may require multiple ethanol or bleach washes or may require longer or shorter durations of washes and rinses. Optimization of this process can be achieved through prior research in peer-reviewed journals in conjunction with trial and error.

Plant material constitutes half the battle. The other half is determining the media formulas. Each stage of the tissue culture process will require different media formulas containing varying concentrations of nutrients, hormones, and antibiotics (Figure 4). If published research already exists for a given species, using that information will expedite the process of determining the optimal media for the species of interest.

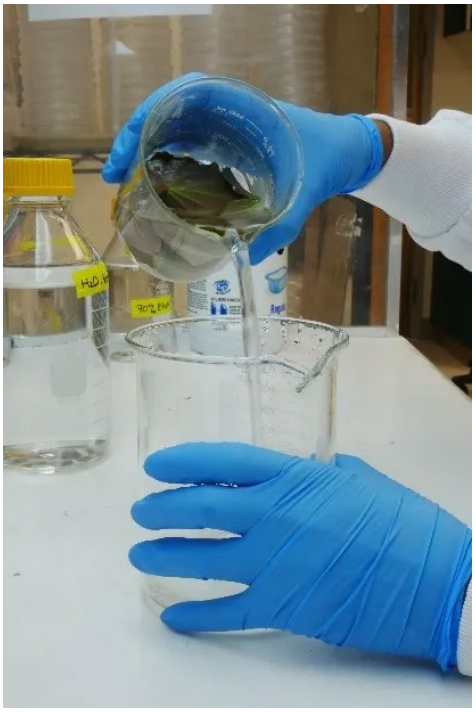

Figure 3. The cleaning of plant material in an ethanol solution. Credit: Wisnu Ardi, UF/IFAS

Figure 4. Various nutrient supplements for media.
Credit: Wisnu Handoyo, UF/IFAS

A sterile work environment is integral to the success of introducing plant species to tissue culture. Proper personal protective equipment (PPE) is also an absolute requirement. Be sure to sterilize the laminar flow hood, all hand tools, and other supplies prior to each use.

At this point, the sterilized plant material is ready to be transferred to premade medium. The components that go into the medium vary significantly by species and stage. Broadly speaking, these components include water, gelling agents, nutrient salts, vitamins, amino acids, carbohydrate sources, and plant growth regulators. A good starting point would be a simple Murashige & Skoog (MS) medium. This is a premade mixture that includes all the macro- and micronutrients a typical plant requires for tissue culture. Add other components such as carbohydrates and gelling agents to the MS medium base to suit the needs of the species of interest. Plant material prepared for tissue culture often cannot produce its own carbohydrates, which is why the addition of sugars is so important. In the case of *Begonia semperflorens*, a 4.4g/L concentration of a standard MS medium, 30g/L of sugar, and 8g/L of agar are sufficient to initiate plant material.

An equally important component is the gelling agent. This is what will transform the media solution from a liquid into a semi-solid, gelatinous substance, perfect for the plant material of choice to rest on and eventually root in. The most common gelling agent is agar, but for situations where the yellow tint of agar could become an issue, there are gellan gum-based gelling agents, such as Phytagel, that produce colorless gel. Higher concentrations of gelling agents will result in a firm medium, while lower concentrations will result in the opposite (Figure 5). For begonia, 10g/L of agar is a suitable starting point. This provides a firm surface for the plant material to rest on, which is preferable in early stages where explants will need to be transferred to new petri dishes as contamination occurs. A consistent theme present throughout the entire tissue culture process is the need to test different media components and concentrations to find the one that works best for the species of interest. To properly homogenize the media solution, it will have to be autoclaved and allowed to cool slightly before plating in petri dishes.

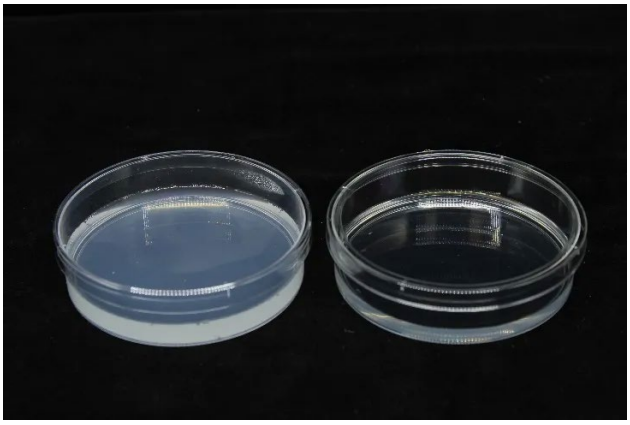

Figure 5. Comparison of agar (left) and phytogel (right).
Credit: Wisnu Handoyo, UF/IFAS

Once cooled, carefully pour the media into the petri dishes (Figure 6) or other types of containers (Figure 7). These come in different sizes and depths to suit the needs of every tissue culture project. Propagators can also pour varying quantities of media into the dishes. Typically, using a light, medium, or heavy pour depends on the stage of the process. A light pour is recommended for plant material that is first being introduced to the media since plant material will most likely be replaced as contamination occurs. A benefit of lighter pours is the reduction of wasted material, which can result in significant cost savings over time.

Figure 6. Delicate pouring of agar solution into a petri dish.
Credit: Wisnu Handoyo, UF/IFAS

Figure 7. Various media containers to accommodate different sizes of plant material.
Credit: Wisnu Handoyo, UF/IFAS

After making the media, plating it in the petri dishes, and allowing it to solidify, it is time to transfer the plant material to the petri dishes. Even with the greatest care and a lengthy sterilization process, contamination is likely to be present in the samples (Figure 8). The last step of Stage I is to ensure all plant material is sterile. This involves constantly checking the petri dishes for signs of contamination and subculturing/transferring the clean plant material to new petri dishes as soon as possible to avoid cross-contamination. This may require multiple cycles of subcultures/transfers to remove all contamination, yielding sterile plant material. Whenever transferring plant material to a new petri dish, seal the dish with simple plastic cling wrap to lock in the moisture and keep out contamination.

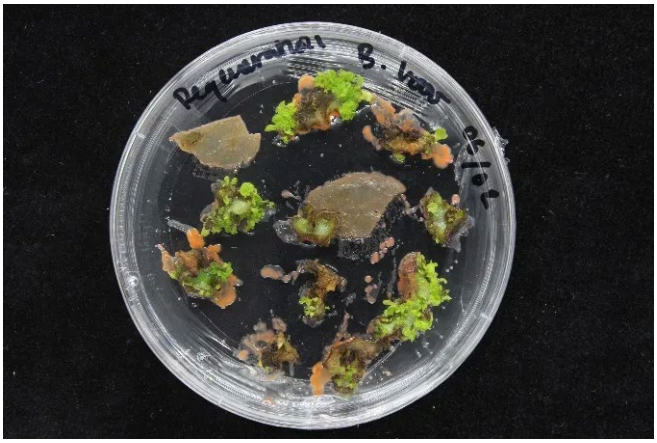

Figure 8. Visible contamination within a tissue culture sample.
Credit: Wisnu Handoyo, UF/IFAS

Stage II: Shoot Multiplication

Once the explants have been successfully integrated into a sterile environment, it is time for Stage II, multiplication. This stage, which is straightforward compared to the previous stages, involves modifying the media to promote shoot development. Note that the development of roots is not important in this stage because the media provides all the nutrients for the explants, and the newly developed shoots will be excised to new petri dishes. The objective is to produce as many explants as needed in a short period of time and utilize as little space as possible. This is the true highlight of tissue culture and micropropagation: the ability to produce many clonal explants in a very small space compared to something like shoot cuttings in a greenhouse. To induce shoot regeneration, use plant growth regulators (Figure 9). Cytokinin is a good option to promote the growth of shoots, but the most efficient concentration needs to be determined for the species of interest. In the case of wax begonia, 1mg/L of benzyl adenine (BA) is sufficient to promote shoot development. Some species may require a combination of cytokinin and auxins. In some cases, roots can form during multiplication, depending on the species.

Figure 9. Multiple young shoots forming on the explants of wax begonia. Media consists of 1mg/L of benzyl adenine (BA) to promote shoot growth.
Credit: Wisnu Handoyo, UF/IFAS

Once new shoots are formed, excise the shoots from the original explant material and transfer them to new petri dishes, where they will undergo the process of forming their own roots. Excising shoots involves using sterile forceps and a scalpel to gently slice the new shoots from the source material, taking care to avoid damaging the shoots with the forceps. Use a new set of sterile forceps and a new scalpel when transitioning to a new petri dish to avoid potential contamination.

Stage III: Root Formation

Once the explants have multiplied, it is time to move forward to Stage III, rooting. In this stage, the explants, which should consist of individual shoots excised to fresh media, will be fed a combination of nutrients and hormones to promote the development of roots, thus transforming them into fully functioning plantlets with their own shoots and roots. First, transfer the explants to a new medium supplemented with auxins to induce rooting in the regenerated shoots. Certain species have more trouble rooting than others, which is why multiple concentrations of varying auxins need to be tested to find the one that suits the species of interest. In the case of *Begonia*, known for its unique ability to develop roots from nearly any plant material, an injection of auxins is not entirely necessary, as opposed to working with other plant genera. A low concentration of 0.1mg/L of 1-Naphthaleneacetic acid (NAA) is a good starting point for most species of begonia. After a few weeks in tissue culture, new roots will form.

Stage IV: Acclimatization

This stage is where tissue culture and micropropagation split paths. Until this point, these plantlets have grown in complete sterile conditions, with all their nutrient needs and ideal environmental conditions met. This stage will prepare the plantlets for life outdoors. They have had no instances of disease, pests, or drought stress and, thus, are extremely fragile. Ideally, environmental conditions should

be modified one at a time to ensure the highest rate of plantlet survival. First, transfer the plantlets to a fine soilless medium, avoiding large pieces of bark, as these could impact the plantlets' ability to survive. Some scientists prefer to autoclave their soil in the beginning to reduce the exposure of plantlets to pests or diseases too early in the acclimatization process, although many may consider this impractical. Once in a substrate, keep the plantlets in the controlled environment room under a humidity dome and allow them to adapt to their new substrate (Figure 10). Humidity is key during this stage. These plantlets have been in a high-humidity environment their entire lives and thus need slow exposure to more natural humidity levels. Some scientists skip using the humidity domes and simply crack the lids of the tissue culture vessel or use filter membranes to allow air exchange. Sometimes, providing mist and shade can help acclimate them to warmer, sunnier conditions. Allow them to grow into their pots and fully acclimatize prior to their final use. If not, they may risk high mortality rates or misleading data in studies. Acclimatization can take one to two weeks, depending on the species. Once the plantlets have adapted to a lower humidity environment, they are ready to be transferred to the greenhouse for continued growth.

Figure 10. Example of a humidity dome in an environmentally controlled room.
Credit: Wisnu Handoyo, UF/IFAS

The entire process, from Stage 0 to Stage IV, can take anywhere from a few weeks to a few months, depending on the amount of clonal material required, the ability of the plant material to regenerate shoots and roots, and the duration of the acclimatization process. In the case of *Begonia semperflorens*, this process can take approximately three months.

Figure 11. Wax begonia clones developed through the micropropagation process.
Credit: Wisnu Handoyo, UF/IFAS

Conclusion

As a review of the steps, first determine the best plant material (explant) for tissue culture integration. Surface sterilize the plant material and transfer it to the tissue culture media. Divide the sterile plant material into hundreds or thousands of explants through multiplication of regenerated shoots. Then, induce rooting to transform those explants into plantlets. Finally, acclimatize those plantlets to natural environmental conditions. There are many moving parts in micropropagation, but once a good protocol is in place, it allows for mass clonal propagation of plants in a short period of time and a limited amount of space. That is the true benefit of tissue culture and micropropagation.

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[2] Julian Ginori, alum (Ph.D. Summer '23) and former graduate research assistant, Department of Environmental Horticulture, UF/IFAS Mid-Florida Research and Education Center, Apopka, FL; Sandra B. Wilson, professor, UF/IFAS Department of Horticultural Sciences, Gainesville, FL; Wisnu Handoyo, graduate research assistant, Department of Environmental Horticulture, UF/IFAS Mid-Florida Research and Education Center, Apopka, FL; Heqiang Huo, associate professor, Department of Horticultural Sciences, UF/IFAS Mid-Florida Research and Education Center, Apopka, FL; UF/IFAS Extension, Gainesville, FL 32611.