Stages of Micropropagation

The development of plantlets in vitro can be divided into three major steps or stages (28). In Stage I, called the explant or establishment stage, a suitable plant part (e.g., explant) is disinfected and cultured aseptically in a culture medium. Stage I material is then utilized for Stage II, which is termed the multiplication phase. The objective of Stage II is to rapidly increase the number of propagules by somatic cell embryogenesis, enhanced axillary branching, or adventitious bud formation. Stage II material may be recycled by subculturing the material back on a proliferation medium or it may be passed to Stage III. Stage III is called the conditioning or pretransplant stage; in some cases special dormancy conditions may have to be satisfied before rooting will occur. Recently, a fourth stage, Stage IV, has been described during which acclimatization of the plantlet to in vivo conditions occurs. In some cases, in vivo rooting may occur during Stage IV.

The stages of micropropagation were first described by Murashige (28) in 1974. He developed three stages of micropropagation for the in vitro multiplication of plants. These stages have now been adopted for a wide variety of plant material propagated both at the research and commercial level. The stages as described by Murashige not only describe the procedural steps of micropropagation, but these steps generally coincide with points in the process where environmental or media changes are required. The three stages described by Murashige include the establishment stage (Stage I) whereby the explant is established under aseptic conditions. Stage II or the multiplication stage is where the number of propagules is increased or multiplied. Stage III or the rooting stage is where the in vitro-derived shoots are rooted and conditioning of the plantlets for transfer out of the culture tube begins. Two other stages can now be added to the process: Stage 0 which involves the preparation of the stock or mother plant from which the primary explant is to be derived and Stage IV which is a stage that involves the transfer of the plantlets to an environment external of the culture tube.
STAGE 0—STOCK PLANT SELECTION AND PREPARATION

Careful attention should be made to make certain the stock plant is a typical variety or cultivar, clearly resembling other plants of the same species or cultivar. The stock plant should be disease-free, preferably maintained in either a growth chamber or greenhouse. The stock plants should be on a regularly maintained pesticide and fertilizer program.

The time of the year in which the explant is taken may effect the results of the micropropagation program. Changes in temperature, day-length, light intensity, and water availability throughout a year will affect the levels of carbohydrates, proteins, and growth substances in the stock plant thus subsequently affecting the response of the explant in vitro. Best results are generally achieved when the explant is taken during the active phase of growth. A possible exception is when the explant is to be derived from a storage organ. Explants can be derived from plant tissue which is in the dormant phase of growth but certain points must be considered. If the material is taken during the dormant phase of growth, the dormancy requirements may have to be met or broken. Plant material taken during the dormant or resting phase may be broken by removing bud scales which may contain bud break inhibitors. Soaking shoot tips in GA₃ or placing the shoot in a refrigerator are techniques which may also be used to break dormancy.

STAGE I—ESTABLISHMENT OF AN ASEPTIC CULTURE

The first step in any successful tissue culture program is the selection of a suitable explant source. Almost any plant tissue or organ can be used as an explant, but the degree of success obtained will depend upon the culture system used, the species being cultured, and the removal of surface contaminants from the explant. The primary goal of Stage I is to obtain a large percentage of explants free from surface pathogens. Disinfecting the surface generally involves washing the tissue, followed by sterilization with one or more disinfectants.

Washing the explant under running tap water for 30 min to 2 hr greatly reduces the amount of contamination on explants derived from field-grown material, highly pubescent tissue, roots, and/or storage organs. This technique has been successful in reducing contamination in the Gesneriaceae and the Lilium genus (15). Washing the explant with soapy water before placing it under running tap water may further reduce the number of pathogens present on the explant or make them more accessible to sterilant. Other presterilization techniques that have been used by the author include soaking the tissue in a mild fungicide...