



Preparing Tissue-Cultured Banana Plantlets for Field Planting

Eden A. Perez and Cerruti R² Hooks

Department of Plant and Environmental Protection Sciences

The use of BBTV-free plantlets as planting material is an important component of an integrated disease management (IDM) program aimed at limiting the spread of banana bunchy top disease caused by banana bunchy top virus (BBTV). A plant may become infected with BBTV if it is fed on by an aphid that contains the virus. Only the banana aphid, *Pentalonia nigronervosa*, is capable of transmitting BBTV. However, it may also spread through the use of infected suckers as planting material and other infected plant tissues used in banana propagation. The time interval between aphid inoculation of BBTV and the initial appearance of disease symptoms (i.e., the incubation period) may be many weeks. Thus, plants that appear symptomless may already contain the virus, and if these symptomless plants are used to establish new plants or plantings, this will help spread BBTV throughout the orchard and to off-farm locations to which the planting material is taken. Using clean planting material is, therefore, the first line of defense against BBTV spread. Scouting for and aggressively rogueing (destroying) infected plants is also a must-do procedure in a field-sanitation program.

This publication describes basic requirements for preparing tissue-cultured banana plantlets in greenhouse nurseries and for field planting. To help growers fight the spread of BBTV and mitigate its negative impact on the banana industry, the CTAHR Agricultural Diagnostic Service Center (ADSC) will have tissue-cultured banana plants available for purchase as part of their seed distribution program.

Micro-propagation

A tissue-culture technique in which propagules are cloned from tissue taken from a single plant is known as micro-propagation. Micro-propagating the banana

shoot tip is the main method used for fast propagation of banana plants. However, once a plantlet has been developed through the tissue-culture technique, care must be taken in moving it from its sterile, artificially controlled environment to the more exposed greenhouse and less protected field conditions.

During the last phase of banana micropropagation, steps are taken to grow individual plantlets and prepare them for adaptation to the external environment. These plantlets are small and not yet capable of surviving in the soil. Mostly, the initiated shoots are in clusters, and roots are absent. To encourage root development and shoot elongation, the clusters are separated and transferred to a special media that promotes root development. This final phase takes 7–10 weeks.

Hardening of plantlets in cultured vessels, and preparing them for transplanting

The hardening, or acclimatization, process begins while the plantlets are still in vitro, i.e., growing in the culture vessels. Acclimatization is the physiological adaptation of an animal or plant to changes in climate or environment, such as light, temperature, or altitude. In this case, banana plantlets are being acclimated from cultured vessels to greenhouse and outdoor environments. Just prior to acclimatization, the plantlets have shoots and roots but are not yet capable of supporting themselves in the soil. The rooted shoots are about 6–8 cm (2.5–3 inches) tall and receive nutrients from an artificial medium that contains major nutrients. To help acclimatize the plantlets, the caps or tops of the culture vessels are removed for a period of at least several days. The recommendation is that a minimum of 10 days be allowed for in vitro acclimatization.

Extra care should be taken when young, rooted



1. Individual banana plantlets ready for planting into plant pots or poly-bags. Photo: E.A. Perez

plantlets are being transferred from the culture vessels to the external environment. Tissue-cultured plantlets are delicate because they have been grown under low-intensity artificial light and high humidity. Thus, they are not adequately “hardened off” and may readily lose water when exposed to ambient conditions. When plantlets are removed from their containers, the artificial, gel-like agar medium is gently washed from the roots. This is important, because the agar contains sucrose and other nutrients that can serve as a medium for growth of disease-causing organisms. The pre-hardened, tissue-cultured plantlets with well branched roots (Photo 1) are now ready for planting into potting media in a greenhouse nursery. Plantlets purchased from ADSC will already be hardened off or acclimatized, unless a special request is made for plants in culture vessels. The hardened-off plants should be quickly prepared for the greenhouse nursery.

Potting media, plant containers, and nursery area

Plastic pots or polyethylene bags (“poly-bags”) are suitable nursery containers. Black poly-bags are widely used because of their low cost, apparent simplicity, and convenience. Small poly-bags 15 cm (6 inches) in diameter and

20 cm (8 inches) tall (Photo 2) are sometimes preferred over plastic pots because they are lighter in weight and still allow vigorous plant growth.

A potting medium providing for the best growth of tissue-cultured banana plantlets should have a good balance between its moisture-holding and drainage characteristics. This allows the roots to obtain sufficient water without “drowning.” A greenhouse potting mixture we use for growing out banana plantlets includes 2 parts of a commercial growing media mixture (Sunshine Professional), 1 part perlite, and 3 parts vermiculite (medium to coarse grade). A slow-release fertilizer is also added to the potting medium prior to planting; alternatively, liquid fertilizer, such as Miracle Grow[®], can be used. One of the advantages of using slow-release fertilizer is that if it is adequately applied to the soil mix, no other fertilizer will be needed before transplanting. However, if a liquid fertilizer is used, multiple applications may be required.

The potting medium should be uniformly moistened with a small amount of water before it is placed into the nursery containers. This will help to station the roots during planting. The plantlet roots should be covered with 1–2 cm of potting soil above the upper part of the roots. Water them immediately after planting. Control of moisture in the medium is a critical factor for suc-



2. Young banana plantlets 4–5 weeks after transplanting from culture vessels. Photo: K.H. Wang

Successful establishment. Automatic misting systems in the greenhouse should be closely monitored during the initial weeks after transplanting to make sure that the potting medium is not becoming too wet. If too wet it could become an environment favorable for algal, fungal, and bacterial growth. Photo 2 shows young banana plantlets 3–4 weeks after transfer from culture vessels to planting pots.

Potted banana plantlets should be placed in a nursery area with partial shading that can initially reduce light by 50 percent. This may be achieved by using shade cloth or fishing net. Reducing the sunlight progressively hardens the plantlets, thus preparing them for exposure to full sunlight, which they will encounter after transplanting. To maintain BBTV-free plants, it is recommended that plants be maintained in an insect-proof environment, such as one covered with a protective screen to prevent exposure to aphids that may be carrying BBTV. In addition to the banana aphid, other aphid species, whiteflies, and spider mites are some common greenhouse pests that may colonize banana plants in the greenhouse. Infestations by these pests can be controlled by spraying an insecticide when their presence is observed. However, after the plants are transplanted into field, these pests are typically not problematic.

Transfer and establishment of plantlets into the field

Plants are generally allowed to acclimatize in the greenhouse for approximately 2 months and to reach a height of about 20 cm (8 inches) before they are transplanted to the field. The poly-bags or pots can easily be removed, and the rooted plants are then ready for transplanting. It is sometimes recommended that the potted plants not be watered prior to transplanting. If the potting soil is a little dry, it may more readily stay attached to the root system during removal of the plant from its container. The growth of tissue-cultured plants may seem to be slow during the initial weeks after transplanting, and the plant may initially produce several suckers. This is due to residual presence of growth regulators that they were exposed to during micro-propagation. The young plants will typically produce a new leaf approximately every five days during their early development (within the first two months). An aggressive scouting program for BBTV will include inspecting these young plants every five days. The length of time after transplanting tissue-cultured plants to harvest depends on the nutrient status of the soil and the cultivar type. In general, plants may be harvestable within 9–10 month after transplanting into the field.

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