Weaning (acclimatization) of *in vitro*-produced banana plants

John Charles Robinson[1], Victor Galán Sáuco[2]*

[1] Du Roi Lab., P.O. Box 1147, Letsitele 0885, South Africa, duroilab@mpu.co.za

[2] Inst. Canario Investig. Agrar. (ICIA), Apartado Correos 60, 38208 La Laguna, Tenerife, Canary Islands, Spain
vgalan@icia.es

* *Correspondence and reprints*

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Abstract — Introduction. The protocol describes a method for promoting the acclimatization and protection of rooted *in vitro* banana plants between rooting in the laboratory and their establishment in a nursery. The principle, key advantages, starting plant material and time required are presented. **Materials and methods**. This part describes the weaning house installation, the weaning medium used to transplant the vitroplants, the two steps for acclimatizing the plantlets: receipt of rooted laboratory plants in flasks and planting, and factors affecting the growth of the plantlets in weaning conditions (light, misting system, temperature, humidity, wind, pest control and nutrition). Possible problems for troubleshooting are listed. **Results**. Under normal conditions, new roots appear 4–5 days after the start of weaning, and new leaves start to grow within 8–10 days. At the end of the weaning phase, young plants are healthy, well developed and they can be established in a nursery.

South Africa / Spain / *Musa* sp. / methods / vitroplants / weaning

Sevrage (acclimatation) de vitroplants de bananier.

Résumé — Introduction. Le protocole décrit une méthode qui favorise l'acclimatation et la protection des vitroplants racinés de bananier entre la phase d'enracinement en laboratoire et leur établissement en pépinière. Le principe, les principaux avantages de la méthode, le matériel végétal de départ et le temps requis sont présentés. **Matériel et méthodes**. Cette partie décrit l'installation de la structure de sevrage, le milieu de sevrage utilisé pour transplanter les vitroplants, ainsi que les deux étapes nécessaires pour acclimater les jeunes plants: réception des plantes de laboratoire racinées en flacons et leur plantation, et facteurs affectant la croissance des jeunes plants en phase de sevrage (lumière, brumisation, température, humidité, vent, contrôle des parasites et nutrition). Des problèmes éventuels sont énumérés. **Résultats**. En conditions normales, les nouvelles racines apparaissent en 4 à 5 jours après le début du sevrage, et les nouvelles feuilles commencent à se développer en 8 à 10 jours. À la fin de la phase de sevrage, les jeunes plantes sont saines, bien développées et elles peuvent être transférées en pépinière.

Afrique du Sud / Espagne / *Musa* sp. / méthode / vitroplant / sevrage

1. Introduction

Application

This protocol aims at promoting the acclimatization and protection of rooted *in vitro* banana plants between rooting in the laboratory and their establishment in a nursery.

Principle

The key to successful weaning is to keep young, sensitive plants free from any form of environmental stress for the entire period. Therefore, the ideal weaning system can be regulated to control the most important environmental factors (light, water, temperature, humidity and wind).

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Figure 1.
Tender new banana vitroplants, just removed from the laboratory flask.

Figure 2.
Newly-transplanted banana vitroplants (right) and nearing completion of the weaning period (left).

Key advantages

The key advantage of this weaning protocol is that the process allows for the protection and stabilization of young rooted plantlets coming out of a sterile laboratory environment, until they are large and hardy enough to be transferred to the harsher environment of a nursery shade house.

Starting material

This prototol uses rooted *in vitro* plants in flasks. The material required comprises very tender plantlets which are actually removed from their protective laboratory flasks inside the weaning house. The plants are typically (2 to 5) cm tall, with brittle white stems, three to five soft green leaves, and several long white primary roots growing from the base of the stem. The plants are packed close together inside the flask, but are separated carefully from their agar gel-rooting medium to start the weaning protocol (*figure 1*).

Time required

In an ideal weaning system, five to eight weeks are necessary from the receipt of rooted plants in the weaning house to having them ready for sending to the hardening nurseries. Under warm tropical conditions, a (5 to 8) cm plant will be produced in five to six weeks, whereas under cooler subtropical conditions seven to eight weeks are required (*figure 2*).

2. Materials and methods

The weaning house

The weaning house can be a normal glasshouse, or else constructed with a semi-circular metal frame covered with clear polyethylene sheeting and with a double-door entrance for hygiene. Inside, the trays should rest on tables raised to working height, and an automatic misting system as well as shade netting should be installed. Under extremely hot external conditions (above 35 °C), it is preferable to have a wet wall at one end, and extractor fans at the other end, to draw cool air through and prevent overheating. Alternatively, adequate ventilation has to be provided. Under cool external conditions (below 18 °C), a heating system should be installed to blow warm air through (*figure 3*).

The weaning medium

The common medium used in weaning trays is peat moss or sphagnum moss, but other types of milled organic material can also be used, *e.g.*, coconut fiber (coir) (*figure 4*). These can also be mixed in various proportions with inert additives such as perlite, vermiculite or styrofoam (*e.g.,* 70% peat moss, 30% vermiculite and 1 $kg \cdot m^{-3}$ dolomitic lime to adjust pH, if necessary). The medium has to be sterilized or pasteurized before use

since *in vitro* plants are highly vulnerable to pathogen infection at this stage, particularly by *Pythium* or *Phytophthora* spp. [1].

Use a weaning medium with an air-filled porosity (AFP) as close as possible to 20%, which is considered optimal. The AFP of a medium should not be below 10% and should not be more than 25%. If AFP is higher than 20%, then the dry-out rate is faster and irrigations have to be more frequent. If the AFP is lower than 20%, the dry-out rate is slower and irrigations can be less frequent. If the AFP is less than 10%, there is a high risk of compaction, water logging and inhibited root growth.

Note: AFP is determined in the laboratory by saturating a sample of the weaning medium with water and draining it, then measuring the volume of water that drains out. The medium has to be free-draining. The volume of water draining out corresponds to the volume of the air pores (AFP), which is expressed as a percentage of the volume of the medium left after draining. A detailed protocol for measuring AFP can be found at http://www.geocities. com/RainForest/Canopy/8771/air_porosity. htm.

Keep water-holding capacity (WHC) of the weaning medium between 40% and 50%.

Note: WHC is determined in the laboratory by weighing a sample of weaning medium wet after free-draining, followed by drying and weighing the medium dry. The mass of water as a percentage of the wet medium mass is the WHC. The lower the AFP the higher the WHC and *vice versa*. A detailed protocol for measuring WHC can be found at http://www.geocities.com/CapeCanaveral/Hall/1410/lab-Soil-06.html.

Keep the pH of the weaning medium between 5.5 and 6.5. If lower than this, buffer with dolomitic lime.

Note: an easy protocol for measuring soil pH can be found at http://www. geocities. com/CapeCanaveral/Hall/1410/lab-Soil-04.html.

Fertilize plants during weaning by one of two options: (A) Pre-mix the weaning medium with a slow-release fertilizer (SRF) containing a NPK ratio of [2:1:2]. An acceptable mix would be 3–5 kg SRF·m^{-3} of medium (3–5 g·L^{-1}), depending on the nutrient release period [usually (70 to 100) days] and the intended duration of weaning. Any surplus release period will benefit the young transplants in the nursery; (B) A soluble fertilizer application system (fertigation) can be installed, but do not exceed 60 mg·L^{-1} nitrogen in the applied solution. Applications are usually made weekly. Such a fertigation program should be recommended for the particular circumstances by a competent nutrition expert.

Figure 3.
Inside a plastic weaning house, showing trays with transplanted banana vitroplants on moveable tables, shade cloth protection, and overhead misting system.

Note: inoculation with arbuscular mycorrhizae may be beneficial for the plant by increasing its growth rate, facilitating nutrient uptake and increasing disease tolerance [2].

Figure 4.
Coconut coir weaning medium showing good air-filled porosity and excellent banana rooting potential, but causing salinity damage on lower leaf.

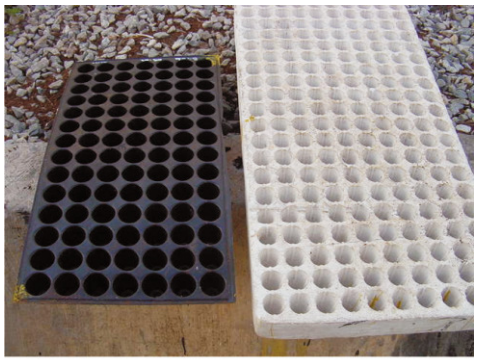

Figure 5.
Two common weaning trays: white molded polystyrene (right) and black plastic (left).

Figure 6.
Rooted banana plants in agar gel, just removed from a laboratory flask (left), separated from the gel (right) with the dipping and cleaning tank (background).

The usual weaning tray is made of 32 density molded polystyrene and contains round or square tapered holes or plugs (30 mm diameter × 60 mm deep) which have a drainage hole at the bottom. Variations in plug size and shape are used according to preference.

Note: there should be approximately 100 to 200 holes per tray and 40 cm^3 per hole, giving a density in weaning of about 550 plants·m^{-2} [3]. Spray plugs in polystyrene trays before use, using an approved sealant such as 'Styroseal' to prevent roots from penetrating the walls of the tray, which would later make plant extraction difficult. Alternatively, use plastic trays which are more hygienic, easier to clean and allow easier extraction of plugs (*figure 5*).

Acclimatization of the plantlets

• Step 1. Receipt of rooted laboratory plants in flasks and planting

Under humid conditions inside the weaning house, take off the lid and remove the clump of agar-gelled plants gently from inside the flask, and separate them. Place individual plants in a bucket of clean water to wash off agar. To assist in planting, trim lengthy roots back to 2 cm. Alternatively, remove all roots neatly before planting [4] (*figure 6*).

Grade the plants into three sizes (*i.e.,* small, medium and large), relative to the average size of plants received. Discard the very small plants up to about 5% of the total plants received. Also, discard any abnormal-looking plants and/or obvious mutations.

Note: careful size grading at this stage permits uniform growth in weaning trays and makes grading at the nursery stage much easier *(figure 7)*.

Dip the whole plant in a fungicide mixture before planting in trays. Captab or a locally used fungicide can be used at recommended concentrations.

At planting, fill the plug holes in the tray with dry medium and level off. Water the tray thoroughly, which causes the level of the medium to drop about one-third down the plug hole.

Insert the plant carefully into the medium in the plug using a probe instrument to create a planting hole. Do not force the plant into the medium. From a separate bucket of wet medium, top up the plug hole to support the plant. Compress the medium lightly around the plant.

Note 1: do not plant small and large plants in the same tray since this variability will be enhanced during subsequent growth.

Note 2: at all stages of the extraction, dipping and planting process, handle the plants extremely carefully since they are fragile, brittle and easily damaged.

After planting, thoroughly water the trays and transfer them to the tray tables in the weaning house.

• Step 2. Inside the weaning house

Control of environmental conditions and appropriate management of the cultural techniques should be as follows (*figure 3*):

– Shade: place the trays under 80% shade netting for the first 3 weeks (20% light transmission), then change the shade netting to 40–50% for the next 3 weeks, or until the end of the weaning period. Arias and Valverde recommend progressing from 70% shade for the first 3 weeks to 30% shade until completion [5]. Plants could even be exposed to full sunlight to harden them before transferring to the nursery [1].

Note: too much light causes burning of the young plants, especially during the first 2 weeks of weaning.

– Misting system: use a permanent automatic misting system to apply 10 s mist every 20 min in hot weather and 10 s every 60 min in cooler weather, via fine mist nozzles. This should be enough to keep the medium moist in a closed greenhouse. Check the correct functioning of the misting unit at regular intervals and ensure the young leaves remain wet at all times during the first 10 days [4]. Excessive leaf drying can easily be identified by curled leaves and burnt edges and tips.

Note: the rooting medium should release a drop of water when squeezed hard between the fingers but should not be saturated, otherwise roots will suffocate and rot, and the plant will remain stunted or die. Do not water manually with a coarse sprinkler head in an effort to keep the leaf surfaces wet in hot weather because water application is excessive and irregular, and the medium becomes saturated. If the medium remains saturated, reduce misting and/or irrigation frequency and increase ventilation. Misting of leaves may become less important than irrigation towards the end of the weaning period [3].

Water quality is important in weaning. Make sure the conductivity of the irrigation water is less than 30 $mS \cdot m^{-1}$ and the concentration of total soluble salts (TSS) is less than 250 $mg \cdot L^{-1}$ [6]. In addition, it is critical the source of irrigation water should be free from any pathogenic nematodes of banana; otherwise, any early nematode infestation in weaning can be multiplied in the nursery or field.

Figure 7.
Uniform transplants in a weaning tray, indicating effective banana plant size-grading at planting.

Note: if these salt levels are exceeded, a marginal leaf burn could be experienced on the tender leaves of young plants.

– Temperature: maintain the growing temperature between 25 °C and 32 °C at all times for the optimum balance between photosynthesis and plant development [1].

Note: *t*emperatures around 22 °C are optimal for photosynthesis and dry matter assimilation, whereas temperatures around 32 °C favor plant development, stem elongation and leaf expansion [7]. If temperatures are continually below 25 °C, the plant becomes short, squat and sturdy with short internodes and small leaves but with a strong root system. Physiologically the plant is healthy and strong but extra time is needed to reach the required nursery transplant size. On the other hand, if temperatures are continually above 32 °C, the stems become elongated, with large floppy leaves and light green coloration, and the root system may become weak. With temperatures approaching 40 °C, respiration is dominant over photosynthesis, reserves are used up, and the plant becomes physiologically weak and prone to dying back [8]. At temperatures above 35 °C, activate the wet wall and extraction fans, or, if there is no

wet wall, lift the sides of the weaning house and increase misting frequency.

– Humidity: maintain relative humidity at more than 90% during the first week of weaning and thereafter within the range of 50% (minimum daytime humidity) to 90% (maximum night-time humidity) at all times, to reduce the rate of leaf drying [4].

Note: if humidity drops below 50%, the chances of rapid leaf drying and plant wilting are increased, especially on new transplants. With a cover of glass or polyethylene sheeting, and frequent misting, it should be possible to keep humidity high.

– Wind: never expose young banana plants to wind stress at any stage during the weaning period.

Note: wind, or even a breeze, removes the boundary layer of high humidity from around the leaf surface. This in turn causes the leaf surface to dry and leaf temperature to increase, resulting in an increased risk of leaf wilting, leaf margin scorching and plant dieback. If vents are opened in the weaning house walls to reduce high temperatures, take care to keep wind movement away from the leaf surfaces, and increase the misting frequency.

– Pest control: apply preventive spraying for aphids, caterpillars or leaf spot fungus, if any of these pests and diseases are prevalent. For caterpillars, use a *Bacillus thuringiensis* formulation and, for leaf spot fungus, spray a systemic fungicide, at the recommended concentration. Repeat these sprays after 6 weeks in the weaning house, or just prior to removal of plants for transferring to the nursery.

Note: the last spray of fungicide protects the young plants from disease development during early establishment in the nursery.

– Nutrition: the slow-release fertilizer or the recommended fertigation program (see under weaning medium) should provide for the nutritional needs of young plants for the duration of their stay in the weaning house. However, if leaves turn pale green or yellow in color, apply extra sprays of a NPK foliar feed with trace elements, at 7–10-day intervals during weaning. For example, a chelated trace element formulation which also contains around 10% nitrogen in the concentrated product can be sprayed at the rate of 1 $g \cdot L^{-1}$ water (*i.e.,* 100 $mg \cdot L^{-1}$ nitrogen in the spray solution).

Note: phytosanitary treatments indicated in this publication are given as examples of possible treatments for pest control. Such treatments must be chosen according to the national regulations.

Troubleshooting

Many problems can occur in the weaning house:

(a) The youngest leaves can be mechanically scarred at their tips. Causes could be (a) that leaves touch the flask lid; (b) that there was rough handling at planting.

Solution: it is superficial damage; plants will grow out of this symptom. Do not allow plants in flasks to push against the lid. Be very careful when removing, dipping and planting the young plantlets.

(b) Leaves wilt and hang down; stem bases turn brown and die. This is due to overwatering causing root dieback.
Solution: water the medium only when it has had a chance to dry. Avoid saturation.

(c) Young leaves turn prematurely yellow and then brown. No new cigar leaves appear. The cause would be a *Pythium* infection originating from either the weaning medium or the irrigation water.
Solution: use sterilized medium and chlorinated water. Alternatively, drench with suitable fungicide, such as propamocarb hydrochloride or mancozeb/metalaxyl-m, at the recommended concentrations.

(d) Plant growth slowing down; leaves become smaller and internodes shorter. This is due to night temperatures becoming too low.
Solution: switch heaters on if minimum temperature drops below 18 °C.

(e) Plants become lanky with large floppy leaves; roots are poorly developed. The cause would be day temperatures becoming too high and plant development becoming too fast.
Solution: activate the wet wall and extraction fans when daytime temperature rises to 35 °C; increase ventilation in the weaning house.

(f) Older leaves get round necrotic spots (*figure 8*). This is due to leaf spot disease. Plants are left too long in the weaning phase or there is overcrowding in trays.
Solution: remove plants earlier from weaning. Alternatively, spray plants with systemic fungicide such as propiconazole at the recommended concentration.

(g) The medium disintegrates at extraction: there is insufficient root growth, or roots become stuck inside the wall of the plug hole.
Solution: leave plants longer to develop more roots; reduce growing temperatures to stimulate roots; spray tray plugs with 'Styroseal' before planting. Change from polystyrene to plastic trays.

(h) A brown ring of necrotic tissue is observed around the leaf margin. This would be a salinity burn, usually due to either saline medium (*e.g.,* coir), or saline irrigation water.
Solution: flush salts from coir medium; use less saline medium (*e.g.*, peat moss); use irrigation water with conductivity less than 30 $mS \cdot m^{-1}$.

(i) Plants are tall and lanky with red stems, bulbous stem bases, pale green/yellow leaves, and root-bound plugs are observed (*figure 9*). The cause is overmaturity due to excessive time in weaning.
Solution: do not leave plants in weaning for longer than 8 weeks. If a delay is required, reduce tunnel temperatures.

(j) There is a plant size variability in the tray at maturity. Some plants are excessively tall and some small and stunted with a poor root system. Plants that are too small have to be replanted. Causes could be (a) poor size-grading at planting; (b) overcrowding in trays; (c) uneven management throughout the tray.
Solution: apply diligent and accurate size-grading at planting; plant fewer transplants in the tray; ensure environmental effects are equalized across the tray.

(k) Brittle white stems of new transplants break or become damaged. Causes could be that (a) plants were too long and lanky at removal from the flask; (b) there was rough handling at planting into the weaning medium.

Figure 8.
Mature weaned banana plant showing *Cerscospora* leaf spotting from overcrowded, humid conditions in the weaning house.

Figure 9.
Banana plants left too long in weaning, resulting in overmature, senescent symptoms and root-bound plugs.

Solution: produce shorter and thicker plants for transplanting; handle with care at transplanting.

(l) White flecks and streaking appear across young leaves. This is due to variegated or masada mutations.
Solution: be vigilant in the weaning tunnel and remove all such mutant plants from the system.

3. Typical results obtained

The standards given below refer to the expected results from weaning of medium-

sized Cavendish cultivars ('Williams' or 'Grand Nain'). Under normal conditions, new roots appear 4–5 days after the start of weaning, and new leaves start to grow within 8–10 days. When weaning is completed, the following plant standards are expected:

– Plants have an average height of 8 cm from the plug level to the junction of the two youngest leaves. Minimum height should be 5 cm and maximum height 12 cm.

– Plants have at least three healthy, dark green leaves on the top half of the pseudostem and, preferably, five leaves at the time of delivery to the nursery.

– The stem of the plant is firm and straight, not soft or flexible. The diameter of the stem at plug level is a minimum of 5 mm.

– The stem of the plant is firmly anchored in the plug of medium and not loose or bent.

– Leaves are normal in shape and color, and they are not folded, misshapen, senescent or flecked with any deficiency, disorder or disease.

– When plants are extracted from the weaning tray, the plugs detach easily without the plant shifting its position or the roots pulling out of the medium. Roots do not grow into the sides of the plug hole and stick there (which causes disintegration of the medium when the plant is extracted).

– Upon extracting the plants, the bottom half of the plugs do not detach from the top half and the roots are totally ramified throughout the medium, binding the latter into a firm 'root ball'.

– Upon extracting the plants, the roots are visible on all sides and they are white and healthy, with root hairs.

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