Field establishment of *in vitro*-produced banana plants

Víctor Galán Saúco[1]*, John Charles Robinson[2]

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

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

Field establishment of *in vitro*-produced banana plants.

Abstract — Introduction. This protocol describes a method for obtaining well-established banana plants in the field, both for open-air and greenhouse cultivation, from hardened tissue culture nursery plants. The principle, starting material and time required are presented. **Materials and methods**. This part describes the process of land preparation, both in the case of planting and replanting, including preplant fertilizer requirements and irrigation recommendations, and the main horticultural practices appropriate both for planting out in the field and for the first months after planting. Special attention is given to correct irrigation, a key issue in the establishment process. Possible problem areas for troubleshooting are listed. **Results**. At the end of the process, vigorous plants with uniform growth and high yield potential are planted out and become optimally established in the commercial field.

South Africa / Spain / *Musa* sp. / methods / vitroplants / plant establishment

Établissement en champ de plants de bananiers produits *in vitro*.

Résumé — Introduction. Ce protocole décrit une méthode pour obtenir des plants de bananiers bien établis en champ, à la fois pour une culture en plein air et en serre, à partir de plantes issues de culture de tissu et endurcies en pépinière. Le principe, le matériel de départ et le temps requis sont présentés. **Matériel et méthodes**. Cette partie décrit le processus de préparation du terrain, dans le cas de plantations ou de replantations, dont la fertilisation avant plantation et les recommandations pour l'irrigation, ainsi que les principales pratiques horticoles appropriées à la plantation en champ et aux premiers mois après plantation. Une attention particulière est donnée à une bonne irrigation, un point clé dans le processus d'établissement. D'éventuels problèmes et leurs remèdes sont énumérés. **Résultats**. À la fin du processus, des plantes vigoureuses de croissance uniforme et à fort potentiel de rendement sont plantés en extérieur et peuvent être établis de façon optimale en exploitation commerciale.

Afrique du Sud / Espagne / *Musa* sp. / méthode / vitroplant / établissement de la plante

* *Correspondence and reprints*

1. Introduction

To achieve the best results with tissue culture banana plants either for commercial banana production or research trials, it is absolutely imperative that correct land preparation is carried out, followed by correct planting out procedures. Both of these are one-off operations that are critical prerequisites in banana production. If done correctly, they will ensure that the plants respond fully to optimal post-plant management. However, if either or both of these operations have been done incorrectly, it is very difficult to rectify the mistakes after establishment and yield potential is immediately reduced. This is always important, but especially when cultivating banana in expensive greenhouses, a practice now common in certain areas of the subtropics, where the very high investment must be followed by quick and high returns [1]. The

Fruits, 2010, vol. 65, p. 43–51

DOI: 10.1051/fruits/2009041
www.fruits-journal.org

following protocols are taken from experience gained in the Canary Islands and South Africa, and have been drawn up for the benefit of both researchers and growers who are planning their next planting program for establishing tissue culture banana plants in the field.

Application

Planting out tissue culture banana plants in the field both in the open air and under greenhouse conditions.

Principle

Optimizing conditions before, during and after field establishment to achieve maximum production potential from tissue culture banana plants.

Starting material

Well-hardened tissue culture banana plants of 20–50 cm height in nursery bags.

Time required

Several months are required for the necessary land preparation to be completed before field establishment. Banana plants can be considered to be field-established at 8 weeks after planting. Some of the protocols listed here will, however, refer to events occurring during the first 6 months after field planting.

2. Materials and methods

Field preparation

• Step 1

Choose and prepare the soil for banana planting well in advance. Choose the best soil for bananas (deep, fertile and well-drained clay/loams), and select the correct orientation (Northern aspect in the Southern hemisphere and *vice versa)*. In cold areas, avoid planting at the bottom of slopes near riverine vegetation, and strictly avoid soils which are poorly-drained [2].

• Step 2

If banana replant land is to be used, first fallow this land or preferably rotate it for a year or two with grasses, legumes or sugarcane. Preferably, do not plant bananas immediately after bananas, mainly to avoid nematode contamination and to rectify cation imbalances. Virgin soil conditions impart the most vigor to a new plantation. Avoid planting in *Fusarium*-infected soils and eliminate all cucurbit plants from the vicinity of the plantation.

• Step 3

Take representative soil samples well before planting, and send them to a laboratory for pH and chemical analysis, soil texture analysis and for preplant fertilizer and lime recommendations.

• Step 4

Take extra soil samples and send them to a laboratory for nematode analysis and for possible nematicide recommendations at planting.

• Step 5

At least 2 months prior to planting, incorporate recommended fertilizers, lime and manure by spreading evenly over the surface and incorporate uniformly by plowing down to 40 cm depth. If large clods are formed after plowing, disc the surface smooth before ripping.
Note: if fertilizers are only applied at planting, place phosphate fertilizer (if recommended from soil analysis) at the bottom of the planting hole and mix them with enough topsoil to avoid direct contact with roots. For nitrogen and potassium fertilizers, apply them as a top-dressing around the planting station, one month after planting.

• Step 6

If installing irrigation, take a water sample from the irrigation source and analyze its suitability for irrigation (pH, conductivity,

total soluble solids, salinity, cations, bicarbonates). If the water is unsuitable for irrigating bananas, choose a new site with suitable irrigation water [3].
Note: good quality irrigation water has below 500 $mg \cdot L^{-1}$ of total soluble solids (TSS) and below 750 $\mu S \cdot cm^{-1}$ (75 $mS \cdot m^{-1}$) conductivity. Water with more than 3000 $\mu S \cdot cm^{-1}$ conductivity, or 200 $mg \cdot L^{-1}$ sodium, presents a severe salinity problem for irrigating bananas.

• Step 7

After plowing, rip (subsoil) and cross-rip the soil to 80–100 cm depth. Use 1-m rip tines to ensure effective ripping to 80 cm. Do not plow or disc after ripping, which may re-compact the surface soil.
Note: soil should be moist for best ripping results. Saturated soil causes a slicing effect during ripping, and dry soil causes large clods to form. Growth of banana roots is severely reduced in compact, unripped land [4].

• Step 8

Mark out in-field irrigation lines and install a new in-field irrigation system (microspinner or sprinklers). If using drip irrigation, delay installing the drip lines until after the planting furrows have been drawn.
Note: young banana plants have critical water requirements [5]. An adequate irrigation system should take into account both the quality and quantity of water available (*e.g.*, for possible leaching requirements in saline soil). This is of critical importance in dry areas or when cultivating under greenhouse conditions.

• Step 9

If using microspinners or sprinklers, irrigate the soil to field water capacity to stimulate new weed growth, then, if allowed, spray herbicides to kill this new weed growth. This can be repeated if time permits, to reduce the weed seed population before planting. Alternatively, eliminate weeds mechanically, but this can be very labor-intensive.

• Step 10

Mark out planting rows. Either furrow planting or planting in holes (on flat land) is practiced depending on land preparation, equipment available and environmental/topographical characteristics.
Note: furrow planting creates better plant stability and deeper rooting after establishment.

• Step 11

Draw a single furrow-making implement along the planting row (20–30 cm depth) with a light tractor. Do not use a heavy double-row furrow implement or heavy tractor, which may re-compact the soil.

• Step 12

Do not use a land plane or disc to level or smooth the soil surface after ripping because it has the effect of re-compacting the soil to a greater or lesser degree.

• Step 13

Install drip irrigation lines at this stage if a drip system is being used.

Planting out in the field

• Step 1

Use the planting density and spatial arrangement most appropriate for the cultivar, soil type and environmental conditions of the site. Densities normally vary between 1700 and 2200 $plants \cdot ha^{-1}$ [2, 6, 7].
Note: tissue culture plants possess a very high uniformity with quicker growth rate and higher yield potential than conventional plants [6, 8, 9], which thus allows their cultivation at higher densities during the first cycle, particularly under greenhouse conditions. However, care should be taken to avoid very high planting densities and, more particularly, spatial arrangements which are conducive to excessive shading. In greenhouse conditions, minimum aisles of 5 m should be left between plant rows to allow air circulation [10].

• Step 2

In the subtropics, plant out preferably in spring or summer when temperature conditions are ideal for banana growing, and young tissue culture plants are physiologically active [11]. With greenhouses in suitable locations, planting out is equally successful throughout the year.

• Step 3

Mark out planting positions in the furrow, dig planting holes at the bottom of the furrow slightly larger than the bag size, and pre-irrigate the top 40 cm of soil to field water capacity before planting [9].

• Step 4

In the event that planting furrows are not used, dig the planting holes much larger and deeper than the bag size (at least to 30 cm depth).

• Step 5

Thoroughly wet the medium in the bags before bringing plants to the field.

• Step 6

Arrange to plant in the early morning or during overcast weather. Avoid planting in the heat of a sunny day, especially in hot areas. Bring plants to the field and place one plant upright next to each planting hole.
Note: do not lay plants on their side in the sun because this can burn the roots through the plastic. These requirements are extremely important for plantings inside polyethylene greenhouses.

• Step 7

Carefully cut away the black plastic bag without disturbing the medium or damaging roots, and immediately place the exposed "rootball" in the bottom of the planting hole. Plants with bags removed must also not be left on their side in the sun because this burns the roots and dries out the medium.

• Step 8

With furrow planting, position the plant in the planting hole so that the surface of the potting medium is 5–10 cm below the soil surface at the bottom of the furrow (about 30 cm below the surrounding soil level). Pack the hole with topsoil around the "rootball", ensuring close contact with the potting medium. About 10 cm of the pseudostem should be covered by soil, which is compressed lightly around the plant to stabilize it.
Note: do not simply position the plant on the bottom of the furrow or in a small depression and then mound soil up around the roots and pseudostem so that the surface of the potting medium is the same level or higher than the natural soil level. The soil mound will easily be washed away with rain or irrigation into the open furrow, thus exposing the roots and destabilizing the plant.

• Step 9

When planting on flat land (no furrow), fill the bottom of the planting hole with loose topsoil and position the plant on this layer so that the surface of the potting medium is well below the level of the surrounding soil (10 cm for 20-cm-high plants, and 15 cm for 30–40-cm-high plants). Firm the soil lightly around the plant.

• Step 10

Soak the planting position with water during and after planting, if possible. A long hose is needed for this. If not available, at least make sure the soil is well irrigated before and after planting.

Immediate after-planting care

Special attention should be given to newly-established tissue culture plants for the first 3 months after transplanting, since this is the period of maximum physiological efficiency [11].

• Step 1

After planting, attempt to wet the leaves and the soil surface at least twice a day for 15 min during very hot conditions, in order to cool the leaves and create a microclimate ("evaporative cooling"). Continue for up to a month, if necessary. This can only be done with permanent microspinners or overhead irrigation. It is not possible to cool leaves with drip irrigation, which is thus a disadvantage of this system for establishing tissue culture plants in hot, dry areas. Do not over-irrigate straight after planting. The leaf area is very small and the soil should have been pre-irrigated. With a microspinner, apply 10 mm twice a week and, with drip irrigation, apply 1 mm to 2 mm every day, for the first 2 months (less water with drip irrigation due to targeted irrigation directly to the small plants). After 2 months, increase the schedule according to prevailing evaporation rates and leaf area development ("crop factors"). If using tensiometers, make sure

the reading does not go higher than 20 kPa in the root zone [5, 12].

• Step 2

When planting in greenhouses, ensure that lateral and top ventilation is provided during summer plantings.

• Step 3

Apply nematicide at planting but only if recommended from the prior analysis of soil samples. Apply registered granular nematicide, at the recommended dosage, and thoroughly mix it with the topsoil. Repeat the application but on the soil surface around each plant, 6 months after planting, in warm conditions. Rotate the types of nematicide used each time.

• Step 4

Start applying fertilizer 2 weeks after planting (20 g nitrogenous fertilizer for each plant such as ammonium nitrate, ammonium sulphate or urea). If fertilizing by hand, apply the nitrogen fertilizer every 2 weeks (20 g for each plant), and a potassium fertilizer (potassium chloride, potassium sulfate) every month (40 g for each plant) for the first 3 months.

Spread the fertilizer well away from the stem of the plant.

If using fertigation, obtain a balanced formula based on the soil chemical analysis, and apply nutrients at least every 2 weeks through the irrigation system. Do not overfertilize, because newly-established plants can easily be burnt.

• Step 5

Completely remove all weed competition by hand-hoeing for the first 3 months after planting. Avoid using herbicides, especially systemic ones, during this period.

• Step 6

After 5 or 6 months, fill in the furrow so that the soil level is uniform in the field, and the anchored rhizome is well below soil level. Heavy rains may fill in the furrow automatically.

• Step 7

Sucker selection is very critical and specific with tissue culture plants, which have

Figure 1.
A young *in vitro* field banana plant with many vigorous suckers emerging in all directions.

numerous vigorous suckers emerging from an early age *(figure 1)*. Cut, but do not destroy, suckers during the first 3 months, to avoid damaging the parent rhizome [13]. New 'peepers' will emerge between cut suckers. Select one of these “second flush” suckers between 4 and 8 months after planting and mark it with a water-based paint to identify it (selection after 4 months for spring planting and selection after 8 months for autumn planting in the subtropics). This selected sucker becomes the second-generation parent. Selected suckers must be similar in size and morphology but, most important, select all suckers in the same direction to maintain continuous uniformity in plantation spatial arrangement [2]. In the southern hemisphere, select suckers to the south-east to avoid interfering with bunches which mostly emerge to the north-west (and *vice versa*). Once selected, it is important to then destroy (desucker) all the neighboring suckers which compete by acting as sinks for available nutrients and reserves. Continue to desucker after selection, as unwanted suckers emerge. The latter increase in size underground and, if left, may force the parent rhizome above soil level, causing “high mat”.

Troubleshooting

(a) Problem: poorly-growing, non-uniform plants, lacking vigor despite good management. Cause: soil compaction due to poor soil preparation.
Solution: difficult after planting; for new lands, subsoil deeply in two directions.

Figure 2.
New *in vitro* banana plantation showing (a) healthy plants in background and (b) plants in poorly-drained soil in foreground. Waterlogged plants are small and stunted with yellow leaves and short internodes from damaged root systems.

Figure 3.
Banana symptoms of transplanting shock due to planting in very hot conditions. Water/heat stress symptoms are leaf folding, burning of lamina on young leaves, and new leaves becoming progressively smaller and thinner.

Figure 4.
Field banana plants established too shallow in the soil, with no furrow protection. Soil is mounded up against the stem which is ineffective.
Note: these excessively lanky plants are also subject to wind breakage.

(b) Problem: root dieback, unstable plants, short internodes, leaf yellowing and stunted growth. Cause: waterlogged soil due to poor drainage on-site (*figure 2*).

Solution: excavate 300-mm-deep drains between every four banana rows; for new lands choose high-lying well-drained soil; plant on 30-cm-high ridges.

(c) Problem: slow resumption of growth; small, stunted leaves become progressively smaller, leaf burning. Cause: transplanting shock (*figure 3*).
Solution: after planting apply effective management; for new plantings, plant early in the morning or in overcast weather; water the field and nursery bags thoroughly before planting; cool plants with irrigation water frequently after planting; control weeds. Try to avoid planting during the hottest period of the year.

(d) Problem: young plants are tall and lanky; stems bend or break in the wind; potting mix and roots become easily exposed. Cause: planting too shallow in the field (*figure 4*).
Solution: plant in a deep hole at the bottom of a 30-cm furrow. Cover 10 cm of the stem base with soil.

(e) Problem: droopy leaves bending at the petiole; laminae fold flat; patches of bleaching or necrotic burning. Cause: water stress due to insufficient water around roots and/or on leaf surfaces after planting.
Solution: irrigate soil profile to field capacity before planting; irrigate every 3 days after planting (sprinklers) or daily (drip irrigation); wet leaf surfaces three times a day in hot weather *(figure 5)*.

(f) Problem: leaves show nutrient deficiencies soon after planting (nitrogen, zinc and boron) (*figure 6*). Cause: temporary deficiencies due to fast initial growth outstripping root extraction potential.
Solution: foliar sprays of zinc and boron 1 and 2 months after planting. Soil-applied nitrogen every 2 weeks after planting (not before 2 weeks).

(g) Problem: leaf burn around margin of leaves; central leaf burn (*figure 7*). Cause: overfertilization in the soil and careless application of granules on top of the plant.
Solution: apply fertilizer on a "little and often" basis and apply well away from the leaf canopy.

(h) Problem: smaller than normal plants; yellow leaves; weeds or grasses growing as high as bananas. Cause: plant competition (*figure 8*).
Solution: destroy weeds before planting; after planting effective hand-hoeing to remove competition.

(i) Problem: (A) pronounced white or brown spots on lower leaves, and (B) distorted leaves with narrow serrated lamina. Cause: herbicide damage by paraquat (A) or glyphosate (B).
Solution: avoid herbicides until lower leaves are 1 m above the ground; spray preferably in the early morning and only when no wind is present, and use a protective shield around the applicator.

(j) Problem: brown necrotic patches on the western side of "cigar" leaves; vertical yellow/brown streaks on the open laminae (*figure 9*). Cause: hot afternoon sun burning the youngest leaf while still wrapped up in a cigar shape.
Solution: damage is minimal and does not occur on older leaves.

(k) Problem: premature dieback and necrosis of the youngest central leaves. Cause: *Pythium* soil-borne fungal infection.
Solution: remove infected plants and replace with healthy plants.

(l) Problem: plants become shorter than the others, with short, wide leaves and narrow internodes. Cause: dwarf mutation
Solution: remove mutant plants and replace with normal healthy plants.

(m) Problem: parent rhizome starts to climb out above ground level, destabilizing the plant. Cause: either (A) planting too shallow, (B) poor soil preparation, or (C) allowing unwanted suckers to grow too large underground.
Solution: loosen the soil thoroughly during land preparation; plant deeply at the bottom of a furrow; remove unwanted suckers early while still small.

3. Typical results obtained

These protocols lead to vigorous, healthy, uniform plants, well-established in the field after two months, and showing no visible signs of either stress, stunting, short internodes, leaf yellowing or marginal necrosis,

Figure 5.
Regular wetting of leaf surfaces on new banana transplants with microspinner irrigation prevents heat stress on young, tender plants.

Figure 6.
Symptoms of early nitrogen deficiency on young field banana transplants (pale yellow leaves, red tinge on petioles).

Figure 7.
Banana leaf margin necrosis due to over-application of granular fertilizer and direct application to funnel of plant.

Figure 8.
Small, stunted banana plants showing direct competition from weed growth after planting.

Figure 9.
Newly-unfolded banana leaf showing vertical yellow bands and necrotic patches. Caused by direct afternoon sunburn on tightly-folded "cigar leaf".

Figure 10.
Left: conventional banana sucker planting material after 1 month in field. Right: *in vitro*-derived plant after 1 month in field. Note vigorous root system and large leaf area on *in vitro* plant, indicating physiological efficiency.

leaf wilting or burning, droopy leaves, plants leaning or falling over, progressively smaller leaves, or excessive competition from weeds. Young *in vitro*-derived plants from field establishment to two months old are much more efficient physiologically than conventional suckers, but they are also sensitive to any stress which can undermine this inherent efficiency (*figure 10*).

Note: as a prerequisite to achieve the best results during field establishment, *in vitro*-produced banana plants must have been produced following the steps given in previous protocols for correct weaning (acclimatization) [14] and nursery hardening [15]. Then, in order to (A) maximize the physiological efficiency of young *in vitro* plants in the field, (B) obtain excellent individual plants as described above, and (C) achieve collective uniformity in the young plantation, it is also essential to consider all the management factors discussed as an integrated program in which all factors have to be operating optimally. Without successful "integrated management", just one factor operating sub-optimally can cause the entire program to suffer since a lack of one factor can easily result in other optimal factors becoming compromised and inefficient. For example, a plantation full of weeds or with high nematode pressure cannot respond fully to optimal fertilizing and irrigation practices.

References

[1] Galán Saúco V., Ait-Oubahou A., Abdelhaq H., Greenhouse Cultivation of Bananas, Chron. Hortic. 44 (2) (2004) 35–37.

[2] Robinson J.C., De Villiers E., The cultivation of banana, ARC-Inst. Trop. Subtrop. Crops, DuRoi Lab., Nelspruit, S. Afr., 2007, 258 pp.

[3] Lahav E., Banana nutrition, in: Gowen S. (Ed.), Bananas and plantains, Chapman and Hall, Lond., U. K., 1995, pp. 258–316.

[4] Delvaux B., Soils, in: Gowen S. (Ed.), Bananas and plantains, Chapman and Hall, Lond., U.K., 1995, pp. 230–257.

[5] Eckstein K., Robinson J.C., Physiological responses of banana *(Musa* AAA; Cavendish subgroup) in the subtropics. VI. Seasonal responses of leaf gas exchange to short-term water stress, J. Hortic. Sci. 71 (1996) 679–692.

[6] Robinson J.C., Handbook of banana growing in South Africa*,* Inst. Trop. Subtrop. Crops, Nelspruit, S. Afr., 1993,128 p.

[7] Robinson J.C., Bananas and plantains, CAB Int., Wallingford, U.K., 1996, 238 pp.

[8] Drew R.A., Smith M.K., Field evaluation of tissue-cultured bananas in South Eastern Queensland, Aust. J. Exp. Agric. 30 (1990) 569–574.

[9] Fraser C., Eckstein K., Plantlet size and planting method for tissue culture banana plants, Acta Hortic. 490 (1998) 159–165.

[10] Galán Saúco V., Los frutales tropicales en los subtrópicos. II Plátano (Banano), Mundi-Prensa, Madr., Spain, 1992, 173 p.

[11] Eckstein K., Robinson J.C., Physiological responses of banana *(Musa* AAA; Cavendish subgroup) in the subtropics. IV. Comparison between tissue culture and conventional planting material during the first months of development, J. Hortic. Sci. 70 (1995) 549–559.

[12] Eckstein K., Fraser C., Botha A., Husselman J., Evaluation of various irrigation systems for highest economical yield and optimum water use for bananas, Acta Hortic. 490 (1998) 147–157.

[13] Robinson J.C., Step by step guidelines for desuckering and sucker selection on young tissue culture banana plants, Tech. Bull. (DuRoi Lab.) 5 (November) (2000) 10–11.

[14] Robinson J.C., Galán Saúco V., Weaning (acclimatization) of *in vitro*-produced banana plants, Fruits 64 (2009) 325–332.

[15] Robinson J.C., Galán Saúco V., Nursery hardening of *in vitro*-produced banana plants, Fruits 64 (2009) 383–392.