The cassava farmer's manual: practical guidelines for cassava seed production in Rapid Multiplication Units (RMU)

Technical editors:
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Embrapa logo

Reniva logo
Multiplication and transfer network for propagating cassava material of genetic and phytosanitary quality.

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Introduction

Cassava (*Manihot esculenta* Crantz) is cultivated throughout the entire Brazilian territory. It is a rustic and widely adapted crop that overcomes the limitations of low rainfall and poor soil fertility present in soil, and climate conditions. It is one of the few crops whose roots can store carbohydrates for periods of up to two years. These characteristics grant cassava great importance in both human and animal consumption. In addition to agro-industrial processes, it is the raw material used in various industrial processes, such as the manufacturing of paper, pharmaceuticals, textiles, cosmetics, mining, and others.

The agronomic success of any crop depends fundamentally on the quality of the propagation material used for the crop establishment. These materials should be robust and pest-free. In cassava this is also true, although it produces botanical seeds, but the typical propagation of cassava is undertaken vegetatively, through pieces of the stems which are commonly known as stem cuttings.

An intrinsic characteristic of cassava, which is also an obstacle to large-scale propagation, is its low rate of multiplication. Each cassava plant produces an average of 10 stem cuttings of 20 cm, in a period of 12 months, which is to say that the propagation rate of cassava is 1:10 and considered low for the planting of commercial crops.

Cassava stem cuttings may accumulate pathogens during successive propagation cycles. This particularity reduces the production of storage roots, the amount of available stem cuttings and their vitality. In critical situations, this effect can impair farming.

Another limiting factor to the availability of good quality stem cuttings to farmers is the impossibility of storing them for long periods. Cassava stems cannot withstand storage periods longer than 90 days without losing their vitality. This issue becomes aggravated in the semi-arid region of Brazil, where cassava harvest is mainly carried out during the driest months, as it is at this time that the roots have the largest amount of stored starch.

Due to the difficulty of obtaining adequate storage conditions, the stem cuttings that are conserved for use in subsequent plantations will be dehydrated and may produce weak plants with low root production. The situation worsens in periods of prolonged droughts, when there is a total loss of stem cuttings. As a consequence, during the rainy season farmers can only obtain stem cuttings with low levels of plant health and low genetic quality, risking the performance of the crop.

The genetic and phytosanitary quality of the stem cutting is the true foundation of the productive process. Thus, it is observed that once these characteristics have been adopted, all of the resulting strands will have the capacity to preserve their plant health and vitality. As a result, productivity increases at an average of 20% to 30%, in addition to an increased supply of stem cuttings for the establishment of new fields. This way, the cloning of stem cuttings in these bases promotes the target audience's recognition of the true value of this basic input.

Overcoming constraints on the quantity of available stem cuttings, regularity of supply, plant health and the preservation of propagating materials is essential to promote better productivity. Cassava agriculture still lacks a production system capable of generating propagating materials in sufficient quantity and with the desired phytosanitary quality.

This necessity led to the creation of a network whose basic premises are not only to produce cassava stem cuttings on a large scale, but also to preserve plant health. Thus, in 2010, based on diagnosis from Embrapa Mandioca e Fruticultura, the Cassava Network for Multiplication and Transfer of Propagative Material with Genetic and Phytosanitary Quality (*Reniva*) was created.

In order to reach these premises, a project was developed with application of rapid multiplication and virus indexing techniques in parent plants, under the general coordination of Embrapa and in partnership with the *Instituto Biofábrica* de Cacau and the *Empresa Baiana de Desenvolvimento Agrícola* - EBDA. *Reniva* was based in a structured partner network for the multiplication and distribution of cassava stem cutting with high genetic and phytosanitary quality, both for small farmers and for large farmers in the main cassava producing regions throughout the country.

Within the scope of the *Reniva* project, the figure of the "Maniveiro" (cassava farmer dedicated to the production of planting material) was established, which constitutes a producer or group of producers who produce seedlings through the rapid multiplication method. With these seedlings, they cultivate areas with a modular size of 1 (one) hectare, equipped with an irrigation system. Good management practices are used to steer the stem cutting production, with permanent technical support. The cassava farmer is therefore seen as the stem cutting producer, while the Rapid Multiplication Unit (RMU) is a seedling production unit composed of nurseries, sprouting and rooting chambers, and the irrigated and seedling-planted area for the harvesting of stems and/or stem cuttings.

Reniva contributes to the establishment of the commercial agribusiness production of cuttings with high genetic and phytosanitary quality in Brazil. For the Brazilian Ministry of Livestock and Food Supply (MAPA), the operation of *Reniva* will soon be backed by specific legislation at the federal level, for the production of cassava seedlings regulated by Normative Instruction. This will enable the establishment of cassava farmers for production of cassava seedlings and stem cuttings, thus contributing to the sustainability of the Brazilian cassava agriculture and promoting the improvement of the quality of life for the thousands of families whose source of income and food security are associated with the cultivation of cassava.

Reniva presents a set of strategies and methodologies that, if well used, can provide stem cuttings for the formation of new areas in sufficient quantity and in times of greatest need. In addition to the regular supply, it is worth highlighting the possibility of producing planting material that is free of pests and diseases.

This manual provides the essential technical instructions for the establishment of *Reniva* in any region of Brazil. The publication is divided into three sections. First, it presents a structural project for the construction of the infrastructure that includes a Rapid Multiplication Unit (RMU), composed of: nursery, sprouting chambers, rooting chambers, work benches and irrigation systems. Next, a detailed analysis is presented for the collection of parent plant samples to analyse the occurrence of virus infections (indexation), so that only those that are effectively virus-free are multiplied at a large scale. The third section describes the rapid multiplication technique of cassava, with instructions for the necessary handling and cutting, so that the farmers themselves are able to clone their field selections and conserve these viable materials throughout the year.

Public and/or private institutions, multipliers and other participants of the cassava production chain who are interested in joining *Reniva* must have an excellent comprehension of the basic principles that govern this type of work, namely:

- Use of basic materials such as parent plants that are proven to be virus-free (indexed).
- Adoption of rapid multiplication techniques to increase multiplication rates.
- Establishment of cassava farmers as responsible for the mass production of cassava stem cuttings and seedlings.

- Establishment of a partnership with Embrapa for technical training, disposal of parent plants and promotion of the work carried out by *Reniva.*
- Availability of permanent technical assistance for both cassava farmers and producers in the scope of the *Reniva*-produced stem cuttings.

Structure of the Rapid Multiplication Unit (RMU)

The modular structure of a RMU is designed for the production of 13,000 seedlings at each four-month cycle. This quantity of seedling per cycle is sufficient for one (01) hectare of planted cassava. If the final average plant population is projected to 12,500 plants, it is estimated to produce 125,000 stem cuttings of 20 cm, after a one-year period.

A modular unit for the production of seedlings and stem cuttings is composed of the following infrastructure:

- Nursery/screened structure measuring 6.0m x 6.0m, with an enclosed patio measuring 6.0m x 6.0 m;
- Six sprouting chambers of 2.0m x 1.0m;
- One rooting chamber of 2.0m x 1.0m;
- One work bench (table with a rectangular base measuring 2.0m x 1.0m);
- Irrigation system for the sprouting chambers and/or seedlings;
- Complete irrigation system for one (01) hectare;
- Area of one (01) hectare for the planting of seedlings.

The RMU should be deployed according to the specifications and spacial distribution described below. Detailed descriptive floor plans of the infrastructure that make up a RMU can be found in the appendices of this manual.

Nursery

Fixed screened on a galvanised tube structure (6m x 6m), with adjacent external patio (6m x 6m). The structure must be made of galvanised metal tubes. Covering materials, side protection and other nursery components are described in the technical information below (Tables 1 and 2).

Table 1. Technical specifications for the installation of a nursery/screened structure, with production capacity of stem cuttings for the planting of one hectare.

Model	Flat Coverage
Length (m)	12
Width (m)	6
Distance between columns (m)	3
Columns	Total of 10, of which 09 are peripheral and 01 is central
Right base (m)	3.20m (0.20m of the perimeter wall and 3.00m of the tubes used as columns)
Total area (m2)	72
Side, front and back coverings	Screen with 50% or 70% shading factor, made of high density polyethylene threads, in black and with UV anti-radiation additives.

Perimetral nursery wall, under coverings	In masonry of ceramic blocks of 6 holes, with plaster, measuring 0.40m high and 0.15m wide. The wall should be 0.20m above ground level.
Columns	In 1 1/4” galvanised tubes, plate 14 or 16, with welded nuts and bolts for locking at the lower end, in accordance with schematic drawing.
Upper and lower perimeter crosspieces	In 1 1/4" galvanised tubes, plate 14 or 16.
Fittings	Made of 1 1/2” galvanised tubes, with welded nuts and bolts for locking, according to illustrative figures. The lower fittings for the settling and slotting of the column tubes should be made of 1” galvanised tubes and should be placed on the perimeter wall, following the sketch. The bases that fix the standpipes to the columns should be made of concrete and measure 0.20m long by 0.10m wide, with the height of the perimeter wall, ensuring adequate support to the structure. The 1” tube for the central tube fitting should be cast on a concrete base of 0.20m x 0.20m, at sufficient depth to provide stability. The height should be levelled with the perimeter wall.
Gate	One (01) gate 1.20m wide by 2.30 high, with a 1” galvanised tube structure, with a central horizontal crosspiece also in galvanised tube. It should be installed in the centre of the screened structure, facing the access to the patio, according to the sketch. The gate will be secured with 03 1/2” gonzo hinges and will have a flat latch bolt welded to the central crosspiece on outside of the nursery. The gate must open to the outside of the screened structure.
Fixing of shading screens	Fixing of the shading screens should cover the entire structure. The juxtaposed fabric bands should be attached to each other with strong polyamide (silk) thread. The thread should also be used to sew the fabric to the structure, securing it.
Greenhouse and patio flooring	Clay floor, levelled at 0.20m below the upper level of the perimeter wall, covered with 8cm of crushed no.1 stone.
External patio (see observations)	Dimensions of 6.0m x 6.0m, with masonry perimeter wall with ceramic blocks, plastered. Approximate dimensions of 0.40m high by 0.15m wide, being at least 0.20m above ground level.
Securing of structure	The screening structure should be cabled with steel or strong wire to prevent damage caused by strong winds. The chosen material should be added to the budget list.

Observations:

1. All iron structures should be treated with anti-rust paint. All welds performed on galvanised tubes should be painted.

2. The electrodes should be purchased only if the producer himself is to make the fittings. In case of hiring a blacksmith, this acquisition is not necessary.
3. The screened structure and the patio should be placed in the north-south direction, respectively, considering its largest dimension as the axis.
4. In the hottest and high-insolation regions, a screen with 70% shading factor should be used. In these regions, the adjacent patio should also be screened. Check the side conditions in advance and, if necessary, define if the adjoining patio will only have a top covering screen, or if it will also have it on the sides. When the screen is placed on all sides, the gate can be placed externally, eliminating the internal partition. To make changes, the amount of material must be reviewed and deemed appropriate. Tubes, fabric, screws and fittings should be resized to suit these expansions.
5. Depending on the yield of the materials and the work undertaken, resizing of the structure and levelling of the ground, other materials such as stone, sand, bricks, blocks, cement and others may require small additions, which should be considered in the budget.

Table 2. Material specifications for construction of a cassava rapid multiplication unit with a screened structure for rooting and acclimatisation (6.0m x 6.0m) and an open patio (6.0m x 6.0m). Total area of 12.0m x 6.0m.

Item	Unit	Quantity of material (for one chamber)
Shading screen with 50% or 70% shading factor, 3.0m wide, made of black high density polyethylene with anti-radiation UV additives.	m2	120
Silk thread to sew and fasten fabric to structure	spool	1
1” (one inch) galvanised tube, plate 14 or 16	6m rod	3
1 1/4” (one and a quarter inch) galvanised tube, plate 14 or 16	6m rod	17
1 1/2” (one and a half inch) galvanised tube, plate 14 or 16	6m rod	2
5/16” x 1 1⁄4” galvanised steel screws, with nuts	unit	45
1⁄2” x 55 mm gonzo hinges	unit	3
3.25mm electrode (for electric welding used by blacksmith)	kg	2
6” galvanised flat latch bolt	unit	1
35mm lock	unit	1
5mm diameter steel cable	m	20
Steel cable clamp	unit	8
Common solid ceramic block	unit	500
6-hole ceramic block	unit	500

Portland-type cement	sc 50 kg	10
Medium sand for masonry	m3	4
No.1 gravel	m3	8
Polyethylene water tank with capacity of 1,500 litres, with lid	unit	1
3/4” internal adapter	unit	1
LR 90° 3/4” elbow union in PVC	unit	1
PVC 3/4” tube	unit	1
PVC 3/4" threaded nipple	unit	1
PVC 3/4” threaded adaptor with ring for water tank	unit	1
Plastic adhesive for PVC tube 17g	unit	1
Thread seal tape with 10.0m	unit	1
3/4" PVC flange	unit	1
3/4” buoy-faucet	unit	1
3/4” brown polyethylene hose x 2mm	100m roll	1
Heavy duty plastic watering can with 10 L capacity	unit	2
Heavy duty plastic watering can with 5 L capacity	unit	2

Figure 1. Prototype of cassava rapid multiplication unit (RMU), 2008.

Figure 2. Standard *Reniva* rapid multiplication unit (RMU) (current model), 2015.

Sprouting chamber

In a seedling production module for one (01) hectare, 6 sprouting chambers are needed (Figures 3 and 4). Each of these are composed of a rectangular base structure 2.0m long, 1.0m wide, 0.10m deep, made of 1 1/4” x 1/8” angle bracket iron. The angle bracket legs are supported by feet made of iron plates 15.0cm x 15.0cm x 0.5mm, welded to each lower end. The legs should also have iron angle bracket clamping 1” x 1/8” on all four sides, welded at the legs at a height of 0.3m. It has a 3/4” x 1/8” flat iron crosspiece, welded crosswise to the length, forming an additional support for the bottom. The sides of the chamber are aluminium or polyamide boards, 9.5cm high, fitted and affixed to the frame with rivets. The bottom of the chamber (seedbed) is a sheet metal plate with perforations of 5.0mm (10.0cm x 10.0cm). The plate is fitted and riveted internally to the base angle brackets.

The chamber cover has a rectangular frame as a base, with dimensions 1.0m wide and 2.0m long, made of 1/4” x 1/8” angle bracket iron. To this frame, 5 equidistant arches should be welded in 3/4” x 1/8” flat iron (0.50m high). To reinforce the structure, a 3/4” x 1/8” flat steel upper locking plate must be welded to the top of the arches, according to the figures. To support the cover when open, a bracket with a total length of 0.57m, made of 3/4” x 1/8” flat iron plate, should be installed and fixed to the medium section of the structures, as shown in the appendix. At the front section, an iron handle should be welded on, in a decentralised position to avoid the spacer mechanism located in the centre of the chamber. The connection between the cover and the base is made by three closed iron gonzo hinges. The cover must be covered with special plastic wrap for agricultural greenhouses, of the diffuser type, with anti-UV treatment and a minimum transparency of 80%. The plastic wrap is attached internally to the base of the cover, using the wooden slats and the rivets (Table 3).

Table 3. Listing of materials for the construction of a cassava sprouting chamber.

Item	Unit	Quantity of material (for one chamber) (1)
1 1/4” x 1/8” angle bracket	metres	25
1” x 1/8” angle bracket	metres	8
3/4” x 1/8” flat iron sheet	metres	15
15.0cm x 15.0cm x 0.5mm thick plate	unit	4
1/2” x 55m gonzo hinge	unit	3
3.25mm electrode (for electric welding used by blacksmith) (2)	kg	1
6.0mm x 35.0mm pop rivet	unit	36
3.0cm wide x 1.0cm thick wood clamp to attach the agricultural plastic to the chamber cover	metres	7
Transparent diffuser agricultural plastic, 200 microns thickness, with anti-UV additive, measuring 5.0m x 3.0m	piece	1
Aluminium or polyamide sheet 1.5mm thick for the chamber sides	m2	0.6 m2
2.0m x 1.0m galvanised sheet, with thickness reference of 24 or 26 (3)	piece	1

Observations:

1. The quantity of the materials listed here must be multiplied by the number of chambers that will be made. Once this has been done, adjust the physical units for material supply, in order to prepare the final list of purchases.
2. The electrodes will be necessary if the structures are to be made by the cassava farmer. If the service is performed by a contracted blacksmith, the welds and anti-corrosion paint should be part of the contracted price.
3. The galvanised sheet is suitable for the sprouting chamber and must be perforated. One can choose other materials for the bottom of the sprouting chambers, the rooting chamber and the work bench, such as concrete, granite or slate slabs. It is important to compare costs, durability and operational conditions. For the rooting chamber and work bench for transplantation we suggest, when possible, to use a reinforced concrete slab, as the zinc sheet has the

disadvantage of raising the temperature considerably when exposed to the sun. The materials for the preparation of cement slabs are not listed in this table.

Figure 3. Cassava sprouting chamber with half-open cover, 2017.

Figure 4. Cassava sprouting chamber with open cover, 2017.

Rooting chamber

The rooting chamber is a table with the same coverage of the sprouting chambers, where the vials with the sprouts will be placed to undertake the rooting in water (Figure 5).

It is a rectangular-based structure 2.0m long, 1.0m wide and 1.1m high, made with 1 1/4" x 1/8" angle bracket iron, with a central rail in 3/4" x 1/8" flat iron sheet, and welded crosswise to length, forming an additional support for the bottom. The bottom of the chamber is a galvanised sheet, fitted internally in the base angle brackets and fixed onto them with rivets. The legs should also have iron angle bracket clamping 1" x 1/8" on all four sides, welded at the legs at a height of 0.3m.

The chamber cover has a rectangular frame at the base, 1.0m wide and 2.0m long, made of 1 1/4" x 1/8" angle bracket iron, with 5 equidistant 3/4" x 1/8" flat iron arches (0.70m high) welded internally to the base angle brackets. To reinforce the cover , a 3/4" x 1/8" flat iron upper locking plate must be welded to the top of the arches. To support the cover when open, a bracket with a total length of 0.57m, made of 3/4" x 1/8" flat iron plate, should be fixed to the medium section of the structures, as shown in the descriptive plans in appendix. At the front section, an iron handle should be welded on in a centralised position. The connection between the cover and the base is made by three closed iron gonzo hinges.

The cover must be covered with special plastic wrap for agricultural greenhouses, with a minimum transparency of 80%. The plastic wrap is attached internally to the base of the cover, using the wooden slats and the rivets (Table 4).

Table 4. Listing of materials for the construction of a cassava rooting chamber 1.0m wide x 2.0m long x 1.10m high.

Item	Unit	Quantity of material (for one chamber) (1)
1 1/4" x 1/8" angle bracket	metres	25
1" x 1/8" angle bracket	metres	8
3/4" x 1/8" flat iron sheet	metres	15
15.0cm x 15.0cm x 0.5mm thick plate	unit	4
1/2" x 55m gonzo hinge	unit	3
3.25mm electrode (for electric welding used by blacksmith)	kg	1
6.0mm x 35.0mm pop rivet	unit	24
3.0cm wide x 1.0cm thick wood clamp	metres	7

Transparent diffuser agricultural plastic, 200 microns thickness, with anti-UV additive, measuring 5.0m x 3.0m	piece	1
2.0m x 1.0m galvanised sheet, with thickness reference of 24 or 26	piece	1

Workbench (table for seedling transplanting)

The workbench for seedling transplanting is a rectangular-based structure 2.0cm long, 1.0m wide and 1.1m high, in 1 1/4” x 1/8” angle bracket iron, with a central rail in 3/4” x 1/8” flat iron sheet, and welded crosswise to length, forming an additional support for the top. The table top should be a galvanised plate, fitted and riveted internally at the base angle brackets. The angle bracket legs are supported by feet made of 15.0cm x 15.0cm x 0.5mm iron plates, welded to each lower end. The legs should also have iron angle bracket clamping 1” x 1/8” on all four sides, welded at the legs at a height of 0.3m.

Table 5. Listing of materials for the construction of a workbench 1.0m wide x 2.0m long x 1.10m high.

Item	Unit	Quantity of material (for one chamber) (1)
1 1/4” x 1/8” angle bracket	metres	25
1” x 1/8” angle bracket	metres	8
3/4” x 1/8” flat iron sheet	metres	1
15.0cm x 15.0cm x 0.5mm thick plate	unit	4
3.25mm electrode (for electric welding used by blacksmith)	kg	1
6.0mm x 35.0mm pop rivet	unit	12
3.0cm wide x 1.0cm thick wood clamp	metres	7
2.0m x 1.0m galvanised sheet, with thickness reference of 24 or 26	piece	1

Irrigation of sprouting chambers

To maintain adequate humidity in the sprouting chambers, ensure regular irrigation. The simplest way is with the use of watering cans, though a simple irrigation system with nebuliser nozzles or micro sprinklers can be used directly inside the chambers (Figure 6).

The best time for irrigation is in the early morning or late afternoon, as there will be time for the free water to evaporate and form a microclimate with ideal relative humidity inside the chambers.

Figure 6. Micro sprinklers for irrigation inside the sprouting chambers, 2017.

Procedure checklist for collection of leaf samples for virus indexing analysis

***Reniva* criteria for selection of cassava parent plants**

- Locate the best area of the crop, in which there are no symptoms or signs of diseases and pests.
- Select the parent plants with good agronomic characteristics (Figure 7).
- Observe that the plants have the typical morphology of the selected variety (petiole colour, lobule shape, terminal apex colour, shaft colour, bark and root colour, root shape and colour, branching type).
- Select plants that have sturdy stems and, when possible, more than one stem per plant.
- Mark only plants with no visual symptoms of viruses, such as the cassava frog skin disease, bacterial blight, anthracnose, rust, root rot, over-budding and over-extending.
- After the collection, inspect the area frequently to check if there have been significant changes in the development and health of the selected parent plants.

Figure 7. Cassava parent plant selected for multiplication, 2017.

Collection of plant material (leaves) for indexing analysis

- Cover the area in a zig-zag trajectory to select plants with no symptoms of the diseases listed in the previous section ("*Reniva* criteria for selection of cassava parent plants").
- Identify each selected plant with a resistant label (plastic, metal or other) whose identification will not be erased easily.
- Fill in the label and firmly attach it to the plant stem. The label must always be legible, as it will be the only identification for the sample. The label should include the following information:

  - Variety name;
  - Sample number (001, 002, 003, …).
- The amount of sampled plants should be enough to provide the amount of stems needed for multiplication. To produce 13,000 seedlings, 75 to 80 stems with a length of 1.0m are required.
- For each selected parent plant, collected at least five (05) intermediate leaves. Collect the leaves preferably in the afternoon, to ensure that they are dry. Condition them in a transparent cellophane (preferred) or plastic bag. Avoid collected moist leaves, as excessive moisture inside the bags can lead to rotting during transport. Identify each one with a paper label, written with a ballpoint pen. Place the sample label inside the plastic bag, together with the leaves, to prevent from losing. The label should include the following information:
-
  - Variety name;
  - Plantation or farm name;
  - Municipality/State;
  - Sample number (001, 002, 003, …)

Attention: Each sample is individual, representing only one plant, which must be perfectly identified. The collections must be done with the aid of plastic bag that should be used as a glove on the hand. Each sampling should use one plastic bag for collection, and another one for storage. Thus, after collecting each leaf, the plastic bag (glove) is discarded to avoid contamination of other samples with the cellular sap of the manually cut shafts. Note that at this stage you should not use any cutting instruments, using only fingertips as clamps to cut the petioles of the leaves.

- When storage is required, the samples can be kept in open containers and taken to a common refrigerator until the next day. Never allow the leaves to freeze. Prepare, in advance, sufficient quantities of PET bottles (600mL) with water to freeze. In the morning, the bottles should be accommodated so as to form a layer at the bottom of the box. The use of PET bottles avoids thawing water to wet the samples. Place a cardboard sheet or 4 to 5 sheets of newspaper over the bottles. Arrange the samples in the box, without pressing them too much. Finally, cover the samples with another sheet of cardboard or newspaper. Close and seal the box.

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Attention: During collection, it is ideal to place the samples in polystyrene boxes, which should be kept closed whenever possible, and protected to avoid direct sunlight. The boxes should be sent to the laboratory as soon as possible after the collection is completed (next day). The leaves must still be turgid when they arrive to their destination, no more than ten days after collection. If necessary, keep the samples in a refrigerator until they are sent to the laboratory.

Figure 8. Stages of cassava leaf sample collection for indexing analysis of disease-causing viruses. Identification of plant (A); collection of leaves (B); packaging (C); identification of sample (D); 2017.

Collection of virus-free cassava stems for rapid multiplication

- After completing the analysis, the laboratory should send a list indicating which samples are virus-free, using the sample identification numbers. The collection of stems can then be carried out.
- With the list, proceed to the collection of shafts. Sterilise the tools to be used (machete, scythe, hoe, and others) previous to the collection, with a cleaning solution[1].
- The shafts, approximately 12 months old, should be taken from the central section of the plant (middle third), without any symptoms of attacks from borers, mealybugs or root rot. Inspect the shafts and roots before beginning the collection.
- The shafts from the same varieties shall form a single batch, tied and specified, with an identification label containing the following information:
  - Variety name;
  - Plantation or farm name;
  - Municipality/State
- Following the collection of the planned number of shafts, the surplus (if any) can be used by the farmer/community to form a new plantation of indexed plants (conventional multiplication). Plants diagnosed as infected should be immediately collected. Their stems should be destroyed, and their roots used normally.
-

Attention: The disposal of infected plants should be the last activity. The tools used to dispose of them must be disinfected again after use.

Figure 9. Collection of stem cuttings of healthy plants (A), root inspection (B), tying the batch (C), identifying the batch (D), 2017.

Precautions during stem transportation

- Wrap and isolate the batches of stems with materials that avoid friction (thin foam, thick cardboard, bubble wrap, cloths). Friction causes damage to the buds. The load must also be accommodated with the same purpose in mind;
- Cover the load to avoid the stems drying from the wind and heat;
- Provide transport that will allow for the shortest time possible between collection and utilisation of shafts.

Rapid multiplication in cassava propagation

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[1] Suggested solution composition: 200mL of sodium hypochlorite (2% bleach) + 200mL of alcohol + 10mL of neutral detergent. Fill up with water until reaching 1L volume. This solution is only usable for 1 hour.

With the objective of increasing the scale of cassava stem cutting production, a rapid multiplication method was developed by the International Centre for Tropical Agriculture (CIAT) (1982), which was later adapted to the Brazilian conditions (SILVA, 2002; MATTOS, et al., 2006: FUKUDA E CARVALHO, 2006). This method is based on the cassava plant's physiological characteristics, which are important for the vegetative propagation and for the success of the method, namely: a) when the stems are cut in small portions, the apical dominance is removed and the buds begin sprouting simultaneously; b) following the removal of the shoots, the buds restart sprouting several times, in intervals of 8 to 10 days; and c) the shoots take root and give rise to new plants, with identical characteristics to the parent plant.

Step-by-step of rapid multiplication:

- **Cutting of the stem cutting in mini cuttings for sprouting**

The cutting of the stem cuttings should be done with a manual or electric saw, leaving sections (mini cuttings) with 2 to 3 buds (Figure 10). It is important that this operation is undertaken with care to avoid the harming of the buds. The cuts must be straight, perpendicular to the direction of the length of the shoots. Irregular or bevelled cuts are not suitable for this technique. The size of the mini cuttings will depend on the distance between the buds (Figure 10).

Figure 10. Mini cassava cuttings with 2 to 3 buds, 2009.

- **Planting of the mini cuttings in sprouting chambers**

The mini cuttings should be placed horizontally, in rows parallel to the smallest width, with 10.0cm spacing. The buds should be facing upwards to facilitate sprouting. The substrate should cover 3/4 of the mini cuttings, leaving only the buds out. For mini cuttings with buds that are very close to each other, a space must be left between them to avoid sticking of the shoots. When the mini cuttings have large spaces between the buds, they should be planted without gaps, touching each other (Figure 11).

The substrate should be of a light texture and preferably with a high content of decomposed organic matter. This will provide an excellent development of the shoots, as well as induce the rooting of the mini cutting.

Figure 11. Mini cassava cuttings planted in rows in sprouting chamber. Mini cuttings with buds close together (A); mini cuttings with buds far apart (B), 2012.

Proper handling of humidity and temperature inside the chamber is essential to promote the proper conditions to stimulate sprouting of the mini cuttings (Figure 12). Enough watering should be done to maintain the adequate level of humidity inside the chamber. The irrigation water must be of good quality. The use of contaminated or brackish water may impair the development of shoots.

Figure 12. Sprouting of mini cassava cuttings with 2 to 3 buds in sprouting chambers, 2011 and 2017.

- **Cutting of the shoots and transfer to rooting chamber**

The shoots must be cut when they reach a height between 10.0cm and 12.0cm, and transferred to the containers in the rooting chamber. When making the cut, leave at least 1cm of the shaft by the buds, so that they are able to sprout again (Figure 13). Subsequent cuts should be performed at 8-day intervals, whenever the shoots reach the ideal size.

During this stage, sharp tools such as a scalpel or stylus should be used to ease the cutting. Avoid using scissors, even pruning shears, as they can damage the shoot tissue. During cutting, these tools should be disinfected with 0.5% sodium hypochlorite (200mL bleach and 800mL water), to avoid contamination of the shoots.

The rooting containers should be of transparent material, such as glasses or disposable cups (volume of 300mL), or even small PET bottles cut at 10.0cm high. Dark containers should be avoided, as they favour the development of algae in the water, impairing the rooting. Each container must be filled with 2/3 water, and should house approximately 10 shoots (Figure 14).

In this stage, the quality of the water is even more important than that used in the sprouting chamber. The shoots must be placed in clean, room-temperature water that has been previously boiled. The containers must be inspected daily to determine the ideal time to change the water. In general, the water should be changed in intervals of 5 to 10 days.

During the rooting of the shoots, the excess leaves are naturally eliminated. It is important to keep the leaves out of the water, to avoid the proliferation of microorganisms.

Figure 13. Detail of cassava shoot cut, leaving 1.0cm of the shaft close to the bud, 2014.

Figure 14. Transparent containers for rooting of cassava shoots. Glasses, 2014 (A) and plastic cups, 2015 (B).

- **Transplanting shoots for acclimatisation**

As they become properly rooted, approximately 15 to 20 days after cutting, the shoots will become ready to be transplanted to polyethylene bags, plastic cups or tubes (approximate volume of 300mL), where they will pass the acclimatisation phase, until they are permanently planted in the field (Figure 15). The acclimatisation phase lasts for about 20 to 35 days, depending on the development of the plants and the environmental conditions.

At the time of transplantation from the rooting chamber to the tubes, root entanglement may occur. In these cases, the seedlings must be carefully separated so as not to damage the roots. The newly-transplanted shoots should be placed in a partially shaded environment to facilitate survival success (Figure 16).

The seedlings will be ready to be transported to the field approximately 70 days after the mini cuttings are planted (Figure 17). Depending on the climatic conditions, the length of time until transplanting may be reduced.

The agricultural inputs required to transplant shoots for acclimatisation into tubes are listed in Table 6. To facilitate transport and avoid damage to the cassava seedlings, it is recommended to drastically prune the aerial part, cutting it with pruning shears at 8.0cm from the base.

Table 6. Agricultural inputs required for the transplanting of cassava seedlings for acclimatisation in tubes.

Item	Unit	Quantity
Substrate for seedling production (1)	kg	1,500

Fertiliser of gradual release of up to 90 days, formula 15-09-12 + micronutrients (150g dose per 50L of mixture) (2)	sc 22.68 kg	1
Fertiliser of gradual release over 90 days, formula 14-16-18 (150g dose per 50L of mixture) (3)	sc 25 kg	1
Coconut fibre powder	kg	1,500
Tubes made of photo-stabilised polypropylene with anti-ultraviolet additive, non-toxic material, black, EPDM, 16cm in length, diameter over 6.5cm, internal substrate volume of 290cm3	thousands	13
Tube tray with 54 cells made of non-toxic polypropylene, black, photo-stabilised with anti-ultraviolet additive, measuring 582mm length-wise, 410mm wide and 165mm high. Box-type tray (2) compatible with 290 cm3 tubes	unit	240

Observations:

1. Suggestion: Formulated with composted pine bark or other sources. Substrates can be adapted with the most viable local offerings, provided they produce a light and porous blend.
2. The box-type trays can be placed directly on the ground and are recommended to avoid workbench costs. Trays on the floor can, however, cause inconveniences due to the discomfort of working with them at this height. If this discomfort is to be avoided, fixed easel structures with four wire strands can be made to keep the trays suspended at waist height (1.1m). For this, common trays can be used, compatible with the recommended tubes, but without the supports. The materials needed for this modification are not listed, as they will depend on the chosen structure.
3. The recommended fertilisers must be commercial brands that meet the technical specifications. One with a 45-day release (15-09-12), and the other with a slower release (14-16-18). The option to use a slower release fertiliser (90 days) is intended to ensure good development of the seedlings after transplanting. Depending on soil fertility and base fertilisation, the slower release fertiliser may be dispensed with.

The substrate used to fill the containers should be composed of: 20L of vegetable substrate, 20L of coconut fibre powder,150g of 14-16-18 fertiliser and 150g of 15-09-12 fertiliser. In order to obtain a better homogenisation, 20L of water can be used for this mixture. The mixing of the substrate should be carried out for immediate use, as the release of nutrients begins when the mixture is moistened.

Figure 15. Rooting cassava shoots. 10 days after cutting (A). 15 days after cutting (B). 20 days after cutting, well rooted and ready for transplanting (C).

Figure 16. Acclimatising cassava seedlings, obtained through rapid multiplication, under a shaded structure (A and B) and in a greenhouse (C).

Figure 17. Pruned cassava seedlings, obtained through rapid multiplication, ready for packaging and transport, 2014.

- **Irrigation of acclimatising seedlings under screened structure**

In order to maintain adequate moisture of the acclimatising seedlings in tubes, sprinklers can be used, as seen in sprouting chamber irrigation. Depending on the scale of the RMU and the available resources, it is recommended to establish sprinkler irrigation systems under the structure cover (Figures 18 and 19). The design of these systems should be done by a technician, evaluating the feasibility and most appropriate solutions.

Figure 18. Cassava seedlings obtained through rapid multiplication, acclimatised under screened structure with sprinkler irrigation, 2014.

Figure 19. Cassava seedlings obtained through rapid multiplication, acclimatised in large scale in greenhouse with sprinkler irrigation, 2014.

- **Transportation of seedlings to the field**

The mode of transportation of the seedlings to the definitive site will depend on the distance from the production site. For small distances, it is possible to transport them in the trays, immediately transferring these to the field and removing them from the tubes. When the planting sites are further away, the seedlings may be removed from the tubes and rolled up together, to ease transportation.

Each roll must accommodate 50 seedlings, which should be arranged on a strip of common plastic canvas, which will in turn be rolled up and secured with adhesive tape. After this roll has been made, the aerial parts should be pruned to facilitate transport and reduce water loss through transpiration (Figure 20).

Figure 20. Packaging of seedlings. Layout of the seedlings on the plastic canvas strip (A). Rolling of seedlings with the canvas (B). Securing the roll with adhesive tape, with pruned aerial parts (C), 2014.

- **Planting seedlings in the field**

The chosen area should have well-drained, fertile soil with medium texture. It must be free of compaction and of the presence of previous cassava cycles which included instances of root rot and bacterial blight. The ideal slope should be of about 5%, allowing for up to 12% of sloping, as long as soil conservation practices have been adopted. When possible, the area should be fenced or surrounded by a fenced environment to avoid animal activity.

Planting should be done in pits or grooves with a depth of 20.0cm to 30.0cm. Mineral and organic fertilisers should be deposited at the bottom of the pits or grooves.

- **Irrigation system for seedling planting area**

The plants that will be destined to the production of propagating material (stem cuttings) must have good growth. Until they reach maturity, between 10 and 14 months, the development of the stem cuttings should not be hampered by periods of water deficiency. To achieve this goal, it is necessary to provide irrigation even in regions where there are few dry periods. These can take the shape of either total irrigation, during months without precipitation, or complementary irrigation, to maintain adequate water supply in periods between rains.

Within the *Reniva* Network, all fields must be irrigated in order to allow the seedlings to be planted at any time of the year, allowing for a year-round production of stem cuttings, regardless of the climatic season. Therefore, the seedlings must be irrigated immediately after planting, which will enable immediate vegetative development.

The choice of method and equipment should be guided by a qualified technician to suit local conditions. It is important to note that micro and drip irrigation, as well as conventional sprinkler systems can be used. The choice will be based on an assessment that considers water supply, power source, local infrastructure, labour availability and available financial resources.

Planting and management instructions for the plants are detailed in the Embrapa publication entitled “Technical recommendations for the production of stem cutting seeds from micropropagated seedlings. The role of the “Maniveiro” - *Reniva* Project” (ROCHA, et al., 2014).

Final considerations

Any region of Brazil can be included in the *Reniva* network. To do so, it is necessary that all four pillars of this strategy be followed in full, i.e. (1) multiply only genetic material that is proven to be virus-free (indexed), (2) have the established figure of the “maniveiro” producing cassava seedlings and shoot from parent plants that are proven to be virus-free, (3) have trained technical assistance to guide cassava farmers and producers included in stem cutting seed production, and (4) establish a partnership with Embrapa in order to receive technical training, technical information on *Reniva,* and virus-indexed parent plants. *Reniva* can and should include both the varieties produced by Embrapa and the creole or traditional varieties that could be low in productivity due to the accumulation of diseases. However, it is important to note that the essential premise of *Reniva* is to ensure high genetic and phytosanitary quality.

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Appendices

Appendix A - Perspective and ground floor of nursery

Perspective

Side view

Figure 1. Central connection, 2008.

Figure 2. “T-shaped” connection, 2008.

Figure 3. Side connection, 2008.

Figure 4. Connecting vertices, 2008.

Appendix B - Sprouting chamber

Ground floor - Sprouting chamber:

Arch in 3/4” x 1/8” outer flat iron

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

Metal sheet

Front view - Sprouting chamber:

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

Fixing rivets for slats and agricultural plastic wrap

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

1 1/4” x 1/8” angle bracket

1 1/4” x 1/8” angle bracket

15cm x 15cm x 5mm foot

Front view - Cover half open - Sprouting chamber:

3/4” x 1/8” outer flat iron

Fixing rivets for slats and agricultural plastic wrap

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

1 1/4” x 1/8” angle bracket

1 1/4” x 1/8” angle bracket

15cm x 15cm x 5mm foot

Side view - Sprouting chamber:

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

Fixing rivets for slats and agricultural plastic wrap

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

1 1/4” x 1/8” angle bracket

Side view - Elevated cover - Sprouting chamber:

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

Fixing rivets for slats and agricultural plastic wrap

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

1 1/4” x 1/8” angle bracket

-B cut - Sprouting chamber:

Outer flat iron

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

1 1/4” x 1/8” angle bracket

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

1 1/4” x 1/8” angle bracket

Soil material

A-A cut - Sprouting chamber:

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

Soil material

1 1/4” x 1/8” angle bracket

Metal sheet

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

2m x 9.5cm x 0.5mm aluminium or polyamide sheet

Appendix C - Rooting chamber

Ground floor - Rooting chamber:

Arch in 3/4” x 1/8” outer flat iron

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab

Front view - Rooting chamber:

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

Fixing rivets for slats and agricultural plastic wrap

1 1/4” x 1/8” angle bracket

15cm x 15cm x 5mm foot

1 1/4” x 1/8” angle bracket

Side view - Rooting chamber:

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

Fixing rivets for slats and agricultural plastic wrap

1 1/4” x 1/8” angle bracket

15cm x 15cm x 5mm foot

Side view - Elevated cover - Sprouting chamber:

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

Fixing rivets for slats and agricultural plastic wrap

1 1/4” x 1/8” angle bracket

A-A cut - Sprouting chamber:

Agricultural plastic wrap

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

Metal sheet

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab

B-B cut - Sprouting chamber:

Outer flat iron

3/4” x 1/8” outer flat iron

Agricultural plastic wrap

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab

Appendix D - Workbench (table for seedling transplanting)

Ground floor - Table:

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab

A-A cut - Table:

1 1/4” x 1/8” angle bracket

3/4” x 1/8” outer flat iron

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab

B-B cut - Table:

1 1/4” x 1/8” angle bracket

1m x 50cm reinforced concrete slab