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Producing rooted cassava plantlets for use in pot experiments

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Abstract

Cassava is normally propagated using stem cuttings of various sizes. Rooted plantlets are however best for pot experiments, due to the short timeframes of these experiments. Unless large pots (more than 5 L capacity) are used, cassava grown in pots is not usually grown for more than 3 – 4 months. Clear responses to nutrient supply in cassava plants cannot be obtained within such short timeframes with mature cassava stem cuttings because of the large amount of nutrient reserves contained in mature cassava stem cuttings. The nutrient reserves delay the observation of responses to soil nutrient supply (fertiliser application). Responses to other treatments are also probably delayed when mature stem cuttings are planted in pot experiments instead of young succulent, nutrient hungry rooted plantlets. This protocol describes how to produce rooted cassava plantlets or shoots for use in pot experiments. The protocol would not have been developed without guidance from Dr R.H. Howeler. The protocol is also based on a protocol he had already prepared in the Cassava Handbook (see bibliography for citation). Please also refer to it, it is available online for free.

Materials

- Machete
- Sharp razor blade
- Disinfectant e.g. bleach (sodium hypochlorite) and alcohol (see where it should be used)
- Buckets (5 L and 10 L)
- Cassava stem cuttings
- Small patch of land to grow cuttings (10 m x 10 m)
- Hand-hoe
- Large sheets of paper
- Distilled water
- Sizable plastic jars; old 5 L containers can be cut and used for this purpose

Before start

Due to the several activities involved before the rooted plantlets are produced, some of which take time, make sure you allocate enough time before the planned start of the pot experiment. The activities involved when producing rooted cassava plantlets are as follows; collection of planting material, production of cassava shoots using rapid multiplication (4 – 6 weeks), and the rooting of cassava shoots into rooted cassava plantlets (4 weeks).

Collecting cassava stem cuttings

1 Cassava stem cuttings of about 1 m to 1.5 m long must be collected from mature healthy looking cassava plants of the respective test cassava variety. A machete will be needed to collect the cuttings. Not so many of these long stem cuttings will be needed as you will cut them into ministem cuttings i.e. short cassava stem cuttings (5 – 10 cm) to be used as planting material. Make sure you collect long stem cuttings that can give you the number of needed plantlets; assume that each ministem cutting will give you a plantlet when doing your calculations. Do not forget to add extras just in-case.
Also make sure that you disinfect the cutting tools e.g. the machete. This should ideally be done as you move from one plant to the next when collecting the long cassava stem cuttings but it could also be done as you move from one field to the next. Disinfecting cutting tools helps eliminate disease transmission from infected plants to cuttings of healthy plants. A 10% bleach solution (e.g. 1 part bleach to 9 parts water) can be prepared and used for this purpose. Cutting tools should be be dipped into the disinfectant solution or thoroughly wiped with it before re-use. Rinse tools in clean water after disinfection. Take disinfection of cutting tools **seriously**, as your cassava plantlets could all turn-out to be diseased and the process of producing them restarted. Some plants may also appear to be healthy making it difficult to identify them as diseased plants, which makes disinfection of cutting tools even more important.

Rapid cassava multiplication

2 Follow the steps listed below:

2.1 ***Site selection for nursery beds***
A small patch of land will be needed to make a nursery bed where the cassava cuttings will be grown. A well-drained, relatively flat piece of land with nutrient poor soils should be selected for this purpose. The site selected should preferably have not been fertilised before and it should have a uniform soil fertility across it. The selected site should not be more than 10 m x 10 m in size; this size creates space for the nursery beds and leaves enough space between and around them (Fig 1). The size of the selected site could be even less or more, as this depends on the number of ministems to be planted. Larger pieces of land may also be needed if you prefer to leave a wider boarder around the nursery beds and the immediate surroundings. The nursery beds should be made as raised beds, with a width of 1 m and they can be of any length.
Some varieties can be very similar to each other, so make a nursery bed for each test cassava variety so that you lessen the probability of mixing up cassava varieties at this stage. Label the nursery beds with the variety names in advance. The nursery bed should be near a source of water to facilitate irrigation.

Fig 1. Nursery beds with planted cassava ministem cuttings

2.2 ***Preparing the cassava ministem cuttings***

Cassava ministem cuttings should be prepared on the day of planting. Cut the long cassava stems into ministem cuttings of approximately 10 cm long. The ministems produced may have a varying number of nodes as cassava grows at different rates. To ensure the successful germination of shoots, each ministem should however have at least a minimum of 2 nodes. Do not neglect to disinfect the cutting tools when preparing ministem cuttings; this should ideally be carried out after each stem has been cut into ministems, and before you move to cut the next cassava stem. The ministem cuttings are now ready for planting.

2.3 ***Planting***

Thoroughly water the nursery beds a day before planting. On the following day, plant the cassava ministems using a spacing of 10 cm x 10 cm. Plant the ministems vertically making sure that at least two-thirds of the cutting in pushed into the soil. This reduces their chance of getting dehydrated hence increasing their ability to survive. When planting, place the older ends of the cuttings into the soil first, as this ensures that

auxiliary buds face upwards and not downwards (Fig 2). Auxiliary buds, which develop into shoots, should be above the nodes and not below the nodes to enable them to grow upwards. After the planting has been completed, water the plants and keep them well-watered by watering them at least twice a day or as weather conditions demand. Leave the cuttings to sprout and for their shoots to grow.

Fig 2. Planted cassava ministem cuttings with auxiliary buds growing into shoots

Note: This is the first step in reducing nutrient reserves from shoots produced from cuttings. As all shoots to be produced undergo this process all their nutrient reserves will be relatively evenly reduced.

Harvesting the cassava shoots

3 The sprouted shoots should be allowed to grow until they are about 15 cm long (Fig 3), after which they can be harvested. You can use any plant cutting tools, including a razor blade to collect the shoots. Do not neglect to disinfect cutting tools when moving from plant to plant. The disinfectant solution should be prepared and placed in a small bucket for this purpose. Alcohol could also be used to disinfect the tools in this case, as the stems and cuttings tools are small in size. Place the collected shoots onto large papers

and keep them shaded and aerated. It is important for cut shoots to be kept from getting dehydrated before they can be placed in rooting water, so do this stage without delays. Carefully observe each plant before harvesting it, to avoid collecting diseased plants. Remember not to mix-up shoots belonging to different cassava test varieties. Make use of labelling! Carry the collected shoots from the field to the place where you will prepare them for rooting.
Note: This step should preferably be carried out in the early morning hours when temperatures are low.

Fig 3. Nursery beds with planted ministems that have produced shoots

Rooting cassava shoots to produce rooted plantlets

4 Follow the steps listed below:

4.1 ***Preparing the shoots for rooting***

Each shoot should have its end re-cut to produce a smooth slanted cut (Fig 4). You can use a sharp razor blade for this purpose, if you do not have fancy cutting tools. Do not forget to disinfect your cutting tools. Re-cutting the shoot ends ensures that any bruised ends that can encourage rotting are removed. Slanted cut ends give the root ends a greater surface area for water absorption. Cut off the stem at a distance of 0.5 cm away from the initial shoot end before you make the slanted cut, if necessary you can go up to 1 cm. The slanted cut should be a 0.5 cm long diagonal cut.
Shoots must not have too many leaves on them when being rooted. Thus, constantly remove any excess leaves from each cassava shoot, leaving behind only 2 - 3 leaves. Leaves should be carefully removed by plucking off only the leaf blades. Petioles should be left to fall off on their own, with time. In other words, do not remove leaves from shoots by plucking off the petioles together with the leaf blade as this may wound the shoots. Shoots must not be wounded as rotting may set in.
Note: The described steps should be carried out immediately before placing shoots in water to root.

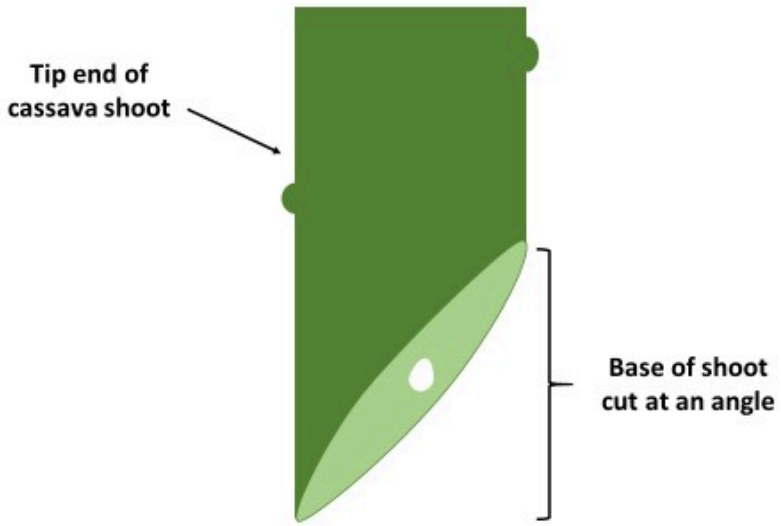

Fig 4. Cut tip end of cassava shoot in preparation for rooting

4.2 ***Preparing the rooting containers***

The jars or containers to be used for rooting should have already been prepared well before this step. Any container can be used. A cut out base of a 10 L water container was used in Fig 5. Enough height (10 - 12 cm) should be left on the container walls as walls give support to shoots and they off course enable the container to hold enough water. The container should however be opaque to block out the sunlight as this discourages

algae growth on the walls and floors of the container. In Fig 6 you can see that this was achieved by having the sides of the transparent containers covered with opaque plastic bags. Prepare a number of these containers so that you will not have to squeeze too many plants into one container. Too many plants in one container makes the rooting water to quickly get bad which in turn enhances shoot rot. Fig 5 shows that right amount of shoot crowding in a container.

Note: Some researchers may advise to root shoots in individual small containers, probably to avoid disease transmission between shoots. Rooting shoots in individual containers would be ideal especially if you can manage to get many small containers or bottles for this purpose.

Fig 5. Shoots being rooted in open plastic containers

Fig 6. Shoots being rooted in open plastic containers with covered sides

4.3 ***Initiating shoot rooting***

Once the shoots have been all prepared for rooting, pour distilled water into each container in readiness for the cuttings. Distilled water is used as it encourages faster rooting. Distilled water is also nutrient free, pH neutral and sterile. If you cannot manage to obtain distilled water you can alternatively use cooled boiled water. Once the distilled water has been placed in the containers, gently place the cassava shoots in them. Do not add too much water into the containers as the shoot ends may float. Test to check which water level is best. Adding only a little water is also undesirable. The shoots should lean on the edge of the containers with their ends below the water surface. Leaves must not be allowed to touch the water in the containers as they may rot in the water.

Note: Rooting young shoots is best carried out in a screenhouse as this environment can help keep away insects and other pests. Thus, this step onwards should be carried out in a screenhouse if you have access to one.

4.4 ***Managing the rooting process***

Monitor the rooting process and observe for any need for making adjustments to the process. Shoots should be continually checked for any signs of rotting, which often begins at the shoot ends. Always check the odour and temperature of the water in each container, as this can give an indication of something going wrong. Bad water odours

and overly warm water temperatures can be a sign of the setting in of anoxic conditions. Temperature changes are best observed when it is cool as this avoids confusion with changes resulting from warm ambient temperatures. Check on the shoots at least twice a day.

Water in rooting containers should be often replaced. Water replacement should ideally be carried out every after 2 days, but sometimes, especially towards the end this can be even done after 3 days. The water in the rooting containers should however be changed each day, to allow shoots to adjust to their new environment during the first week after planting. The frequency of changing water will however be higher when plants are crowded in containers. Remember to always wash the containers before pouring in fresh water.

During root development shoots will continue to grow new leaves even before they begin to root. The rate of new leaf development will vary between varieties just as the rate of root development. Some cassava varieties will also keep elongating their stems as they develop new leaves. Older leaves should constantly be trimmed off to leave behind only 2 to 3 leaves on each shoot. If excess leaves are not removed from shoots, water loss from containers is increased, due to higher evapotranspiration rates. More importantly root development is also delayed as the few available nutrient reserves in the small shoots get expended on shoot growth rather than on root growth.

Fig 7 shows an example of cassava shoots that have just began rooting, while Fig 8 shows cassava shoots with progressed rooting. Roots should be allowed to grow until they are ideally 1 cm long and not more than 1.5 cm long, before the plantlets can be transplanted. Do not wait until roots become overgrown and dense.

Note: Rooting shoots in distilled water helps to further reduce any nutrient reserves in shoots. As all shoots also undergo this process all their nutrient reserves are evenly depleted in readiness for them to show quicker responses to nutrient supply once potted. It takes about 4 weeks for most shoots to become well rooted.

Fig 7. Cassava shoots that have just begun developing their roots

Fig 8. Cassava shoots with progressed but still not sufficient root development

Planting rooted plantlets

5 The pots into which the plantlets will be transplanted into should be already ready once roots are about the desirable length. Transplanting should only be carried out after the soils in the pots have been watered to field capacity and left in this state overnight. On the following day and in the early morning hours you can then begin transplanting. Make a 5 cm deep hole in the moist soil and gently place in the now rooted cassava plantlets (Fig 9). The plantlets will be happier and full of life once in the soil (Fig 10). Lightly water them after they have been planted.

Fig 9. Planting rooted plantlets in soil filled pots

Fig 10. Planting rooted plantlets in soil filled pots

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