2. Hardening of Cassava *In Vitro* Plantlets and Rapid Micro-propagation of Cassava Plants Through Nodal Cuttings

Abstract

Cassava has become suitable for food security and economic development in unfavoured areas of the tropics. Lack of good quality planting material, in larger quantities and at the right time is one of the most important constraints limiting expansion of cassava production in Africa. Plant tissue culture technology has been successfully used to propagate cassava and other plant species like sugarcane, bananas and sweet potato. This has facilitated international exchange of clean clones, conservation of germplasm, and it has also helped alleviate cassava's multiplication constraints at farm level in developed countries. However, in Africa hardening of cassava in vitro plantlets and production of massive plants is a major drawback because this technology is capital-, labor- and energy-intensive. This study describes a successful protocol for hardening and rapid micro-propagation of cassava plantlets under local Kenyan conditions using nodal cuttings, vermiculite, sterile soil and improvised humidity chambers. A total of 1173 plants from 31 putative drought-tolerant and drought-susceptible germplasm accessions were acclimatized using the developed protocol as compared to 722 plants obtained with the use of sub-culturing technique. Overall increase after 210 days with a rate of 13.8 for direct and 8.5 for in-direct micro-propagation were observed. The protocol was also cheaper in terms of consumables as compared to the tissue culture/in-direct method of micro-propagation.

Key words: Cassava; direct micro-propagation; hardening; in-direct micro-propagation; *in-vitro*; Kenya; *Manihot esculenta*; nodal cuttings; tissue culture

2.1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important staple food crops in Africa. In Democratic Republic of Congo (DRC), Ghana, Mozambique, Nigeria, Tanzania and Uganda, it is the number one staple crop (Nweke et al., 2002). Its importance as a food crop in Africa becomes obvious when its annual per-capita consumption is compared to the rest of the world. Whereas the world average annual cassava consumption was 17 kg/capita in 2001, Africa's annual consumption was above 80 kg/capita. Latin America's consumption decreased by half over the past 30 years to slightly more than 20 kg/capita in 2002 (Aerni, 2006). Its productivity, drought and acid soil tolerance, and its ability to grow on marginal soils with minimum inputs makes it a vitally important crop to some of the world's low-income food-deficient countries and a significant famine reserve crop (Cock, 1985; Xia et al., 2005).

Cassava has a high yield potential. According to FAO, 172 million tonnes of cassava were produced worldwide in 2000, of which Africa produced 54%, Asia 28%, while Latin America and the Caribbean produced 19% (Manyong et al., 2004). In Africa, average yields are only 8-10 tons per hectare compared to potential yields of over 80 tonnes under ideal conditions (Taylor and Fauquet, 1997). The gap between the actual and potential yields on farmers fields is around 8-fold. This is a clear indication that the highest potential of cassava production is far from being reached, although when compared to maize, sorghum and rice in environments with no production constraints, cassava can match or exceed the energy production per hectare of these crops (Vries et al., 1967).

Cassava roots are the major portion of economic product in Africa, which are consumed as human food after varying degrees of processing. In addition, they are increasingly being used as a potential substitute for maize in feed concentrates and for wheat in bakery goods. The variety of cassava starch cannot be under-estimated in addition to its role in the production of glue, paper and biodegradable plastics. The roots also serve as a source of cash income for small-holder farmers (Bottema and Henry, 1990; Escobar et al., 2006). Cassava storage roots do not function as propagules like other tuberous roots. The roots serve as a repository of photosynthate

and, thus, they help the plant to recover from defoliation after drought episodes (Han et al., 2001).

Cassava plants are conventionally propagated through stem cuttings. Although this system is commonly used, diseases often accumulate in the propagules resulting in infected stands and reduced yields. Other challenges include high perishability, as cuttings dry up within a few days, high handling and transport costs, low propagation rates compared to grain crops, and inconvenient weight and bulk of the material (Escobar et al., 2006). A collaborative research conducted in 2003 by IITA, the Swiss Federal Institute of Technology (ETH Zurich), the Donald Danforth Plant Science Center (DDPSC, USA), CIAT, the Brazilian Agricultural Research Corporation (Embrapa, Brazil), representatives from local universities, farmer organizations, and multinational companies sponsored by the Swiss Center for International Agriculture (ZIL) revealed that lack of clean planting stakes was the most important constraint facing subsistence farmers in Africa (Figure 2.1).

Tissue culture has been effectively used to eliminate viruses and other systemic diseases from elite cassava vegetative materials (Jorge et al., 2000). This has allowed exchange and conservation of rejuvenated propagation materials, which have higher yields than the same varieties propagated for successive years in the field (Kassianof, 1992). However, one of the major limitations for a wider adoption of this technique in developing countries is the unavailability of a procedure for hardening and multiplication of the tissue culture plantlets before final transplanting in the production sites. Although reports are available on *in vitro* hardening of cassava in the developed world, the protocols are difficult and expensive to implement in developing countries since the technology is capital-, labor- and energy-intensive (IAEA, 2004).

Even though labor is cheap in developing countries, the resources of trained personnel and equipment are often not readily available. In addition, electricity and clean water are costly especially with the plight of climate change and global warming. It is, therefore, necessary to have a low-cost technique for acclimatization and rapid micro-propagation of tissue culture plantlets suited for developing countries.

The present study was conducted within the framework of the project "Identifying the physiological and genetic traits that make cassava one of the most drought-tolerant

crops" implemented since 2005 by the Brazilian Agricultural Research Corporation (Embrapa); the International Center for Tropical Agriculture (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University, USA and University of Goettingen, Germany.

The main objective of the present study was:

- to develop a protocol for hardening and rapid micro-propagation of cassava plantlets under local, low-cost conditions.

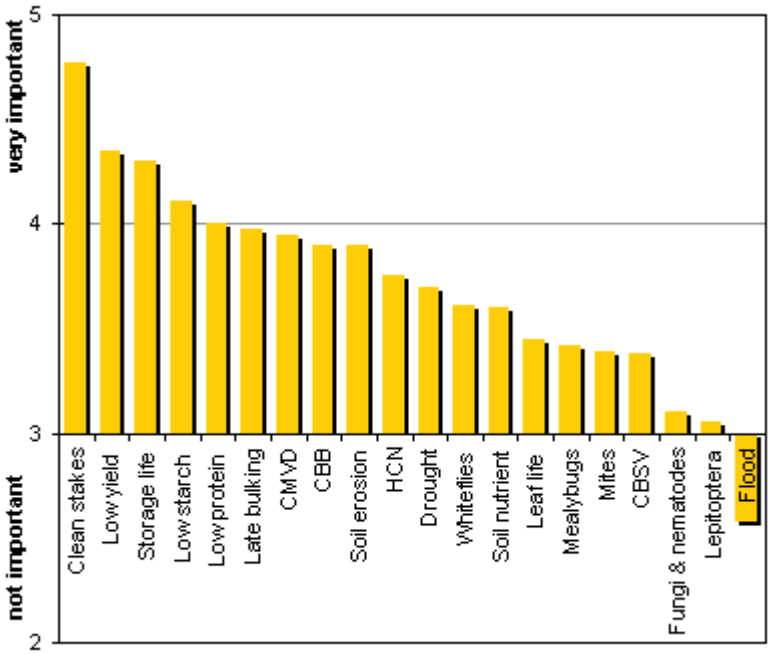

Figure 2.1. Average ratings of the importance of problems in cassava subsistence agriculture in Africa, as assessed in 2003 by researchers from International Institute of Tropical Agriculture (IITA, Nigeria), the Swiss Federal Institute of Technology (ETH, Switzerland), the Donald Danforth Plant Science Center (DDPSC, USA), International Centre for Tropical Agriculture (CIAT, Colombia), the Brazilian Agricultural Research Corporation (Embrapa, Brazil), representatives from local universities, farmer organizations, and multinational companies, on a scale from 1 (not important) to 5 (very important) (Adopted from Aerni, 2006).

2.2. Materials and methods

In vitro plants of 31 putative drought-tolerant and drought-susceptible African cassava germplasm accessions were obtained from IITA, Nigeria (Table 2.1). The plantlets were delivered in polystyrene boxes at Kenya Agricultural Research Institute (KARI, Nairobi). Upon arrival, the plantlets were removed from the boxes (Figure 2.2a). They were counted, genotypes confirmed and kept in the growth room for three days to recover since they had spent one week in the dark during transportation and clearance. On the fourth day, the individuals of every genotype were divided into two, the ones to be sub-cultured to act as a backup (in-direct micro-propagation), and also to compare the multiplication rate with the rapid micro-propagation, and the ones to be hardened and multiplied without sub-culturing (direct micro-propagation) (Table 2.1).

2.2.1. Sub-culturing (in-direct micro-propagation)

The plastic tape that had sealed the bottles was removed. The bottle neck with the plantlets was passed over an open flame and opened aseptically. The plantlet was picked from the culture jar and placed on a Petri-dish with the aid of a sterile forceps. The leaves were chopped off and the stem was cut into small pieces of about 3-4 cm each having at least two nodes. The nodal explants were placed in Kilner jars containing 50 ml basic semisolid culture medium (Murashige and Skoog, 1962) with 2% sucrose and solidified with 2.5% phytagel at pH 5.8 before autoclaving at 121ºC for 20 min. at 15 psi. The bottles were capped with tops and plastic tape. The cultures were kept in the tissue growth room at 27ºC in a 16-h photoperiod. The plantlets were sub-cultured after 2 months. In the 4th month, the plantlets were acclimatized in the green-house.

2.2.2. Transplanting and hardening (direct micro-propagation)

Perforated plastic pots (3" x 4" gauge 100) were filled with sterile, medium-grade vermiculite to three quarters their volume. Vermiculite is cheap and locally available and it promotes maximum root growth since it is well aerated and retains moisture and nutrients. Soil was not used since it gets compact after watering and, hence, damages the absorbent hairs, root cap and roots. The plastic pots containing vermiculite were placed in plastic trays. Plastic tape and bottle caps were removed from the bottles. A spatula was used to disturb the semisolid media taking care not to

damage the roots. The bottles were tapped gently at the bottom in an inclined position so as to extract the plant. Forceps and tweezers were not used so as not to damage the stem. The plantlets were pulled out of the bottle and thoroughly but carefully washed with running tap water to remove adhering medium completely. The plantlets were placed in the perforated pots with vermiculite and labeled (Figure 2.2b). The roots and the stem were handled carefully to prevent physical or physiological damage. The pots were placed on a trough with 1 cm level water. To conserve moisture and maintain a high humidity, each plant was covered with a transparent polythene bag (9" x 13" gauge 300) after pre-spraying with 0.2% Diethane M45 (Mancozeb, 80%; Manganese, 16%; Zinc, 2%; Ethylene bisdithiocarbamate, 62%) fungicide solution and tied with a rubber band at the base (Figure 2.2c). The pots containing the plantlets were not moved during the first month. Watering was done every week through the base of the trough. The temperature in the green-house was maintained between 25ºC and 30ºC throughout the acclimatization period. On the 21st day, one corner of the transparent bag was cut open to enable the plantlets adapt to the micro-environment of the green-house. The other corner was cut on the 24th day. The whole polythene paper was completely removed on the 28th day (Figure 2.2d). During this step, the plantlets were protected from strong dehydrating winds by restricting entrance to the green-house. Between day 30 and 40, the plantlets were transplanted into plastic bags (5" x 8" gauge 100) containing sterile soil (forest soil: sand: humus 45:30:25), since they required more nutrients and space for growth and development (Figure 2.2e and f). A water soluble foliar feed (NPK, 19:19:19) was applied every week up to 90 days at a rate of 1% (1 g l^{-1} of water).

2.2.3. Rapid micro-propagation

After 3 months, each plant of the hardened plants was cut with a sterilised surgical blade in a slanting position into small pieces containing at least two nodes. The cuttings were planted in polythene bags (5" x 8") containing sterile soil and were well labelled (Figure 2.2g). The plant parts were well watered and then covered with a humidified transparent polythene bag (9" x 13") and tied with rubber bands (Figure 2.2h). They were kept in the green-house under high humidity at temperature between 25ºC and 30ºC. On the 7th day, one corner of the polythene bag was chopped off to enable the plants to adapt to the micro-environment of the greenhouse. On the 10th day, the other corner of the covering polythene bag was also chopped off and plants

were maintained that way for 7 more days. On the 14^{th} day, the polythene bag was removed completely. The materials were allowed to grow in the green-house for 1 month and the rapid micro-propagation procedure was repeated again (Figure 2.2i). The plantlets were taken to the field for establishment after 90 days (Figure 2.2i, 2.2j).