Conservation and distribution of cassava genetic resources

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1 Introduction

The conservation of genetic diversity is an essential activity for any crop and underpins current and future crop improvement programmes. Cassava Manihot esculenta Crantz is a vitally important crop in many parts of the world. Significant germplasm collections have been established in international and regional genebanks and have been used extensively by plant breeders. Conservation of genetic resources falls into two main categories: ex situ and in situ. Ex situ includes all germplasm removed from its situation in the wild or on
farmers’ fields. Generally this refers to genebanks at the international, regional, national or local levels. Cultivated cassava is a clonally propagated crop and this is a major factor in how it is conserved in genebanks. As shown in detail below, approaches to conservation are only rarely through true (botanical) seed but rather as a field collection which is regenerated every two years, as in vitro plantlets or, in the future, cryopreservation. This situation is different for wild species of Manihot which are typically propagated through seed and conserved in that form. It seems likely that a significant proportion, at least 50%, of all cassava clonal genotypes is conserved in genebanks. However, the situation with respect to the wild relatives of cassava is very different, with many species represented only very sparsely in genebanks and an unknown but likely small proportion of the diversity of these species being conserved. There is a large potential for in situ conservation approaches with respect to these wild relatives which is hitherto unrealised.

2 Origins and genetic diversity of cassava

Cassava belongs to the family Euphorbiaceae; the possible origin of the genus Manihot is in Mexico/Central America and the cultivated species may have been domesticated from a wild species progenitor possibly along the south-western edge of the Amazon rainforest (Allem, 2002). The highest genetic diversity is seen in Brazil and that within Central America is also high. Cassava is monoecious, with separate male and female reproductive organs on the same plant and this is reflected in the high level of outcrossing observed.

The genus Manihot has the following attributes (from Hershey, 2008):

- Perennial
- Sporadic – do not dominate vegetation in which they occur
- Shade intolerant
- Weak competitors
- Frost sensitive
- Adaptations for drought avoidance and tolerance – include storage roots with starch
- All have linamarin which when cells are damaged produces HCN
- High morphological variability within species

All Manihot species have $2n = 2x = 36$ chromosomes. The classification of Rogers and Appan (1973) has been generally accepted with the genus divided into 98 species in 19 sections (Rogers and Appan, 1973; Rogers and Fleming, 1973; Allem, 2002). A major feature is that Northern and Southern hemisphere species groupings are very distinct (Allem, 1987, 1994, 2002). Allem discovered M. esculenta subspecies flabellifolia in Brazil and subsequent molecular analysis shows M. esculenta lies within the range of variation for this species. The ssp. flabellifolia is a likely progenitor of M. esculenta, possibly with landrace ‘intermediaries’ (Allem, 2002). All the wild species of the genus seem to be mainly seed propagated and the process of domestication is observed mainly in increased root thickening, swollen leaf scars, shortened internodes and thicker stems, serving as carbohydrate reserves. The cultivated species is mainly vegetatively propagated and this has very important implications for its conservation and breeding. Deletre et al. (2016) showed that unconscious selection of seedlings from sexual reproduction, with morphology similar to that of the crop being cultivated, can act to maintain distinct landraces.
3 Ex situ conservation of cassava genetic material

Major international collections of cassava are held at CIAT and IITA, two institutes of the CGIAR. CIAT in Colombia has the most extensive cassava collection. It holds more than 6000 cassava accessions with the great majority of these coming from South America including around 2400 from Colombia and 1500 from Brazil (http://isa.ciat.cgiar.org/urg/cassavacollection.do). IITA conserves over 4000 accessions of the crop mainly from countries of sub-Saharan Africa. More than 90% of this collection originates in West Africa (http://my.iita.org/accession2). The cassava collection at EMBRAPA-Brazil is about 4000 accessions. Other important genebanks are those in CTCRI-India, INIA-Peru, NRCRI-Nigeria, IAN-Paraguay, SRCV-Benin, NARO-Uganda, D.R. Congo and PGRC/CRI-Ghana.

Genebank standards have been published by FAO and updated in 2014 (FAO 2014). They outline the principles and practices of conservation of ex situ conservation. Standard operating procedures (SOPs) should be in place for all genebank activities from acquisition to distribution and including all aspects of data collection and management. These are key components of quality management systems.

At IITA, germplasm is maintained in the field and in vitro and cryobanking of cassava has recently commenced. It also has a small field collection of wild Manihot. Both IITA and CIAT engage in ‘black box’ safety duplication of in vitro plantlets: at CIP (Lima, Peru) for CIAT and the IITA/Africa Rice facility at Cotonou, Republic of Benin, for IITA. The term ‘black box’ means that germplasm is simply maintained as it is without, for example characterisation or distribution. However, this term is somewhat misleading when applied to in vitro duplication of clonal crops since considerable time and effort are required to maintain viable contamination-free plantlets. In the next few years cryobanking at IITA, with germplasm also cryopreserved at another site, will represent a sustainable long-term approach to safety duplication.

4 Field conservation of cassava genetic material

Conservation of genetic resources in the field is a common ex situ approach in cassava. It has the advantage that germplasm can be phenotypically characterised and evaluated and that accessions are maintained in ways that avoid the possibility of genetic or epigenetic changes introduced through tissue culture. The identity of accession, through continuous field propagation from the original, should be maintained if high standards of field procedures and sample tracking are applied. This can be very important in establishing whether accessions (e.g. those held in vitro) are ‘true to type’. However, field conservation also has disadvantages, particularly the resource (labour, land, time) requirements and the potential loss of accessions from pests, diseases, abiotic stress and so on. Cassava performance (and related to risk of loss in the case of field genebanks) has been linked to the type of soil where it is planted; selection of a well-aerated, loose and light sandy loam soil is preferred. Other factors to be considered during land selection for a field genebank include risk of flooding, prevalence of pests and diseases, and unsolicited human intervention. Before the planting of cassava in the field, there are a number of activities that are carried out in land preparation. These include mowing, ploughing, harrowing and ridging. Except where irrigation facilities are available, the planting of cassava is carried out at the outset of the rainy season which is around April in the derived savannah and
some parts of the humid forest regions of Nigeria. The right selection of healthy mother plants for the cassava stem cuttings is key for success in cassava sprout rate. Disease-free stem cuttings with enough energy reserves ensure better sprout rates and these are usually located in the mid-section of healthy and mature cassava plants. A spacing of 1 m by 1 m between plants in ridges and an alleyway of about 1 m to 1.5 m is usually required for maintenance and research purposes and this is done by the methodological marking of the field. Weeds are either removed manually with the use of hoes and cutlass or through the application of herbicides. Manual weeding is laborious while herbicide application may be observed to be expensive in the short term. In most cases, a combination of both approaches is employed for the combating of weeds in cassava fields. The frequency and need for weeding may be different in cassava fields depending on soil and canopy type. A typical approach is the application of pre-emergence herbicide shortly after planting before sprouting of the cassava stems. Post-emergence herbicide application comes any time from the period the planted cassava stems have sprouted to the time they are harvested and this can be carried out from three to four times in a yearly cycle.

Cassava plants are prone to a number of pests and diseases in the field. These include rodents, although generally good weed management is enough to ward them off. Hoppers and whiteflies are also threats and insecticide is usually sufficient for acceptable levels of protection. There are a number of important diseases of cassava fields, especially cassava mosaic disease, cassava bacterial blight, cassava brown streak disease and cassava anthracnose disease. These diseases are best prevented by the use of healthy plant materials that are free from their causative pathogens and the maintenance of high hygienic standards in field cultural practices. Frequent field monitoring is an essential component of cassava field management practices.

Field conservation has the distinct advantage that it allows the collection of a wide range of information about accession description and performance. Most field genebank managers will want to take advantage of this opportunity, not available for in vitro genebanks. It is important that accessions have not only passport data but information on a range of morphological descriptors, that is, characterisation data. Phenotypic characterisation takes place in the field and for cassava at IITA it spans one year. Data are taken up to four times at an interval of three months and is collected from both the aerial and root parts. Data collected are both quantitative (e.g. number of roots) or qualitative, for example, leaf colour. Measurements made on the aerial parts include colour of unexpanded apical leaf, colour of first expanded apical leaf, petiole colour, petiole length, absence/presence of stipules, anthocyanin pigmentation, incidence and severity of diseases. Data taken from the underground parts include storage root form, absence/presence of root peduncle, root surface colour, root surface texture, starch content, harvest index and poundability.

5 Core collections of cassava genetic material

In genebanks which have large numbers of germplasm accessions for a particular crop the concept of a ‘core collection’ has often been applied. A core collection is essentially a fraction of the total number – often around 10% – which captures most of the genetic diversity as shown commonly by morphological descriptors or a combination of morphological characters and molecular markers. A number of such core collections have been developed in cassava. They have proved useful as a focus for evaluation of germplasm for important agronomic traits and as a starting point for molecular characterisation of the
diversity in the collection. At CIAT a core collection of 630 accessions was developed and the diversity and redundancy levels of this core were initially assessed in 1999 using microsatellite, AFLP and isozyme markers (Chavarriaga-Aguirre et al., 1999). This work suggested that morphological characterisation was effective at ensuring ‘genetically representative non-redundant samples’. However, core collections have their limitations, particularly where a subgroup enriched for accessions with a particular trait, for example drought tolerance, is required. Core collections may not adequately capture useful rare alleles of a species. In recent years a number of approaches to developing traits-specific subsets have been described, including the Focused Identification of Germplasm Strategy (FIGS) that draws upon knowledge of the geographic origin and evolutionary history of accessions (Khazaei et al., 2013). It seems likely that such approaches, integrated with the use of genomic data, will become an important part of ensuring greater use of the wealth of cassava germplasm held in genebanks.

6 In vitro conservation of cassava genetic material

The limitations of field conservation mean that the two major international collections at CIAT and IITA have in vitro conservation at the centre of their strategy and operations for cassava germplasm conservation and exchange. Slow growth conservation has many advantages for clonal crops such as cassava. It constitutes a viable alternative to complement and reduce the large land size required for field banks. Plant tissue culture is a powerful tool for a safer and faster way to multiply large quantities of material for distribution, duplication in other genebanks and international exchange (easier plant material transport); and also for breeding purposes. Slow growth storage is, however, for short- to medium-term conservation, after which the plantlets are subcultured when signs of deterioration/necrosis are visible (Gueye et al., 2012). In vitro conservation offers a means of maintaining valuable gene combinations in a small space, protected against pest and disease attack, soil problems and climatic changes; gives possibility of sanitation and with high multiplication potential; and opens the door for further research possibilities (e.g. somatic embryogenesis and protoplast fusion). They are generally more economical and less risky in a long-term perspective, as compared to field collections. Therefore, effort and research have been concentrated on improving the in vitro regeneration (subculture duration), which is the major leverage point for cost-effectiveness.

The conservation of cassava needs a small quantity of material and allows longer duration between two regenerations or subcultures, using slow growth storage. The principle is to place the in vitro plantlets under slow growth conditions, through adaptation of key growth factors (light, temperature, culture medium and growth retardants). A number of cassava genebanks around the world have in vitro tissue culture facilities as either the principal or a complementary conservation system, with the possibility to clean the germplasm from diseases and pests via meristem culture and/or other sanitation methods such as thermo- or chemotherapy. In vitro slow growth conservation methods require technical expertise, facilities and operating budget. According to Hershey (2008), there are about 70 cassava genebanks worldwide but only 12 countries (Ng and Ng, 2002) have in vitro facilities for conservation. The CIAT genebank conserves its collection exclusively in tissue culture. A few genebanks (mainly EMBRAPA, Brazil and CIAT, Colombia) have seed banks to conserve seeds of wild species or breeding material. A few, including CIAT with its extensive collection from the centre of origin for cassava, are also initiating DNA banks.
Many of these laboratories combine *in vitro* techniques targeted for multiple purposes, for example, for pathogen cleaning, rapid multiplication, gene bank conservation and exchange. *In vitro* conservation of cassava is still far less common than field conservation. The largest national *in vitro* collections are held in Brazil and Argentina, while both Thailand and Vietnam are building their *in vitro* genebank inventories. There appears to be very few *in vitro* cassava genebanks in Africa. Apart from the international collections held at CIAT and at IITA (the two CGIAR centres sharing the international mandate for cassava research, breeding and improvement) all other *in vitro* genebanks have national or regional focus (e.g. National Genebank of China for vegetatively propagated crop species, CTCRI-India and EMBRAPA in Brazil). The *in vitro* conservation procedures are available in IITA and CIAT manuals/handbooks (Dumet et al., 2007; Mafia et al., 2009), also accessible on those CG centres’ websites (www.iita.org, https://ciat.cgiar.org/).

### 7 Cryopreservation of cassava genetic material

Cryopreservation, with respect to the conservation of clonal crops, is the maintenance of plant material at ultra-low temperature, normally in liquid nitrogen at −196°C, using cryogenic techniques. At such low temperature, biological activities and metabolism are stopped, eliminating the need to regularly rejuvenate or regenerate the plant. It is currently a supplementary tool to improve conservation of germplasm in a long-term perspective. For species where the techniques are well-developed and reliable, cryopreservation is the most reliable technique for long-term storage of plant genetic resources (Popov et al., 2005). It also ensures the safe long-term conservation of the physiological state of the genetic resources, thus reducing genetic changes through multiple regeneration cycles. Cryopreservation helps to overcome many of the *in vitro* maintenance disadvantages such as labour-intensive subcultures, potential for fungal and bacterial contaminants and tissue ageing (Benson, 2008). Many studies confirmed that it is economically competitive with other conservation systems (Harvengt et al., 2004; Reed et al., 2004; Keller et al., 2008). Thus, cryoconservation techniques have been increasingly used for the long-term storage. In the last 25 years, several cryogenic techniques have been developed, especially those based on vitrification methods (the transition of water directly from the liquid phase into an amorphous or ‘glassy’ phase, whilst avoiding the formation of crystalline ice) such as encapsulation dehydration, pre-culture dehydration and encapsulation/vitrification. The main requirement for using the cryopreservation method is that it should be simple, economical, reproducible and should allow a relatively high regrowth rate (Leunufna and Keller, 2003).

Work on cassava cryoconservation was initiated in the mid-1980s at CIAT and many researchers have improved on the techniques since then (Escobar and Roca, 1997; Escobar et al., 1997). To date, cryogenic techniques used for cassava long-term conservation have met with different levels of success.

Specific cassava protocols were developed testing and optimising a range of different cryogenic methods. The CIAT method is based on rapid freezing of highly proliferating cassava meristem/shoot tips as explant. Cryobanking of cassava shoot tips has been started at the CIAT cassava genebank after adaptation of protocols from other crops in different laboratories (Escobar et al., 1997; Gonzalez-Arnao et al., 2007). IITA tested various cryogenic techniques according to local conditions (Dumet et al., 2013). Working on numerous taxa, cryopreservation via droplet vitrification showed high efficiency with 48 accessions of cassava maintained at IITA. However, work remains to be done on achieving
an adequate recovery level for a majority of the cassava collection. There appear to be a certain number of recalcitrant accessions, on which research should continue on improving recovery, before committing to large-scale cryopreservation of any genebank.

Cryopreservation is also used for other purposes, such as the cryopreservation of embryogenic tissues for genetic transformation and for botanic seeds.

8 Conservation of cassava genetic material as true seed

Because all known cultivars of cassava, including all landrace varieties in farmers’ fields and in genebanks, are highly heterozygous, the plants that result from cassava true seed segregate for many traits. As a result, none of the progeny from a given plant, even if self-pollinated, will be genetically identical to the parent plant. This is in contrast to the normal vegetative propagation of cassava, where indefinite generations of propagation will yield identical genotypes. In terms of genebanks for cassava, this means that cloned offspring from any single individual of a genetically uniform variety can faithfully represent the genotype of that variety in a genebank. This is the principal under which field and in vitro genebanks are managed.

On the other hand, seeds can preserve the genes of the parents, if a large enough population of seeds is maintained. There are, however, several challenges to seed conservation for genebanks. First, cassava tends to produce few seeds, and some varieties are not known to flower at all (or produce seeds). Producing adequate-sized populations for purposes of gene conservation would be a challenge for some landrace varieties. Secondly, as mentioned above, seed conservation would not allow reproducing the original variety from which they were derived. Since it is often the specific gene combinations that give special value to farmers’ varieties, this value would not be recaptured without extensive breeding. Thirdly, in order to retain just the set of genes existing in a particular variety, without contamination with genes from other varieties, it is necessary to do self-pollination of each variety or accession of interest. Since cassava suffers inbreeding depression in the $S_1$ generation, this reduced vigour can create additional challenges for maintaining and regenerating subsequent generations. However, it would also be possible to inter-cross $S_1$ plants from a given clonal variety, to recover some vigour in subsequent generations while not expanding the gene population beyond what was present in the original variety.

In summary, seed conservation is an option for cassava genebanks, but, given the complexity of management, will not likely be done by more than a few major genebanks, if done at all on a systematic basis.

From the point of view of seed viability, cassava should not present any difficulties. Seed of cultivated cassava can be stored under the same conditions as for many seed-propagated crops. Ellis et al. (1981a) stated that, ‘there can now be no doubt that cassava seeds are orthodox’ and ‘no problems are envisaged with long-term cassava seed storage’ (Ellis et al., 1981a,b). However, conservation of cultivated cassava as seed is rare and at a low level.

9 Data collection and management in genebanks

Different and distinct stages are involved in managing genetic resources. Each stage generates different types of data, including information related to the identity of the accession and where collected (passport data), and the agro-morphological characterisation...
of the plants (characterisation and evaluation data). Full genebank data should be available and publicly accessible through well-managed databases. Genebanks information should be integrated into the global portal for plant genetic resources implemented for national and international genebanks.

Passport data are used as one of genebanks’ ways to manage diversity. This has helped genebank curators and users to group the materials conserved in a more logical and comprehensive order. The minimum descriptors used can serve as a linkage between genebank data and external data sources such as breeders’ evaluation data. One can link geographical passport descriptors to spatial data sources and relate to climatic data. IITA cassava passport data are available in both its public accessions database (http://my.iita.org/accession2/) and in Genesys (https://www.genesys-pgr.org/welcome).

Similar to a person’s passport information page, accession passport data constitutes the basic information used for the general management of accessions: its entry into the genebank collections and description parameters related to where the accessions were originally collected. Materials enter the collection through collection expeditions, from breeding programmes, or acquisition from other institutes. In each case, the material will already have some identifier assigned by the collector, breeder or other institute. Accession name is the vernacular name of the material and is commonly captured by the collector or assigned by the breeder. Genebank accessions obtained through collecting missions should maintain data about the site and dates of the collecting and collector information. Lines developed by breeding programmes of the institute may be included in the collection. Information provided by the breeders should include the pedigree, ancestral information of the material, along with names and identifiers used by the breeding programme and the codes and names of institutes that developed the material. Materials coming from other institutes and genebanks must be accompanied by accession passport data as documented in the source genebank.

Accession documentation should capture any identifiers provided by the source institute. This data allows for validation and curation of passport data between the genebanks and allows researchers to obtain material from either collection. The Multi-crop Passport Descriptors (MCPD V.2) (http://www.bioversityinternational.org/e-library/publications/detail/faobioversity-multi-crop-passportdescriptors-v2-mcpd-v2/) are a revision of the original FAO/IPGRI publication released in 2001, expanded to accommodate emerging needs, such as the broader use of GPS tools, and the implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (http://www.planttreaty.org) Multilateral System for access and benefit sharing.

The Crop Trust (https://www.croptrust.org/) manages an online data portal for accession-level data management-GENEYS. GENEYS is a global portal to information about Plant Genetic Resources for Food and Agriculture. It is a gateway from which information about germplasm accessions and genebanks around the world can be easily found and accessions ordered (https://www.genesys-pgr.org/welcome).

10 Germplasm distribution

International exchange of germplasm is a vital part of the system of genetic resource conservation and utilisation. A key aspect of this is arrangements for phytosanitary testing
and import/export approvals. In cassava this has historically been a constraint and challenge to safe germplasm movement and this is still the case. The second major aspect of germplasm distribution is the system of international law and regulation governing access and transfer. Here a major component is the ITPGRFA. A key element of this Treaty is the development of a multilateral system for the conservation and distribution of a number of major crops listed in Annex 1 of the Treaty. These Annex 1 crops can be held by genebanks and distributed to those requesting accessions (for purposes of research, breeding and training for agriculture) using a Standard Material Transfer Agreement. Effectively this multilateral system obviates the need for bilateral negotiations for germplasm access and distribution under the Convention for Biological Diversity. Cassava is listed under Annex 1 of the Treaty but the related wild species are excluded. In addition, the genebanks of the CGIAR (e.g. CIAT and IITA) have a special status under the Treaty (Article 15) in which accessions are designated as held ‘in trust’ for future generations.

11 **In situ** conservation of cassava genetic material

Whilst *ex situ* conservation of cassava genetic resources is clearly key for their continued survival and utilisation, it may not be possible to conserve all landraces in this way and it is unlikely that a significant proportion of the wild relatives will be conserved *ex situ* in the near future. So it is important that conservation in the wild or in farmers’ fields – that is, *in situ* conservation – is developed as an alternative and ideally complementary approach. This also has the advantage of allowing continued evolution of germplasm in response to changing environment, including climate, and abiotic and biotic stress.

Hershey (2008) estimated that about 27 000 landraces of cassava exist *in situ* and that less than half of these are in genebanks (the precise number is difficult to ascertain since the extent of duplication of accessions within genebanks has not yet been fully evaluated). There are a number of significant threats to cassava landraces *in situ*. These include loss of habitat, pests and diseases, and perhaps most significantly, replacement of landraces with improved varieties that may have a more narrow genetic base. In some cases the best option may be to secure these landraces in genebanks. However, in other cases the conservation *in situ* can be equally or more practical. Maxted and Kell (2009) considered the case of cassava wild relatives and outlined the priorities for their *in situ* conservation in terms of species and geographical area. The wild relatives are particularly important in the light of evidence that a number of them can cross easily with the cultivated species and that interspecific hybridisation has been important in the evolution and breeding of cassava (Bredeson et al., 2016).

Currently there is very little ongoing effort to systematically conserve cassava landraces *in situ*, and there is a strong case for more research and more investment to be deployed, including the use of molecular tools to survey diversity existing both *in situ* and in genebanks.

12 **Molecular genetic studies of cassava diversity**

As with many crops the tools of molecular genetics are being deployed with increasing power and scope in cassava and they have many potential applications in diversity analyses and genebank management as well as pre-breeding and breeding.
In recent years considerable progress has been made with respect to use of single-nucleotide polymorphism (SNPs) to study diversity in cassava and application of next-generation sequencing to both marker development and identification of quantitative trait loci or the position of important single genes (Ferguson et al., 2012).

Wang et al. (2014) sequenced two genotypes: W14 *M. esculenta* ssp. *flabellifolia* and KU50 a bred variety common in Southeast Asia with higher root yield and starch content. They confirmed that the ‘level of heterozygosity in cassava is among the highest found in sequenced plant genomes’. They also found a greatly reduced linamarin and lotaustralin content in KU50 relative to W14 and speculated that the domestication of cassava around 7000–12 000 years involved changes in the pattern of carbon flux.

Sequencing studies also reveal synteny with other sequenced Euphorbiaceae genomes, for example, *Ricinus communis* and *Jatropha curcas*. Bredeson et al. (2016) produced a high-quality assembly of the cassava genome sequence and compared it to wild accessions and related species. They found evidence of significant interspecific hybridisation in the evolutionary history of cassava.

Peña-Venegas et al. (2014) studied diversity amongst cassava cultivated by different ethnic groups in the Amazon and Moura et al. (2016) used microsatellites to distinguish between two groups of sugary cassava accessions held in Brazilian genebanks. This work helps demonstrate the increasingly important role of molecular genetic approaches for genebank management. Rabbi et al. (2015) used genome-wide SNP markers derived from genotyping by sequencing methods to track variety ancestry and adoption. Again, such approaches offer a powerful tool for the identification of duplicates in collections and for variety identification. Pariyo et al. (2013) used an earlier generation of molecular markers, simple sequence repeats (SSRs), to relate genetic diversity in cassava from South, East and Central Africa to resistance to cassava brown streak disease.

In recent years there have also been efforts to improve genetic mapping in cassava and Soto et al. (2015) described a high-density map related to a physical map and the whole-genome sequence.

13 Where to look for further information

Further information can be found in the References section below and from the websites of IITA and CIAT in particular.

14 Acknowledgements

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15 References


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