International Master Programme at
the Swedish Biodiversity Centre

Master's thesis
No. 68
Uppsala 2009

Morphological characterisation and cryopreservation of sweet potato, *Ipomoea batatas* (L.) Lam., accessions at the NPGRC of South Africa

Machoene Tshidi Manamela

Supervisors

Eva Thörn

Andre Lezar

CBM MASTER THESES SERIES

CBM Swedish Biodiversity Centre

Abstract

Morphological characterisation of germplasm helps in the identification of duplicate accessions, unique traits and the development of core collection. Cryopreservation serves as a complementary conservation method to field and *in-vitro* methods. This study aimed at evaluating the diversity of *Ipomoea batatas* landrace collection maintained in tissue culture at the National Plant Genetic Resources Centre (NPGRC) of South Africa. The response of the selected sweet potato accessions to cryopreservation as the complementary conservation method was also tested. Vegetative and storage root characters of the 51 sweet potato accessions were characterized using International Potato Centre descriptors for sweet potato and analyzed with Numerical Taxonomy System-pc software. Encapsulation-vitrification and encapsulation-dehydration protocols were tested for cryopreservation on selected accessions. The unweighted pair-group method using an arithmetic average (UPGMA) defined 22 clusters at a distance coefficient of approximately one. Four Principal Components cumulatively explained 46 percent of the variation, whereas four Principal Coordinate axes, explained 50 percent. Preliminary results presented here show that the NPGRC of South Africa conserves high morphological diversity of sweet potato landraces in the field and tissue culture. But, the results need to be verified with replicates.

Keywords: cryopreservation, duplicate accessions, germplasm, morphological characterisation, NPGRC, phenotypic diversity, sweet potato

Contents

Introduction

The root and tuber crops such as potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), indigenous African potato (*Plectranthus esculentus*) and cassava (*Manihot esculenta*), amongst other crops, play an important role in food security, especially in Africa (Allemann *et al.* 2004). Sweet potato is grown for its enlarged storage roots used for human consumption and animal feed. The vines are sometimes consumed as green leafy vegetables and to a lesser extent as animal feed. About 100 developing countries in Asia and Africa grow sweet potato, with China as the largest global producer (Food and Agriculture Organization 2008). In Africa, the largest producer of sweet potato is Uganda (Gibson *et al.* 2000).

In South Africa, sweet potato is of considerable economic value with marketing chains well-organised for local and export market. However, potato production and processing is larger than that of sweet potato (Allemann *et al.* 2004). The annual production of sweet potato was approximately 50 000 tons by 2007 and about 20 000 tons sold on the major fresh produce market in South Africa (Department of Agriculture 2007). It should be noted that sweet potato is also produced and sold by informal sectors, of which the figures are not included in the official production figures. Furthermore, it is grown as subsistence crop by resource-poor farmers and plays an important role in food security and poverty alleviation. Moreover, the crop can be sold on small scale to generate an income, contributing to poverty alleviation (Laurie 2004).

Taxonomy and morphology of sweet potato

Sweet potato is a dicotyledonous root tuber crop belonging to the morning-glory family, Convulvulaceae. As compared to potato which is a tuber, sweet potato has an extended storage root accumulating more edible component volume. The colour of the stem and leaves varies from green to totally purple due to the presence of anthocyanin pigmentation, with the petiole from 5-30 cm (Laurie & Niederwieser 2004). Sweet potato general leaf outline vary from round to almost divided with the margins ranging from no lateral lobes to deeply lobed. The shape and size of the storage root can be between round and long irregular or curved depending on the variety and environmental factors (Woolfe 1992). The smooth storage root skin ranges from white to dark purple and the flesh colour vary from white to orange in various distributions (Laurie & Niederwieser 2004).

Origin of sweet potato

The exact origin of sweet potato is still not well-documented. However, the historical evidence suggests that the crop originate from the New World i.e. Central or South American lowlands. South American indigenous communities

have probably cultivated the crop since 3 000 BC (Woolfe 1992). Therefore, sweet potato is believed to have originated from the region between Yucatan Peninsula of Mexico and Orinoco River in Venezuela and spread to the rest of the world by the explorers (Zhang *et al.* 2004), and introduced to Europe and Asia and later spread to Africa by the 16th century (Allemann *et al.* 2004).

The wild progenitors of sweet potato have not been documented. It is believed that cultivated varieties of sweet potato, which are hexaploid, are a result of hybridization between tetraploid primitive and diploid weedy sweet potatoes (Sauer 1993). It is possible that wild hexaploid are available but according to the history, cultivars were independently domesticated in different regions. However, their origin is not known.

Cultivation of sweet potato

Sweet potato requires warm days and nights for optimal yields. It is sensitive to low temperatures and grows best in the tropical and warm temperate regions wherever there is sufficient sunlight and water. It grows favourably under well aerated and moderate to slightly acidic, sandy to sandy-loam soils but has the ability to tolerate harsh soil and climatic conditions and still give satisfactory yield (van den Berg & Laurie 2004). Sweet potato can be cultivated asexually from vine cuttings or sexual from seed and can be ready for harvesting from four to six months depending on the temperature and variety (Woolfe 1992). In South Africa, sweet potato is propagated vegetatively from transplants produced by bedding mother roots or from rooted cuttings (van den Berg & Laurie 2004). Sweet potato can grow anywhere in South Africa where good cultivation practices are applied.

Gibson *et al.* (2000) mentioned that landraces are adapted to their local areas and have developed resistance to local pests and diseases, gaining recognition from the resource-poor farmers. However, in most cases these landraces produce low yields that lower the sweet potato production (Allemann *et al.* 2004) in South Africa. Similarly, Laurie *et al.* (2008) reported low yields and yield instability due to the use of old landraces which were found to lower production by resource-poor farmers.

The most common virus of sweet potato is transmitted by aphids. Sweet Potato Plant Improvement Scheme was established by the Agricultural Research (ARC)-Roodeplaat in order to produce virus-free varieties with improved yield. Thus facilitating sustainable sweet potato production and contributing to food security in South Africa (Laurie *et al.* 2008). According to Laurie & van den Berg (2002) in Allemann *et al.* (2004), much work has also been done at ARC-Roodeplaat to improved cultivars that will replace the low-yielding landraces. This however raises a concern because most of the

landraces are gradually replaced by the improved varieties. Furthermore, the resource-poor farmers may not afford the improved varieties.

Importance of sweet potato

Globally, sweet potato ranks seventh in production after wheat, rice, maize, potato, barley and cassava (International Potato Centre [CIP] 2008). The largest collection of sweet potato is maintained by CIP, with about 4 950 landraces, 21 wild varieties and six improved varieties (System-wide Information Network for Genetic Resources [SINGER] 2009). These collections were donated from other genebanks all over the world. In Eastern and Southern Africa, sweet potato is third to cassava and potato among the major food root crops, both in cultivation and consumption (Ewell & Mutuura 2003) and thus plays an important role in food security and nutrition in Africa. Sweet potato is considered a food security crop in sub-Saharan Africa where it is mainly grown on subsistence scale and provides compliant source of food before other crops mature.

Laurie (2004) wrote that in some African countries, starchy crops such as sweet potato are the staple food whereas other countries utilise it as an additional or security food crop.
Sweet potato is a good source of carbohydrates, proteins, fibre, iron and moderately rich in vitamin C (Woolfe 1992). The storage root of sweet potato provides considerable amounts of carbohydrates compared to other root crops but has lower protein and fat contents. The orange-fleshed sweet potato has high levels of beta-carotene which is a forerunner of Vitamin A, contributing much to human health and nutrition especially for children (Woolfe 1992).

Characterisation of crop germplasm

The Southern African Development Community (SADC) Plant Genetic Resources Centre was established in 1989 under the Food, Agriculture and Natural Resources (FANR) Directorate. Its activities include promotion and co-ordination of a regional network of plant genetic resources management through the National Plant Genetic Resources Centres (NPGRCs). The main activities of NPGRCs are the collection, documentation, conservation, evaluation and utilisation of regional plant genetic resources, thus contributing towards ensuring food security of the people in the region (SADC 2007). Conservation of plant genetic resources has many components that include collecting, documentation, characterization, evaluation and maintenance (Reeds *et al.* 2004) which are vital in the management of plant genetic resources.

Morphological characterisation of plant species is important in the identification of duplicate accessions, detection of unique traits and also the structure of the population to be conserved, thus saving on the storage space and simplifying selection by plant breeders (Reed *et al.* 2004). Morphological

diversity is assessed by measuring variation in phenotypic traits such as flower colour, shape of the leave and growth habit (Rao 2004). Phenotypic traits have long been used in selecting crops that best suit needs of farmers and also led to domestication of useful plants (Gepts 2004). Morphological characterisation supplemented by molecular characterisation provides information for comparison of individual accession/variety thereby facilitating germplasm improvement and effectiveness of the collection. Smith (1998) in Allemann *et al.* (2004) alluded that molecular characterisation is important to avoid duplication in genebanks and protect the special cultivars.

Molecular markers such as Amplified Fragment Length Polymorphism have been used (Zhang *et al.* 2004) to identify duplicates in sweet potato germplasm. With this kind of analyses the structure of genetic diversity in different genotypes can be investigated. Accessions that are morphological identical and produce the same banding pattern of proteins in electrophoresis or DNA fingerprints are considered duplicate (Huamán *et al.* 1999). Thus, it facilitates the elimination of duplicates and establishment of a core collection. A core collection contains a subset of accessions from the entire collection that represents most of the available genetic diversity of a particular germplasm. Brown (1995) in Huamán *et al.* (1999) advocated that a core collection can provide genebank managers with information on which accessions to keep in field collection. Thus, it becomes cost-effective since the duplicate accessions are eliminated.

The descriptors for sweet potato developed by CIP *et al.* (1991) have been widely used to assess morphological variation in sweet potato collections. Descriptors list provides a standard scale that can be used in the conservation of germplasm, especially by curators of genebanks. The descriptors list is particularly important for crops that are listed in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which has to be adopted by all the member states (CIP *et al.* 1991). Mok & Schmiediche (1998) used the CIP descriptors to identify sweet potato landraces in Indonesia. The most important accessions identified were then maintained in the field for subsequent evaluation and used in breeding programs. With the help of the same descriptors, Veasey *et al.* (2007) found that sweet potato landraces maintained by farmers in Vale do Ribeira exhibited high morphological variability. Similarly, Tairo *et al.* (2008) reported low genetic diversity in sweet potato using these descriptors potato in Tanzania. This clearly illustrates that these descriptors are widely applicable and are reliable in assessing morphological diversity of sweet potato accession.

Conservation methods of sweet potato

Conservation of plant genetic resources is important for improving food security and nutrition for the present and future human population especially

the resource poor farmers dealing in subsistence farming (Engelmann 1991). High crop diversity ensures adequate food supply and traits to improve yield, quality, resistance to pests and diseases and adaptation to changing environmental conditions. The NPGRCs serve as *ex-situ* safety mechanisms for conservation of plant genetic resources as well as restoration, and rehabilitation in areas affected by natural calamities. Although the most widely used method of conservation is seed genebanks at low temperatures (Engelmann & Engels 2002), it is suitable only for seed bearing crops (orthodox seeds) that can withstand up to five percent or less reduction in moisture content, neglecting those that cannot withstand the reduction in moisture (recalcitrant seeds) and also crops that do not produce seeds (Engelmann 1991).

Sweet potato is propagated vegetatively; its collections are conserved as clones in field gene banks and as *in-vitro* plantlets in the laboratory (Engelmann 2004). The NPGRC of South Africa conserves approximately 5 000 landrace accessions of crops listed in Annex 1 of the ITPGRFA, including 51 accessions of sweet potato (Department of Agriculture 2009). Three of these sweet potato accessions are as well maintained at CIP gene bank. However, information on passport data of these accessions is not available at CIP (SINGER 2009). It should be noted that wild accessions of sweet potato can be found in the centre of origin of sweet potato. The NPGRC of South Africa houses the traditional varieties (landraces) of sweet potato. The knowledge of the plant genetic resources kept at various gene banks is of great value for the development of crop breeding programs and also for the coordination of collections at different gene banks in the world.

It is important to conserve these landraces in order to have better understanding and reliable information (Gepts 2004) about the plant genetic diversity available in South Africa. Furthermore, due to the constant change in climate and evolvement of new pests and pathogens, new genes are required by plant breeders for crop variety improvement (Engelmann & Engels 2002). Conservation of these landraces can ensure food security, especially to resource-poor farmers who cannot afford the improved crop varieties. Collection and conservation strategies of landraces need to be strengthened to avoid erosion of landraces. The new genes required for breeding may be found in these landraces. The commonly used methods of sweet potato conservation at NPGRCs are discussed below.

Field-genebank or on-farm conservation

Being vegetatively propagated, sweet potato is conserved on-farm or in field-genebanks (Engelmann 2004). This method presents major setbacks which limit its effectiveness and threatens the safety of sweet potato. The collections are exposed to natural disasters, attacks by pathogens in addition to the method being expensive and laborious. There is also noticeable decrease of

land for *in-situ* conservation due to urbanisation and human settlement. Therefore, safety duplicate collection *in-vitro* is recommended as a complementary method to field collection (Engelmann 1991).

In-vitro (tissue-culture) conservation

A combination of *in-situ* and *ex-situ* conservation techniques provides a reliable and appropriate complementary conservation strategy for plant genetic resources. Reed *et al.* (2004) recommended sufficient replicates of accessions to be kept in field and *in-vitro*. In the latter method the material can be maintained for short to medium term in a pathogen free environment, thereby facilitating safer collections. However, their maintenance is also expensive due to the repeated sub-culturing, which involves transferring to a fresh media (Rao 2004). Whereas growth retardants can be used to increase duration between sub culturing, it has been reported to change the phenotypic characters of the plants (Engelmann 2004). Furthermore, Rao (2004) noted that repeated sub-culturing pose the risk of losing the accessions due to contamination, human error or somaclonal variation, more so when growth retardants are used.

Cryopreservation

Several authors recommended cryopreservation as an alternative safety method to field genebank and tissue-culture. Cryopreservation requires low maintenance and limited space (Engelmann 2004) and the material can be stored for an extended period, than all the other preservation methods but expensive to acquire the equipments required for this method. It is done in liquid nitrogen (LN) at -196^{0}C whereby the metabolic function of the plant cell is reduced (Towill & Jarret 1992). However, cryopreservation cannot replace field and tissue culture methods but serves as a safety duplicate. Basing on the pros and cons of the different preservation methods, Reed *et al.* (2004) recommended that collections should be conserved with all the available strategies.

Both classical and vitrification techniques have been attempted (Sharma *et al.* 2006). In classical technique the plant materials are dehydrated by a slow cooling step in programmable freezers whereas vitrification technique is based on dehydration using chemicals before exposure to LN. The most favoured protocols for cryopreservation of tuber crops such as sweet potato, potato, cassava and Chinese yam are vitrification-based protocols. Examples of these are; vitrification, encapsulation-vitrification and encapsulation-dehydration (Pennycook & Towill 2000; Hirai & Sakai 1999, 2001; Charoensub *et al.* 2004 and Sharma *et al.* 1996). In all the protocols explants must be sufficiently dehydrated in order to avoid ice crystallization yet enough water is retained to sustain the explants.

Encapsulation means enclosing an explant in a jelly like form. The explant is suspended in a culture medium containing sodium alginate (two-three percent, species dependant) and sucrose (0.08-0.4 M, species dependant) without calcium, then sucked with a pipette and poured drop-wise in liquid medium enriched with calcium chloride (0.1M) (Sharma *et al.* 2006). When the drop enclosing the explant comes into contact with calcium chloride, it polymerizes and finally forms a bead in which an explant is enclosed. Encapsulation helps in protecting the explant against pre-treatments which could be detrimental, yet necessary to ensure survival after freezing (Sharma *et al.* 2006).

In vitrification, freezable water in the explants is dehydrated using a combination of chemicals with cryoprotectant properties. The high concentration of cryoprotectants causes the freezable water in the cell to vitrify, and is converted from liquid to amorphous phase without crystallization (Sharma *et al.* 2006). The most commonly used cryoprotective solution is plant vitrification solution two (PVS2), that consists of 30 percent glycerol (w/v), 15 percent (w/v) ethylene glycol (EG) and 15 percent (w/v) dimethylsulfoxide (DMSO) and 0.4M sucrose (Sakai *et al.* 1990). Furthermore, due to the toxicity, the temperature and duration of explants in this solution require optimization depending on genotype.

Towill & Jarret (1992) and Pennycook & Towill (2000) reported that cryopreservation of sweet potato shoot tips by vitrification was successful and recommended the applicability of this protocol at all locations where a suitable cryogen is available. However, further studies are required to increase the rate of survival and to identify factors that affect the high levels of within and between treatment variability for explants regeneration. Hirai & Sakai (1999) achieved 70 percent shoot formation using encapsulation vitrification protocol on shoot tips of potato whereas Hirai &Sakai (2001) reported 64 percent of shoot formation for potato, lily, strawberry and Chinese yam. The highest survival (80 percent) of cassava shoot tips after encapsulation-vitrification was reported by Charoensub *et al.* (2004).
Another 80 percent regeneration rate after encapsulation-vitrification of sweet potato shoot tips was also reported by Hirai & Sakai (2003). The success of encapsulation-dehydration in embryogenic tissues of sweet potato was reported by Sharma *et al.* (2006). They obtained approximately 63 percent regeneration rate of embryogenic tissues of sweet potato. The encapsulation-dehydration requires longer time for the development of embryogenic tissues from meristems as well as during regeneration of a complete plant from embryogenic tissues. Each protocol is genotype dependant and modification in each step needs to be done in order to increase regeneration rate of the cryopreserved material.

Justification of research

Sweet potato production is of importance for nutritional and economic values in South Africa. It serves as a security crop for poverty alleviation and food security, especially for small-scale farmers in the rural areas (Niederwieser 2004). Though it is not largely produced in formal commercial markets, it is a major source of income for the informal sectors. Beta-carotene together with other essential elements and nutrients found in the crop contribute to human health and nutrition for the present generation (Woolfe 1992) and will continue to do so in the future generations if conserved and utilised sustainably.

The changing climate in addition to the new diseases and pests, necessitate the genetic improvement of crop in order to counteract these effects. Traditional varieties of crops harbour genes required by plant breeders for further breeding advancement. It is important to conserve these traditional varieties of crops to ensure their availability when needed for breeding purposes.Sweet potato is one of the three crops that are vegetatively propagated and maintained as clones in tissue culture at the NPGRC of South Africa. There has been an increasing concern that sweet potato landraces in South Africa are gradually replaced by improved varieties. As mandated by FANR, all crop genetic resources need to be collected, characterised, documented and utilised to ensure efficiency of the collection.

Currently, the NPGRC of South Africa conserves 51 landrace accessions of sweet potato collected from small-scale farmers. There is little information on trait representation and description within the collection. Therefore, data needs to be collected in order to improve the usability of the accessions. Of these 51 accessions, there is a need to investigate diversity of the collection towards gap analysis and core collection development. This will improve the efficiency of the collection by eliminating duplicate accessions and also identify gaps of unique traits that are not represented in the collection. The NPGRC of South Africa maintains these sweet potato accessions in tissue-culture at 18^0C with repeated sub-culturing. To avoid somaclonal variations, no growth retardants are used. Viruses are controlled through the use of heat therapy. Several authors highlighted the drawbacks of conservation in field genebanks and tissue-culture (Rao 2004; Engelmann 2004).

Therefore, attempts have been made to explore cryopreservation on sweet potato as a complementary safety method for long-term conservation. Although some cryopreservation research has been reported on different explants of sweet potato, the available protocols (Hirai & Sakai 2003; Sharma *et al.* 2006) need to be tested for the sweet potato landraces at NPGRC laboratory. This study aimed at:

i. Evaluating the diversity that exists in sweet potato landrace collection at NPGRC of South Africa in order to eliminate duplicate accessions and establish a core collection and

ii. Testing encapsulation-vitrification and encapsulation-dehydration protocols as the complementary preservation method to tissue culture and field genebank methods.

Materials and Methods

Morphological Characterisation

Plant Collection

A total of 51 sweet potato landrace accessions were available at NPGRC of South Africa. These had been collected from different locations in South Africa by NPGRC between 2003 and 2004. The locations in terms of longitudinal and latitudinal co-ordinates were also captured and points were extrapolated on the map (Fig. 1). The criterion of the collection was to cover most of the available morphological diversity from farmers. The mandate of the NPGRC is to collect crop genetic resources, focussing more on the landraces. The standard collection form developed by the Southern African Development Community Plant Genetic Resources Centre (SPGRC) was used to capture passport data.

The passport data captured during the collection included amongst other: accession number, depositor, collection date, province, district and village (Appendix I). However, the available passport data included 35 accessions from the sweet potato collection and no data were available for 16 accessions. This was due to that earlier accessions were collected without passport data. Nonetheless, this study is not attempting to link certain character states to certain geographical areas. Thus the fact that some accessions do not have any passport data is not considered in this study, but these accessions still remain valuable as genetic resources.

Planting of accessions in the glasshouse

Each accession was planted in a fibre glass green house in 30 cm pots containing potting mixture made of shredded pine bark, in a randomized block of 30 cm between plants. The plants had to be re-established from tissue culture and the material was not enough to plant the replicates during this study. Multifeed fertilizer was applied once per week in a concentration of 28 g/8 L. Plants were watered by hand on every third day. The day temperature in the glasshouse was 27°C-30°C. The plants were raised during summer months and thus it was presumed that the night temperature inside the glasshouse should not have dropped below 20°C. Taro was grown on the edges of the 51 accessions in order to minimise the edge effect.

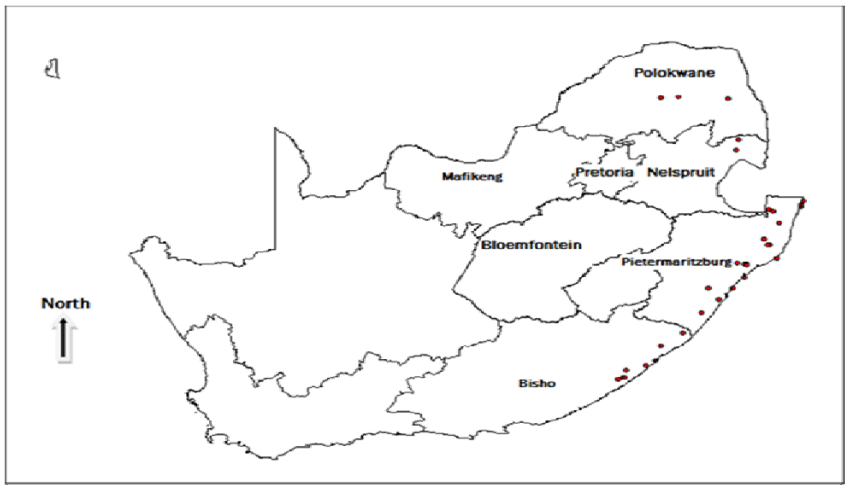

Figure 1. A map of South Africa showing the areas (dots) where 51 accessions of sweet potato were collected

Data collection

Morphological characters of all the 51 accessions of sweet potato were scored using the standard descriptors of sweet potato described by the CIP *et al.* (1991) (Appendix II). A set of 16 vegetative characters were scored three months after replanting in the glasshouse and 15 storage root characters (Figs. 2-8) were characterised after nine months in the glasshouse. According to the requirements of the CIP descriptors; vines and leaf characters were recorded as the average expression of character observed in a section of the main stem located in the middle portion of several main stems. Three leaves per plant in the middle portion of the stem were recorded. Medium to large sized storage roots were considered the most representative expression of the character. Between medium to large sized storage roots were measured and the average score was recorded.

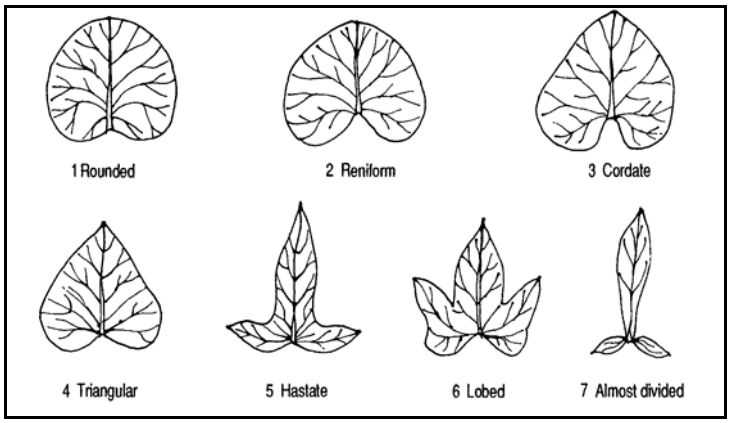

Figure 2. General leaf outline of sweet potato as indicated in the Descriptors for sweet potato (CIP *et al* 1991)

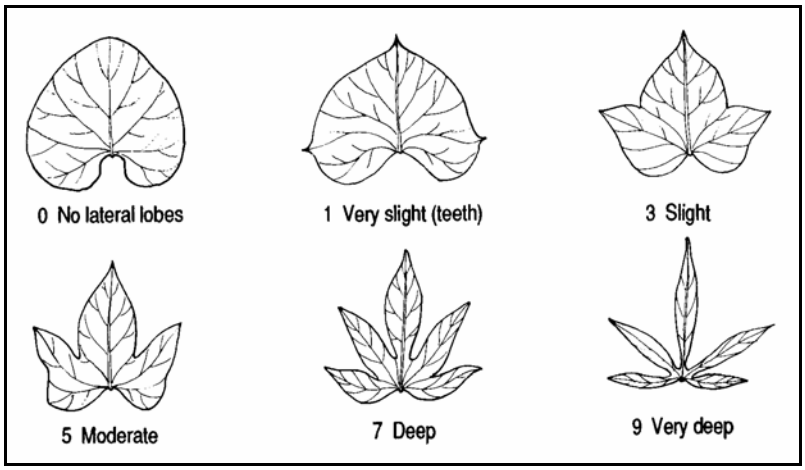

Figure 3. Leaf lobe types of sweet potato as shown in the Descriptors for sweet potato (CIP et al 1991)

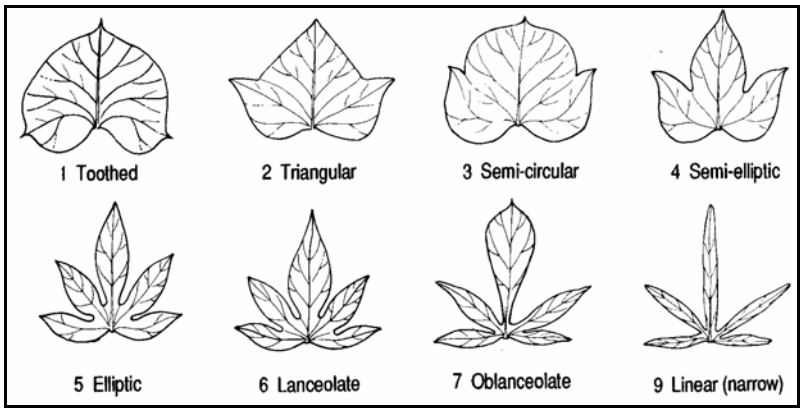

Figure 4. Shapes of the central leaf lobe of sweet potato as indicated in the Descriptors for sweet potato (CIP et al 1991)

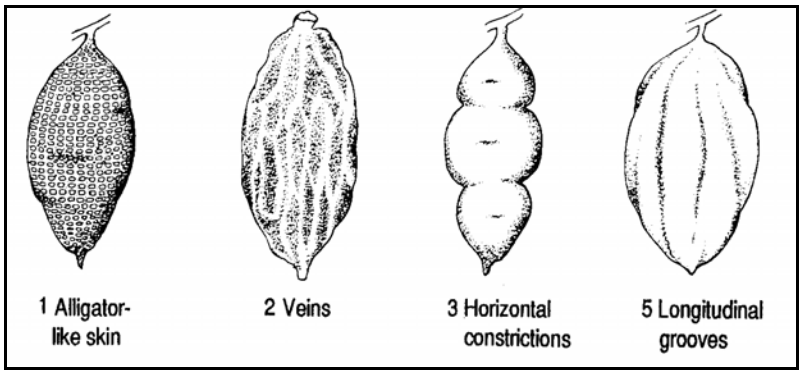

Figure 5. Storage root surface defects of sweet potato according to the Descriptors for sweet potato (CIP *et al* 1991)

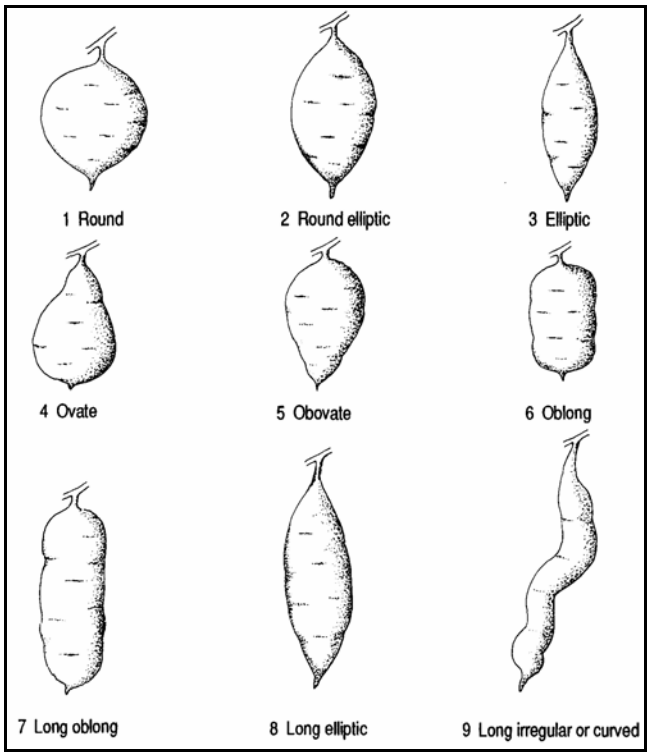

Figure 6. Shapes of the storage roots of sweet potato according to the Descriptors for sweet potato (CIP *et al* 1991)

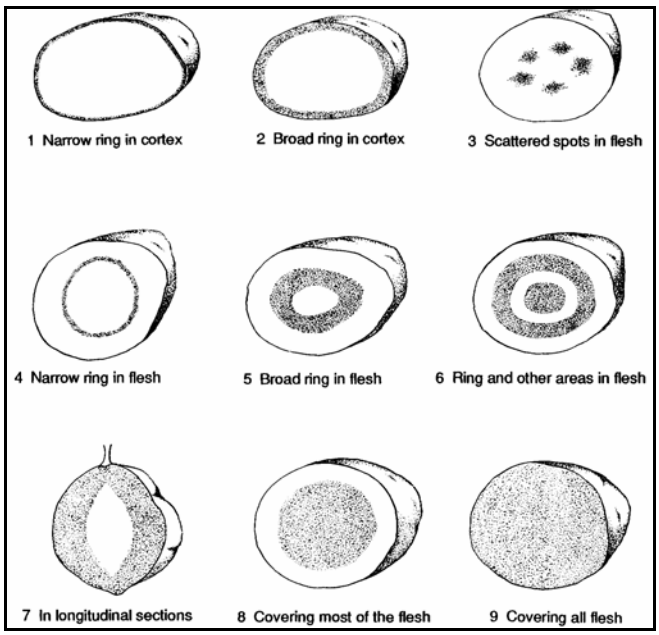

Figure 7. Distribution of secondary flesh colour of sweet potato as shown in the Descriptors for sweet potato (CIP *et al.* 1991)

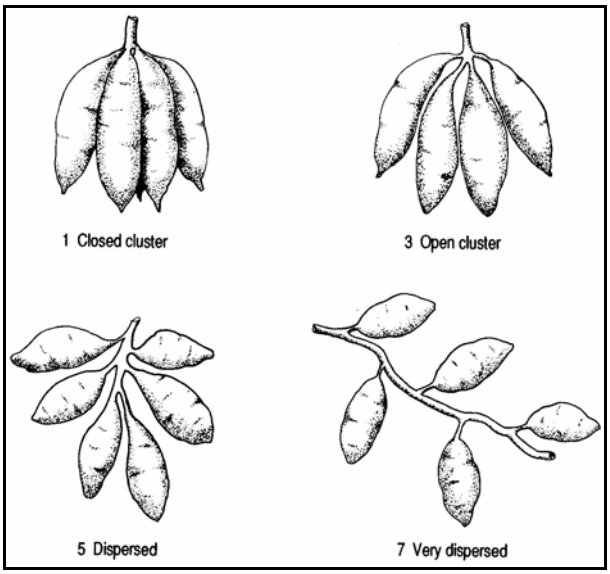

Figure 8. Storage root formation of sweet potato as shown in the Descriptors for sweet potato (CIP *et al.* 1991)

Data Analysis

All the data were analysed for the variation in each character (univariate analysis). Multivariate analyses were also done using the Numerical Taxonomy System-pc (NTSys-pc) software (Rohlf 2000) to determine variations among the different accessions. The data for each morphological character was first transformed using the STAND procedure in NTSys-pc in order to eliminate the effects of different scales of measurement. To compare the dissimilarity between accessions the distance coefficient was computed from the transformed data and the information was summarised in dendograms using Unweighted Pair Group Method using arithmetic Average (UPGMA) parameters in NTSys-pc.

Principal Component Analysis (PCA) was performed to determine the correlation on characters and the most significant traits contributing to variation in the collection, through the generation of Eigen vectors and Eigen values. The Eigen vectors with values > 0.7 and < -0.7 indicate the significance of a particular character to each component. Principal Coordinate Analysis (PCoA) was also conducted using the DCenter, Eigen and Graphics programs as described in Rohlf (2000) to complement Cluster Analysis. Projection was done to compare objects (containing accessions) and the projection file was combined with the Eigen vector (containing characters) file using the Matrix plot option in NT Sys-pc to explain the character-based grouping of accessions.

Cryopreservation procedure

Micropopagation

Six accessions namely; A2910, A2318, A1738, A5799, A9, and A2936 were selected from 51 accessions maintained in tissue culture at NPGRC of South Africa. Accession A5799 was selected because it was totally purple and looked different from the rest. Other selected accessions showed no signs of contaminations and had a faster multiplication rate in tissue culture. Nodal segments with a piece of stem were micro-propagated in the sterile solid medium consisting 30 gl^{-1} sucrose, 4.42 gl^{-1} Murashige & Skoog (MS) salts with vitamins and 2.2 gl^{-1} gelrite (pH 5.6) The medium was autoclaved at 121°C for 20 minutes for sterilization. Ten nodal segments were cut from each plant and sub-cultured on the above- prepared medium at 25°C and incubated under 16 hour photoperiod for six-eight weeks.

Encapsulation-vitrification protocol

A protocol on encapsulation-vitrification described by Hirai & Sakai (2003; Appendix III) was used on three accessions (A 2316, A2910 and A5799) out of the above six accessions. It is not easy to handle shoot tips of many accessions at once. Thus, only three accessions were evaluated in this protocol. The procedure was replicated twice in order to eliminate any artefacts. Shoot tips were removed as control experiments after each step in encapsulation-vitrification (Fig. 9), and cultured on the same recovery medium one, to be used for regeneration ability after freezing in liquid nitrogen.

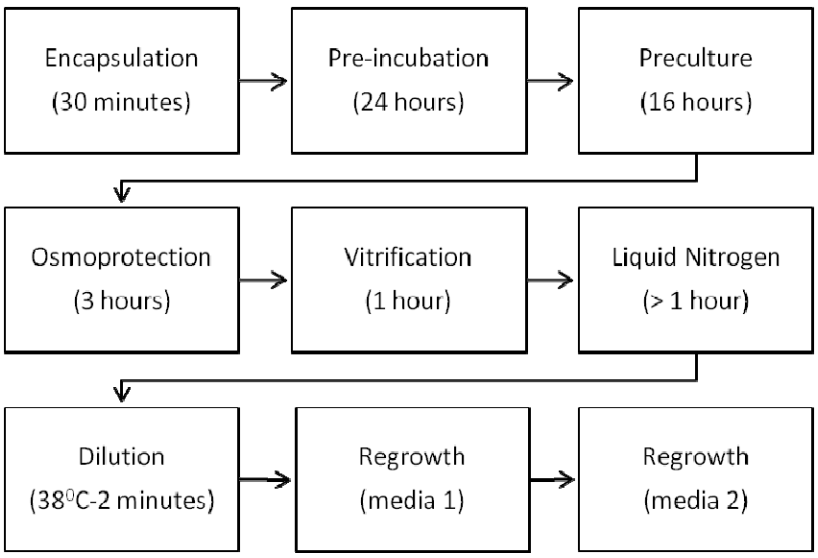

Figure 9. Encapsulation-vitrification protocol tested on shoot tips of accessions 2316, 2910 & 5799 of sweet potato according to Hirai & Sakai (2003)

Five shoot tips per accession were grown on recovery medium one after each stage of the protocol in order to test the regeneration ability. After LN treatment five shoot tips per accession were tested for regeneration on recovery medium one for seven days in the dark at 25^0C, while another five shoot tips per accession were incubated at 16 hour photoperiod at 25^0C for seven days. After seven days the shoot tips were then transferred to recovery medium two and incubated for 21 days at 16 hour photoperiod. Regeneration ability was expressed as the number of shoot tips forming shoots after 21 days.

Encapsulation-dehydration protocol

A protocol on encapsulation-dehydration (Fig. 10) described by Sharma *et al.* (2006 Appendix IV) was evaluated. Embryogenic tissues of the same accessions used in encapsulation-vitrification were used. Embryogenic tissues were removed after each procedure to test the regeneration ability.

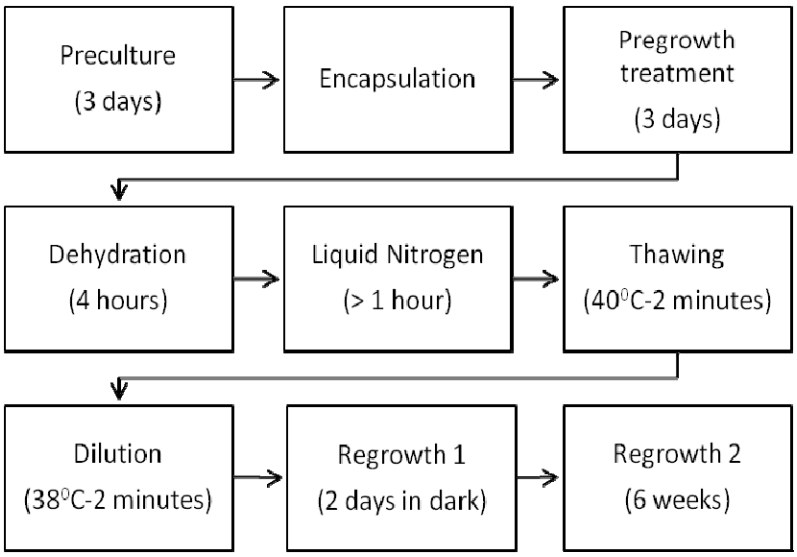

Figure 10. Encapsulation-dehydration protocol tested on embryogenic tissues of accessions 2316, 2910 & 5799 of sweet potato according to Sharma *et al*. (2006)

In pre-culture and pre-incubation procedures, Ten tissues per accession were tested on recovery medium whereas five tissues per accession were tested after dehydration procedure. Ten tissues per accession were cultured on recovery medium after LN and then incubated for two days under the same conditions as in encapsulation-vitrification. The embryogenic tissues were then transferred to the same but fresh medium and incubated at 16 hour photoperiod for six weeks. Regeneration ability was expressed as the number of embryogenic tissues forming somatic embryos and later plantlets after three weeks of culture on the recovery medium.

Results

Morphological Characterisation

Uni-variate Analysis

Many morphological characters were scored in this study; only characters with most variability were considered in this analysis (Table 1). The traits that significantly (< 60 percent) contributed to variability were vine internode length (VIL), leaf lobe type (LLT), leaf lobe number, shape of central leaf lobe, abaxial leaf vein pigmentation, storage root surface defects, storage root cortex thickness and predominant storage root skin colour.

Table 1. Morphological characters & their scores that contributed much variability in the univariate analysis of 51 sweet potato accessions at NPGRC of South Africa. Percentage of accessions in each score is also shown.

Characters	Score	% of accessions	Characters	Score	% of accessions
VIL	1	27	MLLT	0	25
	3	55		1	6
	5	14		3	2
	7	4		5	18
	9	0		7	39
MLLN	0	25		9	10
	1	6	SRCT	1	7
	3	2		3	30
	5	18		5	35
	7	39		7	28
	9	10		9	0
SCLL	0	23		0	0
	1	4	SRSD	0	0
	2	0		1	17
	3	0		2	20
	4	24		3	59
	5	2		4	0
	6	43		5	4
	7	2		6	0
	8	0		7	0
	9	2		8	0
ALVP	1	0	PSRSC	1	0
	2	12		2	50
	3	25		3	0
	4	2		4	0
	5	12		5	0
	6	4		6	37
	7	12		7	0
	8	31		8	13
	9	2		9	0

*The numbers in the score column represent the scores in each character (refer to Appendix II for the explanation of scores) and the percentages represent the percent of accessions in each score. VIL (vine internode length); MLLT (mature leaf lobe type); MLLN (mature leaf lobe number); SCLL (shape of central leaf lobe); ALVP (abxial leaf vein pigmentation; SRCT (storage root cortex thickness); SRSD (storage root surface defects; PSRSC (predominant storage root skin colour)

Multivariate Analysis-Clustering

The 51 sweet potato accessions grouped into 22 clusters at a distance coefficient of approximately 1-dotted line (Fig. 11). The cluster identification membership is shown in Table 2.

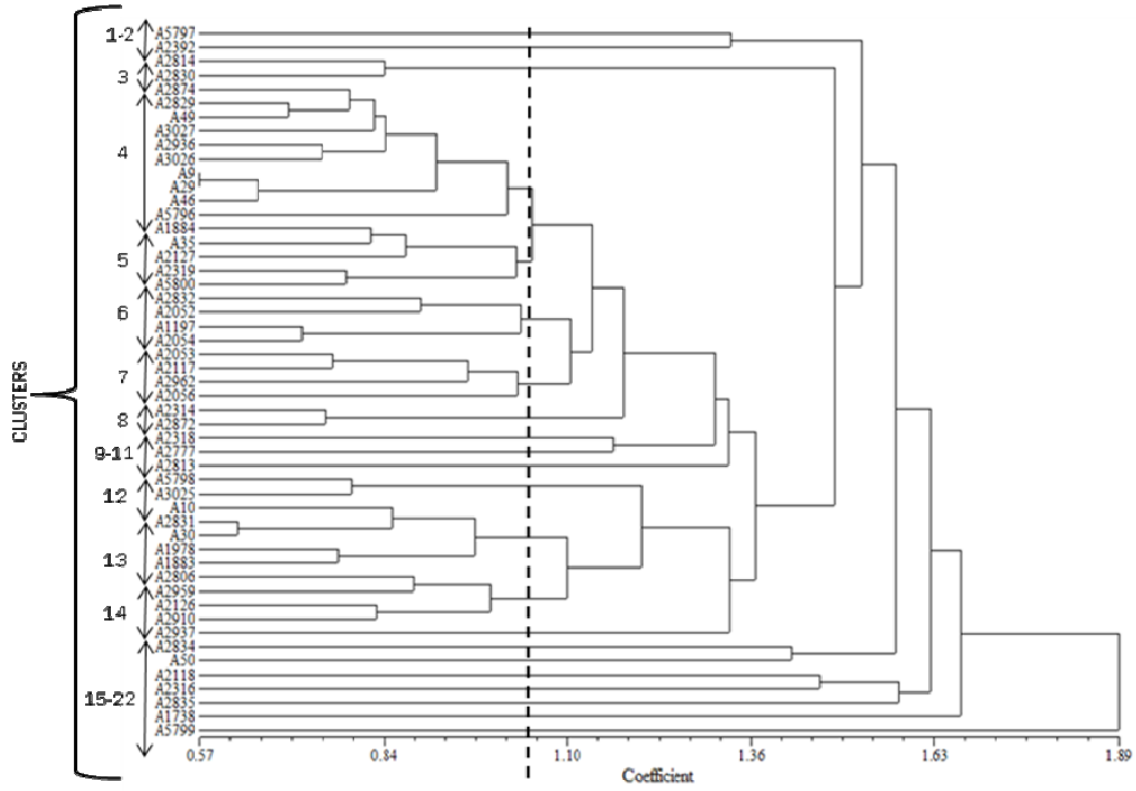

Figure 11. Hierarchical clustering using the distance coefficient. The y-axis shows the 51 accessions and 22 clusters; x-axis indicates the distance coefficient between clusters. 22 clusters at coefficient approximately 1 are shown.

Multivariate Analysis-Principal Component Analysis (PCA)

NTSys-pc indicated the Eigenvalues measuring the degree of contribution of each component to the total variance of the collection. The first PC axis accounted for 16 percent of the total multivariate variation, while the second accounted for 11 percent and the third for ten percent (Table 3). The cumulative variation reached 38 percent in the first three PC axes and 76 percent in the tenth PC axes.

Table 2. Cluster identification membership of 51 accessions of sweet potato, showing the list of accessions in each cluster and characters that made them group together

Cluster number	Cluster Identification of 51 accessions	List of Accessions
1	Ovate storage root	5797
2	Elliptic storage root, Linear-narrow central leaf lobe	2392
3	Abaxial leaf vein pigmentation green	2814, 2830
4	Green mature foliage leaf, Medium mature leaf, Five leaf lobes	2874, 2829, 49, 3027, 2936, 3026, 9, 29, 46, 5796
5	Deep leaf lobe, Five leaf lobes, Lanceolate central leaf lobe	1884, 35, 2127, 2319, 5800
6	Storage root with shallow horizontal constrictions, Very dispersed storage roots	2832, 2052, 1197, 2054
7	Vine tip pubescence absent, Erect plant	2053, 2117, 2962, 2056
8	Predominant vine pigmentation totally purple, Mature leaf lobe moderate	2314, 2872
9	Mature foliage leaf green with purple vein upper	2318
10	Large mature leaf	2777
11	Pink secondary storage root skin, Immature foliage mostly purple	2813
12	Vine tip pubescence heavy	5798, 3025
13	Triangular leaf outline, No lateral lobes, one mature leaf lobe	10, 2831, 30, 1978, 1883
14	Triangular leaf outline, one mature leaf lobe	2806, 2959, 2126, 2910
15	Oblong storage root	2937
16	Green with purple veins upper	2834
17	Short storage root stalk, Yellow Predominant Storage Root Flesh,	50
18	Slightly purple immature foliage leaf, Open cluster storage root, Yellow-green mature foliage leaf, secondary vine pigmentation with purple nodes.	2118
19	Long storage root stalk	2316
20	Slight mature leaf lobe, orange predominant storage root flesh	2835
21	Purple-red storage root skin colour, long vine internode	1738
22	Lower surface and veins of abaxial leaf totally purple	5799

Table 3: Eigenvalues, percentage of variation & cumulative percentage variation of 30 Principal Components of the 30 characters scored on the 51 sweet potato landrace accessions maintained by NPGRC.

Character	Eigenvalue	Percent	Cumulative	Characters	Eigenvalue	Percent	Cumulative
1	4.80	16.01	16.01	16	0.61	2.05	91.36
2	3.59	11.98	27.99	17	0.50	1.67	93.04
3	3.11	10.37	38.37	18	0.46	1.53	94.58
4	2.32	7.73	46.11	19	0.30	1.03	95.61
5	2.07	6.90	53.01	20	0.28	0.94	96.55
6	1.84	6.14	59.15	21	0.25	0.84	97.40
7	1.55	5.16	64.32	22	0.19	0.65	98.06
8	1.28	4.27	68.59	23	0.15	0.51	98.57
9	1.18	3.95	72.55	24	0.12	0.41	98.98
10	1.07	3.59	76.14	25	0.09	0.31	99.30
11	0.95	3.18	79.33	26	0.08	0.29	99.60
12	0.85	2.83	82.17	27	0.05	0.16	99.76
13	0.77	2.57	84.74	28	0.03	0.11	99.88
14	0.71	2.38	87.12	29	0.02	0.08	99.97
15	0.65	2.18	89.31	30	0.01	0.02	100

*The numbers in the character column represent the 30 characters scored on the 51 accessions of sweet potato (Refer to Appendix II for the explanation of the characters)

The first PC axis differentiated among accessions the leaf lobe number (-0.91), leaf lobe type (-0.88), central leaf lobe shape (-0.87) and leaf outline (-0.82). The second PC axis separated predominant vine pigmentation colour (0.76) whereas the third PC axis split the number of storage roots per plant (-0.78) (Table 4). The characters with < -0.7 and > 0.7 contribute much in each component.

Table 4. Correlation coefficient of 30 morphological characters scored on 51 accessions of sweet potato at NPGRC of South Africa showing Eigenvalues of the first four Principal Components (PC). The characters with < -0.7 and > 0.7 contribute much in each component

Character	PC 1	PC 2	PC 3	PC 4	Characters	PC 1	PC 2	PC 3	PC 4
1	0.49	0.02	0.66	0.10	16	-0.22	0.69	-0.37	-0.06
2	0.55	0.05	0.45	-0.08	17	-0.00	0.35	0.01	0.49
3	-0.46	-0.15	-0.37	0.00	18	0.14	-0.03	0.36	0.44
4	-0.07	**0.76**	-0.24	-0.07	19	-0.05	0.30	0.06	0.09
5	-0.15	-0.26	-0.05	-0.27	20	0.18	0.37	0.19	0.31
6	0.19	0.34	-0.17	-0.52	21	0.31	-0.10	-0.15	-0.46
7	**-0.82**	-0.21	0.13	-0.09	22	0.19	0.24	0.39	0.29
8	**-0.88**	-0.26	0.17	0.00	23	-0.25	0.64	0.46	-0.20
9	**-0.91**	-0.19	0.11	0.06	24	-0.20	0.38	0.57	-0.56
10	**-0.87**	-0.16	0.19	0.02	25	-0.08	0.11	0.28	-0.61
11	0.15	0.17	-0.16	0.16	26	0.38	-0.27	-0.32	-0.39
12	-0.19	0.67	-0.10	0.21	27	0.24	-0.23	0.34	-0.13
13	-0.21	0.26	0.12	0.04	28	-0.17	0.18	-0.21	-0.05
14	-0.11	0.56	-0.29	-0.19	29	-0.19	-0.05	-0.09	0.28
15	-0.45	0.29	0.09	-0.07	30	0.19	-0.04	**-0.78**	0.04

*The numbers in the character column represent the 30 characters scored on the 51 accessions of sweet potato (Refer to Appendix II for the explanation of the characters)

The characters that influenced the first splitting of the 51 accessions were leaf lobe number and vine internode length (Fig. 12). Cluster 21 with only one accession (1738) displayed a long vine internode, while five leaf lobes were observed in Clusters four & five and only one leaf lobe in Clusters 13 & 14. The second split was attributed to predominant vine pigmentation colour and storage root formation while the third split was due to the plant type and number of storage roots per plant. Cluster eight presented totally purple predominant vine. The storage root of Cluster 18 was in an open cluster formation as compared to Cluster six with a much dispersed formation. An erect plant was observed in Cluster seven. These characters had a high magnitude in causing the splitting of accessions.

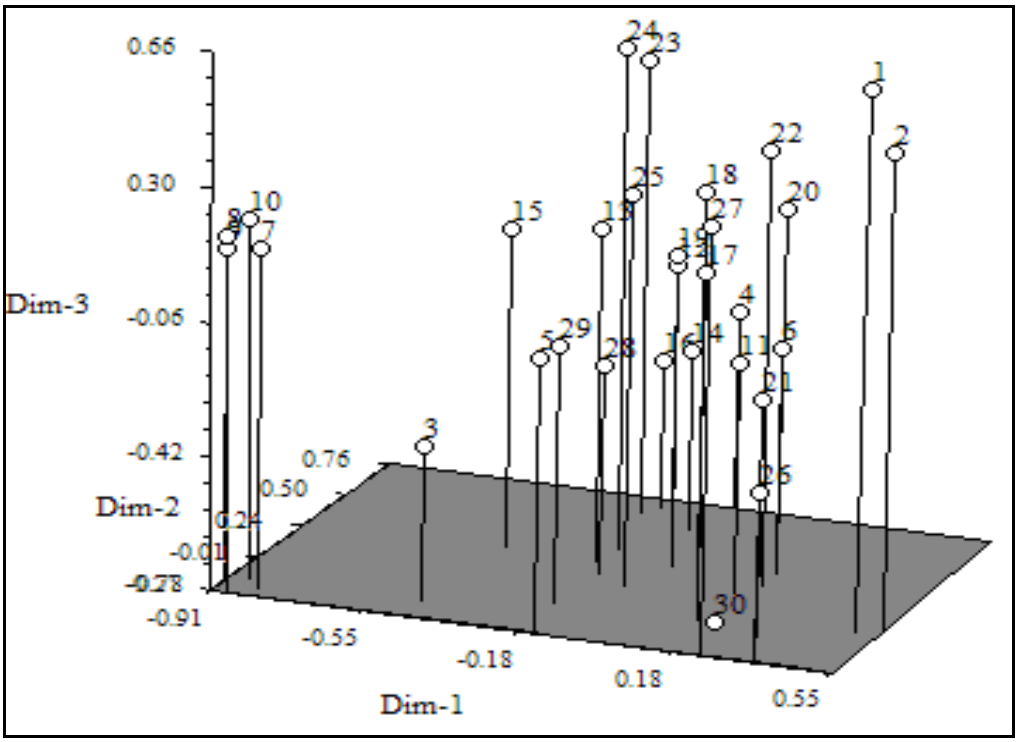

Figure 12. PCA showing the contribution of characters to the variation in 51 sweet potato accessions at NPGRC. Dim-one; two & three refer to three Principal components and their Eigenvalues Numbers, 1-30 refer to the morphological characters scored (Refer to Table 5)

Table 5. Explanation of character numbers (1-30) shown in Figs. 12 & 14

Character No.	Trait	Character No.	Trait
1	Plant type	16	Petiole pigmentation
2	Vine internode length	17	Storage root shape
3	Vine internode diameter	18	Storage root surface defects
4	Predominant vine pigmentation colour	19	Storage root cortex thickness
5	Secondary vine pigmentation colour	20	Predominant storage root skin colour
6	Vine tip pubescence	21	Intensity of predominant storage root skin colour
7	General outline of the mature leaf	22	Secondary storage root skin colour
8	Mature leaf lobes type	23	Predominant storage root flesh colour
9	mature leaf lobes number	24	Secondary storage root flesh colour
10	Shape of central leaf lobe	25	Distribution of secondary storage root colour
11	Mature leaf size	26	Storage root formation
12	Abaxial leaf vein pigmentation	27	Storage root stalk
13	Mature foliage leaf colour	28	Variability of storage root shape
14	Immature foliage colour	29	Variability of storage root size
15	Petiole length	30	Number of storage roots per plant

Multivariate Analysis-Principal Coordinate Analysis (PCoA)

The 51 accessions of sweet potatoes were separated into 22 clusters by PCoA axes one and two. In fact, this separation was in agreement with the grouping of accessions in Fig. 11. The first PCoA contributed 17 percent and the second 13 percent to the grouping of accessions (Fig. 13). In addition the first four components cumulatively reached 50 percent.

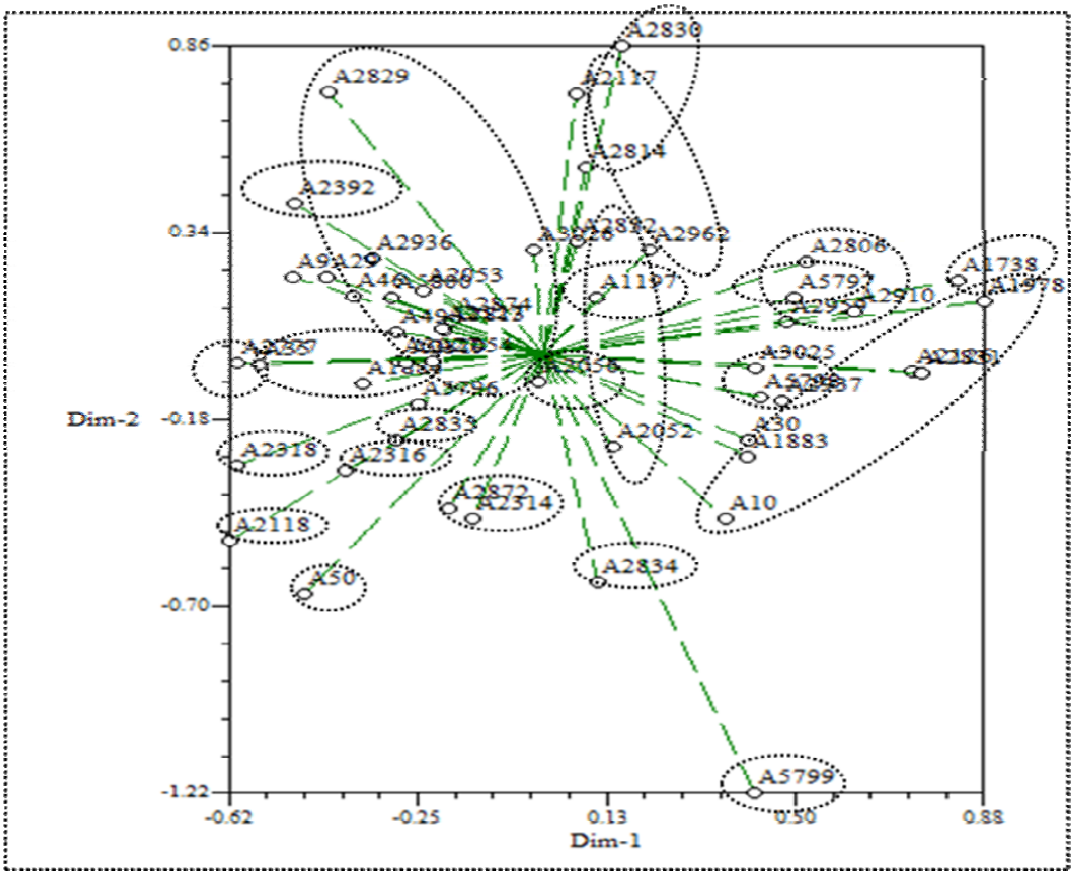

Figure 13. Plot of the first two Principal Coordinate axes (dim-1 & 2) of 51 accessions of sweet potato. The 22 groups are shown, confirming the groups observed in Cluster analysis (Fig. 11)

Multivariate Analysis-Matrix Plot

The projection matrix performed confirmed the grouping identified in Fig. 11. A combination of characters caused the grouping of the 51 accessions characterised.

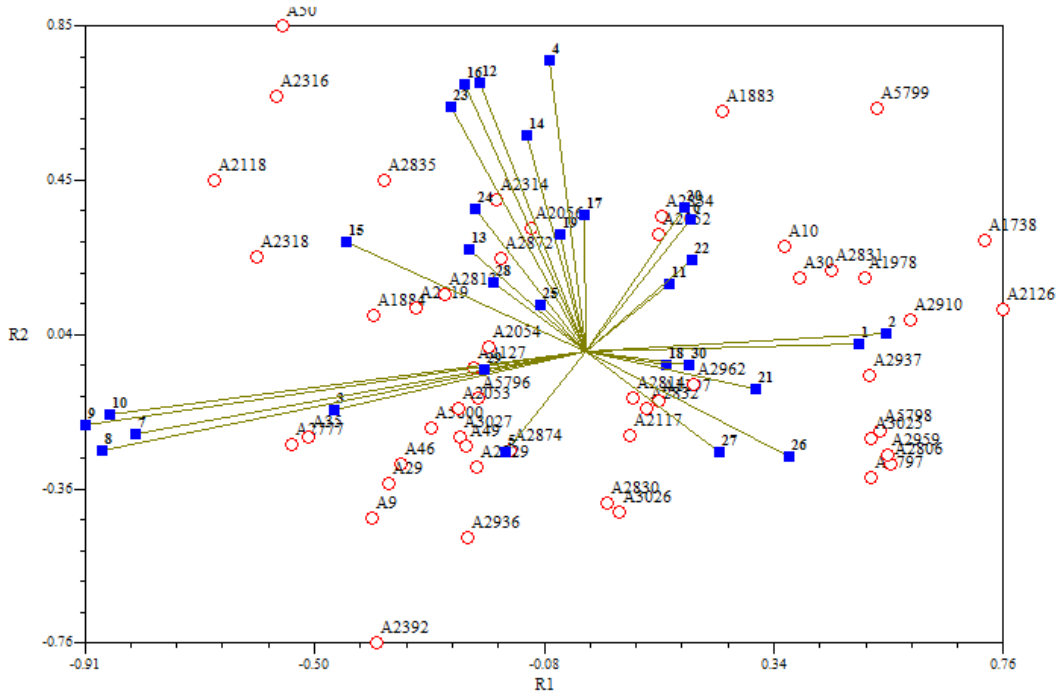

Figure 14. Matrix plot showing characters that caused the grouping of 51 accessions of sweet potato collection at NPGRC. Circles (○) represent 51 sweet potato accessions and the squares (■) represent 30 morphological characters scored (Refer to Table 5)

Cryopreservation

Encapsulation-Vitrification

All the shoot tips tested after pre-incubation and pre-culture procedures showed 100 percent regeneration ability (Fig. 15).

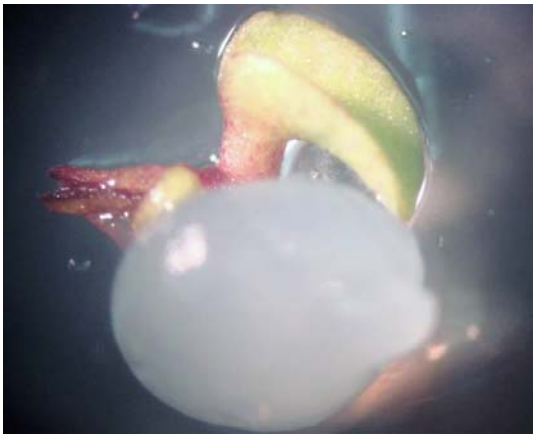

Figure 15. A shoot tip of sweet potato showing regeneration ability after exposure to pre-incubation and pre-culture treatments. The photo was taken under the microscope at 3 x magnification. Source: Tshidi Manamela

Only one shoot tip survived after exposure to osmoprotection solution and PVS2, whereas no regeneration ability was observed after immersion in LN for one hour.

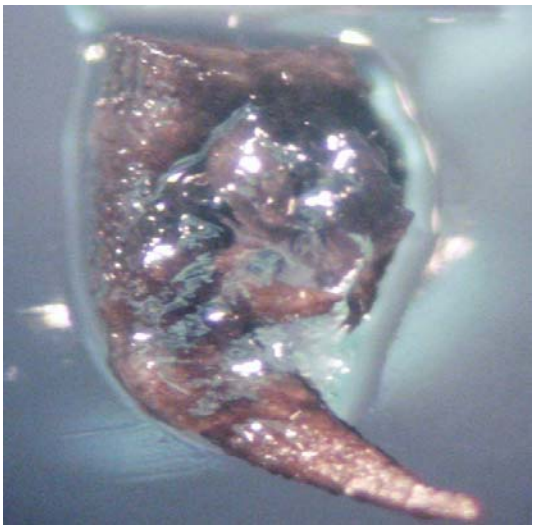

Figure 16. A dead shoot tip of sweet potato after pre-treatment with osmoprotection solution. The photo was taken under the microscope at 3 x magnification. Source: Tshidi Manamela

Encapsulation-Dehydration

The results showed no survival of embryogenic tissues at any stage of encapsulation-dehydration method.

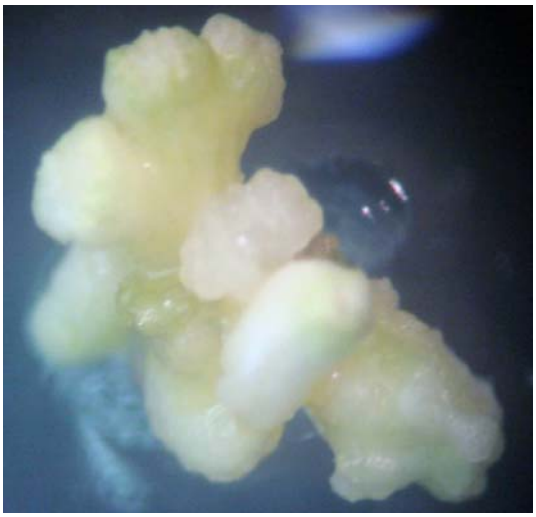

Figure. 17. Embryogenic tissues of accession 2910 prior to encapsulation-dehydration. The photo was taken under the microscope at 3 x magnification. Source: Tshidi Manamela

Discussion

The 51 accessions of sweet potato at the NPGRC of South Africa grouped into 22 clusters (Fig 11) at a distant coefficient of approximately one, which indicate a high level of morphological diversity in the collection. The coefficient of one was selected based on similar studies done by other scientists (Veasey *et al.* 2007). And also, according to Mohammadi & Prasanna (2003) the distant coefficient that shows the largest number of groups should be considered. In this study, coefficient one gave a meaningful number of clusters. Accessions 9 and 29 were almost similar in all the characters, except for the vine internode diameter, secondary vine pigmentation colour, type of mature leaf lobe and petiole length.

Huaman (1999) identified duplicates of the same cultivar ranging from 1-99 at CIP. Furthermore, Veasey *et al.* (2007) observed seven duplicates at a similarity index ranging from 0.12-1.00, which they considered as indicative of high diversity among sweet potato accessions. Tairo *et al.* (2008) reported a distance coefficient of between 0-0.57, indicating a very low diversity among sweet potato accessions in Tanzania. In this study, the distance coefficient varying from 0.57-1.89 gives an indicator of high morphological diversity in sweet potato collection of the NPGRC. But, the results need to be verified with a larger number of replicates.

The total number of variables (30 characters scored) determines the number of components and thus number of PC axes (30). NTSys-pc indicated the Eigenvalues measuring the degree of contribution of each component to the total variance of the collection. Sneath & Sokal (1973) highlighted that the first three PC components (Fig. 12) showing high Eigenvectors should be considered as significant because they can explain as much as up to half of the total variation in a collection. Higher coefficients or Eigenvector values for a certain character indicate the relatedness of that character to the specific PC axes (Sneath & Sokal 1973). Rohlf (2001) advocated that the Eigenvectors (characters in each component) are significant at values > 0.7 and <-0.7. These characters; leaf lobe number, leaf lobe type, shape of the central leaf lobe and predominant vine pigmentation colour were observed to repeatedly contribute to most variability in other analyses performed in this study.

Similarly with PCA, the total numbers of accessions studied determine the total number of PCo axes. In this case, 51 accessions of sweet potato generated 51 PCo axes. PCoA, according to Rohlf (2000) complements cluster analysis. The latter is more sensitive to closely related objects, whereas PCoA is more informative in terms of distances among major groups. The groupings of accessions as observed in Cluster Analysis were confirmed by PCoA. However, PCoA does not calculate both the accessions and characters at once. In this

case, projection matrix supplemented PCoA because both matrices (accessions & characters) were projected simultaneously to determine which characters caused the accessions to group together.The projection matrix performed confirmed the grouping identified in Fig. 11. Characters such as mature leaf lobe number, general outline of the mature leaf, mature leaf lobe type, central leaf lobe shape, vine internode diameter, secondary vine pigmentation colour and variability of storage root size caused the accessions in Clusters two & ten and Clusters four & five to group together (Fig. 14).

On the other hand predominant vine pigmentation colour, vine tip pubescence, abaxial leaf vein pigmentation colour, immature foliage leaf colour, mature foliage leaf colour, mature leaf size, petiole length, petiole pigmentation, storage root shape, storage root cortex thickness, predominant storage root skin colour, secondary storage root skin colour, predominant storage root flesh colour, secondary storage root flesh colour, distribution of storage root flesh colour, and number of storage roots per plant were responsible for the groupings in Clusters 6, 8 9, 11, 17, 18, 19 & 20. It would seem that colour was the dominating aspect in these characters. Clusters 1, 3, 7, 12, 13, 14, 15, 16, 21 & 22 were linked to plant type, vine internode length, storage root surface defects, and intensity of predominant storage root colour, storage root formation, storage root stalk and variability of storage root size.

In this study, the traits that contributed to high variability (< 60 percent) in the univariate analysis were vine internode length, leaf lobe type, leaf lobe number, shape of central leaf lobe, abaxial leaf vein pigmentation, storage root surface defects, storage root cortex thickness and predominant storage root skin colour. In contrast with Table 1, sweet potato collection in Vale do Ribeira presented 39 percent of its accessions with a very slight leaf lobe type (Veasy *et al.* 2007). They also observed 45 percent almost half of their collection with five leaf lobes, similarly to this study with 49 percent having five leaf lobes.

NTSys-pc software is widely used (Tairo *et al.* 2008; Huamán 1991) for the analysis of characterisation data. The different analyses of morphological data performed in this study are complementary to each. Hierarchical clustering method is relevant for analysing the phenotypic and genetic diversity of germplasm. Clustering method grouped accessions of sweet potato based on the characters they possessed. Accessions with similar characters were grouped in the same cluster. PCoA & PCA complemented clustering method in that it confirmed the groups and it is not sensitive to closely related objects (Mohammadi & Prasanna 2003). And also, PCA depicted the relationship among the characters scored and their contribution to the total variance of the 51 sweet potato accessions. These analyses combined gave a fairly reliable output that can be used to make safe conclusions if there were replicates.

Sweet potato is a clonally propagated crop and thus there is a risk of many duplicates in the collection. Nonetheless, all the analysis performed on the morphological characterisation data obtained from sweet potato landrace accessions available at NPGRC, displayed high variation that exist in the collection. This needs however to be verified with another study with replicates for each accession. The main limitation of this study was absence of replicate plants which means that only one plant per accession was scored. In addition, the variations that exist within accessions of sweet potato were not assessed.

Several plants of each accession need to be grown in a randomized test-design before safe conclusions can be made. Environmental factors such as light, temperature, water and pH of the soil affect anthocyanin production and the size of the leaves and storage roots. However, all accessions used in this study were grown under the same condition and treated exactly the same as is the requirement for performing characterization. Taro was grown on the edges of the 51 accessions in order to minimise the edge effect and ensure the uniform condition of the growth environment in the glasshouse. In morphological characterisation, plants on the edge of the planting site are usually not characterised because they may have been influenced by the external environmental conditions. Characters that are scored are highly heritable and not influenced much by the environment. The CIP descriptors for sweet potato are completely based on qualitative characters and the fact that sweet potato is a clonal crop may not have influenced the characterisation results.

On the other hand, it may also be possible that the data from the replicates would give somewhat different result output. Therefore, further analysis of the same set of landraces needs to be performed in order to verify these findings. Furthermore these findings need further verifications from biochemical or molecular analysis. The NPGRC of South Africa currently does not have facilities for these kinds of analysis. In my knowledge the results obtained in this study will therefore be used for subsequent collection missions.
The conservation method employed presently at the NPGRC is tissue culture and field genebank methods. According to several authors (Rao 2004; Engelmann 2004) these methods have both the positive sides and some drawbacks. Cryopreservation has been reported to be the alternative conservation method that complements tissue culture and field genebanks (Pennycook & Towill 2000; Charoensub *et al.* 2004) and the material can be conserved for long term.

Encapsulation-vitrification as reported by Hirai & Sakai (2003) presented 80 percent survival rate on shoot tips of sweet potato. According to Hirai & Sakai (2003) pre-incubation followed by pre-culture produces significantly higher survival rates. It was further mentioned that pre-incubation alone or pre-culture without pre-incubation produced very low recovery rates. These

findings were confirmed by Pennycook & Towill (2000) who obtained the same results in their study. Hamalgyi & Deliu (2007) confirmed that sucrose concentration should be optimised according to the individual species so that a high recovery rate can be obtained. Temperature and duration for exposure of explants to PVS2 are important factors affecting regeneration of cryopreserved material because of the toxicity of this solution (Halmagyi & Deliu 2007).

The duration of one hour of encapsulated shoot tips in PVS2 in the study of Hirai & Sakai 2003, did not affect the regeneration ability of sweet potato shoot tips as compared to this study. Therefore, the optimal duration of encapsulated shoot tips need to be modified for sweet potato accessions at the NPGRC of South Africa. Several authors advocated that photo oxidation have a negative effect on the cryopreserved material and recommended the incubation of the cryopreserved material for at least two days in the dark to counteract the effect of light (Sharma *et al.* 2006).

The protocol described by Hirai & Sakai (2003) did not mention incubation in the dark. However, in this study shoot tips were incubated both in the dark and 16 /8 h photoperiod after LN treatment. No survival was observed after LN. Different concentrations of sucrose and glycerol in osmoprotection solution need to be tested and the concentration for optimal survival can then be implemented in further experiments. Furthermore, duration of shoot tips in PVS2 should be optimized for high regeneration ability.
According to encapsulation-dehydration protocol, no survival was observed at any of the steps.

High or low sucrose levels may reduce the survival rate of explants as is the case in encapsulation-vitrification procedure. My hypothesis is that the recovery medium described by Sharma *et al.* (2006) needs evaluation as the embryogenic tissues died in the germination tests performed after each step of encapsulation-dehydration. The concentration of sucrose in the medium used for the production of embryogenic tissues was lower (0.12 M) than in the recovery medium (0.15 M). All the meristems of the accessions used for this experiment produced embryogenic tissues whereas no growth was noted in the recovery medium after LN treatment. Therefore, further experiments are required to investigate and modify the regeneration medium of this protocol.

Conclusions and recommendations

i. Preliminary results presented here show that the NPGRC of South Africa conserves high morphological diversity of sweet potato landraces in the field and tissue culture.

ii. From the 51 accessions characterized unique traits were identified as shown in the cluster identification membership. This means that there are still gaps in trait representation in the sweet potato collection at NPGRC of South Africa. The subsequent collections of sweet potato should therefore, focus on the unique traits.

iii. Encapsulation-vitrification and encapsulation-dehydration protocols in the present form were not successful for cryo protection of sweet potato of the NPGRC. We recommend that the recovery medium used in the encapsulation-dehydration protocol be investigated.

iv. Furthermore, the size of the explants, the concentration and temperature of the pre-treatment solutions should be modified. Further experiments and optimization of these protocols may prove successful for the studied sweet potato accessions.

v. Further morphological analysis but also biochemical or molecular analysis of the same set of landraces needs to be performed to ascertain the findings of this study. Sweet potato landraces are gradually replaced by the improved varieties.

vi. We recommend the collection of most of the landrace diversity available and for conservation strategies to be strengthened.

Acknowledgment

I am grateful to my supervisor Mr Andre Lezar (Agricultural Research Council, Pretoria) who provided guidance throughout this study. The SADC Plant Genetic Resources Centre and Swedish International Development Agency are appreciated for their sponsorship and Swedish Biodiversity Centre for facilitating the Programme. I am thankful to my employer, Department of Agriculture for believing that skills and knowledge I have acquired in this Programme will contribute toward achieving our objectives. I thank the Agricultural Research Council-Vegetables and Ornamental Plant Institute, Pretoria for allowing me to use their facilities. My sincere gratitude is extended to my family for their understanding, motivation and for taking care of my daughter, Itumeleng during my absence. Special thanks go to Prof. Sadhna Alström (Swedish University of Agricultural Science, Sweden); Ms Jane Njuguna and Mr John Tumuhairwe (PhD students) for reviewing my work. I appreciate my colleagues and friends at the Department of Agriculture, South Africa, for their time during data collection. Ms Lizzy Mdluli and Merriam Mthombeni (ARC-VOPI), I will never forget your assistance during laboratory work. I also thank my co-supervisor, Dr Eva Thörn (Swedish University of Agricultural Science, Sweden). Ultimately, honour to God the Almighty who gave me strength, patience and courage to complete this study.

Refrences

Allemann, J., Laurie, S. M., Thiart, S. & Vorster H.J. 2004. Sustainable production of root and tuber crops (potato, sweet potato, indigenous potato, cassava) in southern Africa. *South African Journal of Botany* 70: 60-66.

Charoensub, R., Hirai, D. & Sakai A. 2004. Cryopreservation of *in-vitro*-grown shoot tips of cassava by encapsulation-vitrification method. *Cryoletters* 25: 51-58.

CIP, AVRDC, IBPGRC, Huamán, Z. (ed.) 1991. *Descriptors for sweet potato.* International Board for Plant Genetic Resources. Rome, Italy.

Department of Agriculture (ed.). 2009. *Abstract of Agricultural Statistics.* Department of Agriculture, Pretoria.

Ewell, P.T. & Mutuura J. 2004. Sweet potato in the food system of eastern and southern Africa. *International Society for Horticultural Science Acta Horticulturae* 380: 405-412.

Engelmann, F. 1991. *In vitro* conservation of tropical plant germplasm-a review. *Evolution* 87:227-243.

Engelmann, F. & Engels, J. M. M. 2002. Technologies and strategies for *ex situ* conservation. In Engels, J. M. M., Rao, V.R., Brown, A. H. D. & Jackson, M. T. (eds) *Managing Plant Genetic Diversity*, 89-104. CAB International and IPGRI, Wallingford and Rome.

Engelmann, F. 2004. Plant cryopreservation: Progress and prospects. *In vitro Cellular and Development Plant Biology* 40: 427-433

Food and Agriculture Organization. 2008. *FAO Statistical Yearbook.* http://faostat.fao.org [accessed May 2009].

Gepts, P. 2004. Crop domestication as a long selection experiment. In Jules, J. (ed.). *Plant Breeding Reviews*, Vol 24: 1-23. John Wiley & Sons, Carlifornia

Gibson, R. W., Jeremiah, S. C., Aritua, V., Msabaha, R. P., Mpembe, I. & Ndunguru J. 2000. Sweet potato virus disease in Sub-Saharan Africa: Evidence that neglect of seedlings in the traditional farming system hinders the development of superior resistant landraces. *Journal of Phytopathology* 148: 441-447.

Halmagyi, A. & Deliu C. 2007. Cryopreservation of carnation (*Dianthus caryophyllus* L.) shoot tips by encapsulation-vitrification. *Scientia Horticulturae* 113: 300-306.

Hirai, D. & Sakai A. 1999. Cryopreservation of *in vitro*-grown meristems of potato (*Solanum tuberosum* L.) by encapsulation-vitrification. *Potato Research* 42: 153-160.

Hirai, D. & Sakai A. 2001. Recovery growth of plants cryopreserved by encapsulation-vitrification. *Bulletin of Hokkaido Prefectural Agricultural Experiment Stations* 80: 55-64.

Hirai, D. & Sakai A. 2003. Simplified cryopreservation of sweet potato [*Ipomoea batatas* (L.) Lam.] by optimizing conditions for osmoprotection. *Plant Cell Report* 21: 961–966.

Huamán, Z., Aquilar, C. & Ortiz R. 1999. Selecting a Peruvian sweet potato core collection on the basis of morphological, ecogeographical, and disease and pest reaction data. *Theoretical Applied Genetics* 98: 840-844.

International Potato Centre (CIP). 2008. *Sweet potato.* http://www.cipotato.org/sweetpotato [6 April 2009].

Laurie, S. M. 2004. Sweet potato in perspective. In Niederwieser, J. G. (ed.) *Guide to sweet potato production in South Africa,* Vol 1: 1-6. Agricultural Research Council. South Africa, Pretoria.

Laurie, S. M. & Niederwieser, J. G. 2004. The sweet potato plant. In Niederwieser, J. G. (ed.) *Guide to sweet potato production in South Africa*, Vol 1: 7-14. Agricultural Research Council. South Africa, Pretoria.

Laurie, S. M. & Magoro M. D. 2008. Evaluation and release of new sweet potato varieties through farmer participatory selection. *African Journal of Agricultural Research* 3: 672-676.

Mohammadi, S. A. & Prasanna B. M. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and consideration. *Crop Science* 43: 1235-1248.

Mok, I. G. & Schmiediche P. 1998. Collecting, chatacterizing and maintaining sweet potato germplasm in Indonesia. *Plant Genetic Resources Newsletter* 118: 12-18.

Pennycook, J. C. & Towill L. E. 2000. Cryopreservation of shoot tips from in vitro plants of sweet potato [Ipomoea batatas (L.) Lam.] by vitrification. *Plant Cell Reports* 19: 733-737.

Rao, N. K. 2004. Plant genetic resources: advancing conservation and use through biotechnology. *African Journal of Biotechnology* 3: 136-145.

Reed, B. M., Engelmann, F., Dullo, M. E. & Engels, J. M. M. (eds) 2004. *Technical guidelines for the management of field and invitro germplasm collection.* IPGRI Handbooks for Genebanks N0 7. International Plant Genetic Resources Institute. Rome, Italy.

Rohlf, J. F. (ed.). 2000. *Numerical Taxonomy and Multivariate Analysis System.* Version 2.1 (NTSys-pc software). Exeter Software, New York.

Sakai, A., Kobayashi, S., & Oiyama, I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Report* 9: 30-33.

Sauer, J. D (ed). 1993. *Historical geography of crop plants: a selected roster.* CRC Press. Los Angeles, California.

Sharma, S. K., Sharma, N., Agrawal, A., Malik, S. K., Mandal, B. B., Chaudhury, R., Tyagi, R. K., Pandey, R., Gupta, S. & Hussain, Z. (eds) 2006. *A Laboratory Manual for In Vitro and Cryopreservation Techniques for Conservation of Plant Genetic Resources.* National Bureau of Plant Genetic Resources. New Delhi, India.

Sneath, P. H. A & Sokal, R. R. (eds) 1973. *Numerical Taxonomy: the principles and practice of numerical classification*. Stony Brook, New York.

Southern African Development Community. 2007. The SADC Plant Genetic Resources Centre. http://www.sadc.int/fanr/agricresearch/spgrc/ [accessed 6 April 2009].

Tairo, F., Mneney, E. & Kullaya A. 2008. Morphological and agronomical characterisation of sweet potato [Ipomoea batatas (L.) Lam.] germplasm collection from Tanzania. *African Journal of Plant Science* 2: 077-085.

Towill, L. E. & Jarret, R. L. 1992. Cryopreservation of sweet potato (*Ipomoea batatas* [L.] Lam.) shoot tips by vitrification. *Plant cell Reports* 11: 175-178

Woolfe, J.A. (ed.) 1992. *Sweetpotato: an untapped food resource*. Cambridge Universal Press and the International Potato Centre (CIP). Cambridge, UK.

Van den Berg, A.A. & Laurie S.M. 2004.Cultivation. In Niederwieser, J.G. (ed.) *Guide to sweet potato production in South Africa*, Vol 1: 15-26. Agricultural Research Council, Pretoria.

Veasey, E. A., Silva, J. R. Q., Borges, A., Bressan, E. A. & Peroni N. 2007. Phenology and morphological diversity of sweet potato (*Ipomoea batatas*) landraces of the Vale do Ribeira. *Scientia Agricola* 64: 416-427.

Zhang, D. P., Rossel, G., Kriegner, A. & Hijmans R. 2004. AFLP assessment of diversity in sweet potato from Latin America and the Pacific region: Its implications on the dispersal of the crop. *Genetic Resources and Crop Evolution* 51: 115-120.

Appendix I. Passport data of 35 accessions from the 51 accessions maintained at the NPGRC. Of these 51 accessions, passport data for the 16 accessions was not available for this study

Acc.no.	Collection date	Depositor	District	Latitude	Longitude	Province	Town	Village
9								
10								
29								
30								
35								
46								
49								
50								
1197								
1738	2003/05/19	Mrs. Noku Mavithane	OR Tambo	-31.38	29.43	Eastern Cape-Province	Ngqeleni	Elucingweni
1883	2003/06/09	Mr Simion Meyiwa		-29.64	31.16	Kwazulu Natal	Umbumbulu	Ndlandlama
1884	2003/06/09	Mr Simion Meyiwa		-29.64	31.16	Kwazulu Natal	Umbumbulu	Ndlandlama
1978	2003/06/13	Mrs Phumzile Njapha	Umbumbulu	-29.64	30.58	Kwazulu Natal	Isiphingo	Emphusheni
2052	2003/07/10	Mrs Sibongile Mdlalose	Eshowe	-28.85	31.30	Kwazulu Natal	Eshowe	Ntumeni
2053	2003/07/10	Mrs Sibongile Mdlalose	Eshowe	-28.85	31.30	Kwazulu Natal	Eshowe	Ntumeni
2054	2003/07/10	Mrs Sibongile Mdlalose	Eshowe	-28.85	31.30	Kwazulu Natal	Eshowe	Ntumeni
2056	2003/07/10	Mrs Gladys Mahaye	Eshowe	-28.88	31.45	Kwazulu Natal	Eshowe	Esiqwaqweni
2117	2003/07/11	Mrs Eva Khanyile	Eshowe	-28.90	31.50	Kwazulu Natal	Eshowe	Mbomboshane
2118	2003/07/11	Mrs Eva Khanyile	Eshowe	-28.90	31.50	Kwazulu Natal	Eshowe	Mbomboshane
2126	2003/07/11	Mrs Jostina Mtshali	Eshowe	-28.91	31.50	Kwazulu Natal	Eshowe	Berea
2127	2003/07/11	Mrs Jostina Mtshali	Eshowe	-28.91	31.50	Kwazulu Natal	Eshowe	Berea
2314	2003/07/29	Mr Elmond Sigundla	Mbombela	-25.09	31.32	Mpumalanga	Nelspruit	Nyongani
2316	2003/07/29	Mr Elmond Sigundla	Mbombela	-25.09	31.32	Mpumalanga	Nelspruit	Nyongani
2318	2003/07/29	Mr Elmond Sigundla	Mbombela	-25.09	31.32	Mpumalanga	Nelspruit	Nyongani
2319	2003/07/29	Mr Elmond	Mbombela	-25.09	31.32	Mpumalanga	Nelspruit	Nyongani

		Sigundla						
2392								
2777								
2806	2004/05/04	Kwangwanase Farmers Union	Umhlabu-yalingana	-26.98	32.89	Kwazulu Natal	Mangozi	Kwangwanase
2813	2004/05/04	Ekuthuleni Comm Garden	Umhlubu-yalingana	-27.28	32.17	Kwazulu Natal	Mangozi	Kwangwanase
2814	2004/05/04	Ekuthuleni Comm Garden	Umhlubuya-lingana	-27.28	32.17	Kwazulu Natal	Mangozi	Kwangwanase
2829	2004/05/05	Zizamele Community Garden	Umhlubu-yalingana	-27.14	32.85	Kwazulu Natal	Mangozi	Thandizwe
2830	2004/05/05	Zizamele Community Garden	Umhlubu-yalingana	-27.14	32.85	Kwazulu Natal	Mangozi	Thandizwe
2831	2004/05/05	Zizamele Community Garden	Umhlubu-yalingana	-27.14	32.85	Kwazulu Natal	Mangozi	Thandizwe
2832	2004/05/05	Zizamele Community Garden	Umhlubu-yalingana	-27.14	32.85	Kwazulu Natal	Mangozi	Thandizwe
2834	2004/05/05	Zizamele Community Garden	Umhlubu-yalingana	-27.14	32.85	Kwazulu Natal	Mangozi	Thandizwe
2835	2004/05/05	Mr MD Phoseka	Umhlubu-yalingana	-27.09	32.83	Kwazulu Natal	Mangozi	Thandizwe
2872	2004/05/06	Mrs Corina Mngomezulu	Ingwavuma	-27.23	32.05	Kwazulu Natal	Ingwavuma	Kwa-Lindizwe
2874	2004/05/06	Mrs Corina Mngomezulu	Ingwavuma	-27.23	32.05	Kwazulu Natal	Ingwavuma	Kwa-Lindizwe
2910	2004/05/07	Mr Dakamiya mathenjwa	Umhlubu-yalingana	-27.65	32.30	Kwazulu Natal	Jozini	Nkangala
2936	2004/05/10	Mrs Irene Ntshangase	Hlabisa	-28.31	32.01	Kwazulu Natal	Hlabisa	Mgovuzo
2937	2004/05/10	Mrs Irene Ntshangase	Hlabisa	-28.31	32.01	Kwazulu Natal	Hlabisa	Mgovuzo
2959	2004/05/10	Mrs Mariette Mathula	Hlabisa	-28.14	31.94	Kwazulu Natal	Hlabisa	Kwanqunwana
2962	2004/05/10	Mrs Mariette Mathula	Hlabisa	-28.14	31.94	Kwazulu Natal	Hlabisa	Kwanqunwana
3025	2004/05/13	Mrs MS Msibi	Umhlathuza	-28.72	32.26	Kwazulu Natal	Richards Bay	
3026	2004/05/13	Mrs MS Msibi	Umhlathuza	-28.72	32.26	Kwazulu Natal	Richards Bay	
3027	2004/05/13	Mrs MS Msibi	Umhlathuza	-28.72	32.26	Kwazulu Natal	Richards Bay	
5796								
5797								
5798								
5799								
5800								

Appendix II. International Potato Centre (CIP 1991) descriptors for sweet potato showing characters and their scores

Character	Character scores
1. Plant type	3=Erect, 5=Semi-erect, 7=Spreading,9=Extra spreading
2. Vine internode length	1=Very short, 3=Short, 5=Intermediate, 7=Long, 9= Very long
3. Vine internode diameter	1 = Very thin, 3=Thin, 5=Intermediate, 7=Thick, 9=Very thick
4. Predominant vine pigmentation colour	1=Green, 3=Green with few purple spots, 4=Green with many purple spots, 5=Green with many dark purple spots, 6=Mostly purple
5. Secondary vine pigmentation colour	1=Green base, 2=Green tip, 3=Green nodes, 4=Purple base, 0=Absent,
6. Vine tip pubescence	0=Absent, 3=Sparse, 5=Moderate, 7=Heavy
7. General outline of the mature leaf	1=Rounded, 2=Reniform, 3=Cordate, 4=Triangular, 5=Hastate
8. Mature leaf lobes type	0=No lateral lobes,1=Very slight, 3=Slight, 5=Moderate, 7=Deep
9. Mature leaf lobes number	1,3,5,7,9
10. Shape of central leaf lobe	0=Absent, 1=Toothed, 2=Triangular, 3=Semi-circular, 4=Semi-elliptic
11. Mature leaf size	3=Small, 5=Medium, 7=Large, 9=Very Large
12. Abaxial leaf vein pigmentation	1=Yellow, 2=Green, 3=Purple spot base of main rib, 4=Purple spot in several veins, 5=Main rib partially purple
13. Mature foliage leaf clolour	1=Yellow green, 2=Green, 3=Green with purple edge, 4=Greyish green, 5=Green with purple veins upper
14. Immature foliage colour	1=Yellow green, 2=Green, 3=Green with purple edge, 4=Greyish green, 5=Green with purple veins upper
15. Petiole length	1=Very short, 3=Short, 5=Intermediate, 7=Long, 9=Very long
16. Petiole pigmentation	1=Green, 2=Green with purple near stem, 3=Green with purple near leaf, 4=Green with purple both ends, 5=Green with purple spots through petiole
17. Storage root shape	1=Round, 2=Round elliptic, 3=Elliptic, 4=Ovate, 5=Obovate
18. Storage root surface defects	0=Absent, 1=Alligatorskin-like, 2=Veins, 3=Shallow horizontal constrictions, 4=Deep horizontal constrictions
19. Storage root cortex thickness	1=Very thin, 3=Thin, 5=Intermediate, 7=Thick, 9=Very Thick,
20. Predominant storage root skin colour	1=White, 2=Cream, 3=Yellow, 4=Orange, 5=Brownish orange
21. Intensity of predominant storage root skin colour	1=Pale, 2=Intermediate, 3=Dark
22. Secondary storage root skin colour	0=Absent, 1=White, 2=Cream, 3=Yellow, 4=Orange
23. Predominant storage root flesh colour	1=White, 2=Cream, 3=Dark cream, 4=Pale yellow, 5=Dark yellow
24. Secondary storage root flesh colour	0=Absent, 1=White, 2=Cream, 3=Yellow, 4=Orange
25. Distribution of secondary storage root colour	0=Absent, 1=Narrow ring in cortex, 2=Broad ring in cortex, 3=Scattered spots in flesh, 4=Narrow ring in flesh
26. Storage root formation	1=Closed cluster, 3=Open cluster, 5=Dispersed, 7=Very dispersed,
27. Storage root stalk	0=Sessile or absent, 1=Very short, 3=Short, 5=Intermediate, 7=Long
28. Variability of storage root shape	3=Uniform, 5=Slightly variable, 7=Moderately variable
29. Variability of storage root size	3=Uniform, 5=Slightly variable, 7=Moderately variable
30. Number of storage roots per plant	Quantitative

Appendix III. Encapsulation-vitrification protocol by Hirai & Sakai (2003)

Plant Material

Excised apical buds with one or two nodal segments from the plantlets and culture on 20 ml basal MS (Murashige and Skoog 1962) medium supplemented with 30 g/l sucrose, 1 g/l casamino acids and 2 g/l Gellan-gum, in tissue culture dishes (90 mm x 20 mm) at 25°C under a 16 h photoperiod at 50 μmol m^{-2} s^{-1} for 14 days (stock culture). Adjust medium pH to 5.7 and autoclave at 121°C for 7 min. For micropropagation, transfer nodal segments consisting of a piece of stem about 8 mm long to basal medium supplemented with 0.5 mg/l 6-benzyl aminopurine (BA) and incubate as above. Remove leaves from nodal segments before their culture for the uniform growth of axillary buds. After 10–14 days incubate excised shoot tips with three to four leaf primordia (about 1 mm long).

Encapsulation, pre-culture and osmoprotection

Suspend the excised shoot tips in a bead solution consisting of Ca2+-free MS inorganic medium supplemented with 2 percent (w/v) Naalginate (100–150 cp; Wako, Japan) and 30 g/l sucrose. With a sterile pipette dispense a droplet of bead solution containing a single shoot tip into MS medium supplemented with 30 g/l sucrose and 0.1 M CaCl2 (encapsulation solution). Leave shoot tips in the encapsulation solution for 30 min at 25°C to allow completion of the gelation process (to about 4 mm in diameter). Pre-incubate beads in 40 ml liquid basal MS medium supplemented with 30 g/l sucrose and 1 g/l casamino acid in a 100 ml Erlenmeyer flask for 24 h on a rotary shaker (90 rpm). Then transfer them to a sucrose-enriched (0.3 M) liquid basal medium and preculture for 16 h. Pre-incubate and pre-culture under the same conditions as for stock culture.

Osmoprotection and Vitrification

Incubate precultured beads in liquid MS medium supplemented with different concentrations (0.4–1.8 M) of sucrose with or without 2 M glycerol for 3 h on a rotary shaker (60 rpm) at 25°C. Dehydrate between 10 and 15 encapsulated, precultured and osmoprotected shoot tips in 20 ml Plant Vitrification Solution 2 (PVS2) in a 50 ml Erlenmeyer flask on a rotary shaker (60 rpm) at 25°C for various periods of time (0–100 min). PVS2 contains 30 percent (w/v) glycerol, 15 percent (w/v) ethylene glycol, 15 percent (w/v) Dimethylsulphoxide and 0.4 M sucrose in MS medium (pH 5.8). Transfer beads to a 2 ml cryotube and suspend in 0.5 ml PVS2. Immerse cryotubes directly into Liquid Nitrogen for a minimum of 1 hour.

Dilution process

After immersion in LN, rapidly warm cryotubes in a water bath at 38°C for 2 min, then drain PVS2 from the cryotubes and replace twice at 10 min intervals with 1 ml 1.2M sucrose solution (dilution solution).

Viability and plant regrowth

Place the LN-treated beads on recovery medium 1, consisting of basal medium supplemented with 0.5 mg/l BA and 1 mg/l gibberellic acid 3 (GA3) for seven days. After seven days transfer to recovery medium 2, consisting of basal medium supplemented with 0.5 mg/l GA3.

Appendix IV. Encapsulation-dehydration by Sharma *et al*. (2006)

Plant material

Induce Somatic embryos by culturing axillary bud meristems on MS medium supplemented with sucrose 40g/l and 2, 4-D (dichlorophenoxyaceticacid) 1mg/l. After one & half or two months, subculture embryogenic tissues on the same but freshly prepared media for its multiplication. The multiplied embryogenic aggregates along with embryogenic tissues are used for cryopreservation.

Preculture

Excise about 1.5 to 2.0 mm embryogenic aggregates and transfer them to semisolid Murashige & Skoog (MS) medium supplemented with 0.3 M sucrose for three days before encapsulation.

Encapsulation

After 3 days, suspend the embryogenic tissues in culture medium without calcium containing 2.5percent (weight/volume) sodium alginate and 0.15 M sucrose. Pump the medium with embryogenic aggregates into a pipette, poured drop by drop in liquid MS medium enriched with 100 mM calcium chloride. This will allow the alginate to polymerize into calcium alginate and to form beads in which embryogenic tissue is enclosed.

Pregrowth treatment

Pre-treat the encapsulated tissue as beads for three days in liquid MS medium enriched with 0.5M sucrose. During this step the explants get dehydrated due to the high osmotic pressure of the pregrowth medium. The concentration of intracellular solutes is also increased due to sucrose uptake by cells.

Dehydration

Further dehydrated the beads under the laminar air flow for 4 hours on a sterile Petri dish. This will ensure survival after freezing.

Freezing

After dehydration transfer the beads to a 1.8 ml polypropylene cryovial and plunge directly in LN for at least 1 hour.

Thawing and re-growth

After 1 hour thaw the beads by placing the cryotubes directly into a water bath at 40 ^{0}C for 2 min. Then transfer the beads to semisolid MS medium supplemented with 1 mg/l 2,4D + 0.15 M sucrose and incubated in dark for

two days. Remove embryogenic aggregates from the beads and subculture on the same but fresh semi-solid MSmedium and incubated for another six weeks for further growth of embryogenic tissue.

Regeneration

The regrown and multiplied somatic embryos from above will initiate regeneration of plantlets after three weeks of culturing on MS basal medium.