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Status and Prospects of Biotechnology Applications to Conservation, Propagation and Genetic Improvement of Yam

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ABSTRACT

Yam plays a critical role in providing good quantity and quality yield and source of income to combat the challenge of food insecurity in the tropics. Although significant advances in science and technology have improved efficient management and improvement of their genetic resources, the mission of fully exploiting their potentials is yet to be maximally achieved. Lack of formal seed systems increases germplasm losses and reduces yields. Research is needed in the control of tuber dormancy in yam. Biotechnological approaches, especially *in vitro* culture of meristem/shoot tip combined with thermo/cryotherapy are promising methods to clean yam seeds of pathogens like viruses. The multiplication of clean yam seeds so obtained can be propagated

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using improved systems such as temporary immersion bioreactors, aeroponics and photoautotrophic systems but knowledge gaps still exist on their use. Although the relative importance of clean materials, survival on farmers' fields, production cost and multiplication ratio should be determined and considered in deciding the propagation technique to adopt, conventional and improved tissue culture techniques will be indispensable in the production of clean seed yams. Marker-assisted selection, embryo culture, genetic transformation and genome sequencing have been initiated to support conventional genetic improvement, but investigations into the presence of transposable elements, site targeted mutagenesis, somatic embryogenesis and haploid plant production will be necessary to fast track the genetic improvement. These biotechnological approaches will not only enhance the use of disease-free, quality-declared planting materials but also facilitate germplasm exchange and speed up genetic improvement while providing excellent means for conservation.

Introduction

Yam (*Dioscorea* spp.) is a tuber-producing plant and is popular in the humid and sub-humid tropics, particularly in Africa, West Indies, parts of Asia and South and Central America. Knuth (1924) estimated that there are about 600 species in the genus *Dioscorea*. Yam species are annual or perennial vines and climbers with annual or perennial underground tubers. The Guinea yam (*Dioscorea rotundata* Poir. and *D. cayenensis* Lam.), are most preferred in Africa but in the Caribbean and Pacific, *D. alata* L. and *D. esculenta* (Lour.) Burk. are preferred. *D. bulbifera* L. also forms small aerial tubers or bulbils in the leaf axils. *D. hispida* Dennst. (Asia), *D. Dumetorum* (Knuth.) Pax. (Africa) and *D. trifida* L. (Central America and the Caribbean), *D. opposite* Thunb. and *D. japonica* Thunb. (China and Japan) are other edible species. Yam is a primary source of income in West Africa, from where 94% of global yam production emanates. The edible portion is the underground tuber which is a major source of carbohydrate. Nigeria produces 71% (FAO 2006) of global yam production, equivalent to 37 million tonne. The consumption of yam on a per capita basis on the West African coast is highest in Togo, followed by Cote d'Ivoire, Ghana, Benin Republic and Nigeria (Onwueme 1978, Kalu and Erhabor 1992). The most preferred form of eating yam is the boiled and pounded form. It is also fried in palm oil or roasted and eaten with oil, while yam flakes and chips are taken as snack (Orkwor 1998).

Yam production is constrained by abiotic factors, influence of pests and diseases and scarcity of planting material. In addition, tuber dormancy, which prevents year-round production and uncontrolled sprouting after dormancy break, which causes storage losses, also hampers productivity and reduces incomes and profits (Craufurd et al. 2001, Lang 1996). Consequently, its

genetic resources should be used to support crop improvement programmes followed by production, propagation and distribution of quality planting materials to farmers for optimal yields. Biotechnology is the more reliable tool to achieve this. This chapter presents the state-of-the-art, constraints and way forward in yam genetic resources (GR) conservation, propagation and improvement.

Losses in Yam GR

Yam is susceptible to genetic erosion and variety loss. The losses are caused by multiple factors and are often related to their vegetative mode of propagation. The combination of population increase, negative consequences of climate change, and increased incidence of pest and diseases and natural disasters is a serious threat. *D. alata* anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sac. (Mignouna et al. 2001, Pérez et al. 2003) caused losses in excess of 90% (Winch et al. 1984). *D. alata* and *D. rotundata* vine and foliage are also attacked by the beetle *Crioceri slivida,* the cricket *Gymnogryllus lucens* eats the tubers and cut the vines while the beetle *Heterolygusmeles* damages the tubers (Emehute et al. 1998). The nematode *Scutellonema bradys* (Steiner and Lehew) damages the tubers by forming superficial lesions, and this facilitates entrance of fungi. Chlorosis, vein-banding, flecking and leaf pluckering in *D. alata* indicate infection by the water yam virus (Thottaphilly 1992) while the yam mosaic virus was isolated from *D. rotundata* (Thottaphilly 1992) and *D. alata* (IITA 1993). In cases of severe infection, the whole plant appears stunted. Storage rots are caused principally by *Botryodiplodia theobromae* (Emehute et al. 1998), *Penicillium oxalicum*, *P. sclerotigenum*, *Aspergillus niger, Rhizoctonia solani* and *Fusarium oxysporum* (Ikotun 1983).

Climate and soil also affect yam production (Orkwor and Asadu 1998). Idah, an ancient town in Igala Kingdom in Nigeria, bordered by the River Niger produces about 20% of yams consumed in the country. However, in 2012, homes and farmlands were washed away according to the All Farmers Association of Nigeria (AFAN) and crops worth more than N400 million were lost in nine local government areas of Kogi state (Patrick 2012). At the other extreme, desertification is fast encroaching on Lake Chad basin of Northern Nigeria where farming activities have virtually ceased. The recent flood has been attributed to changes in rainfall pattern due to climate change, which is a serious challenge to agriculture and livelihood in sub-Saharan Africa. This is because agriculture is mostly rainfed in contrast to advanced countries where irrigation and out-of-season farming is common place and coping with climate change more practical. Consequently, food security of millions of people, whose lives rely on these staple, is also endangered.

Conservation of Yam GR

Safeguarding genetic resources of yam is critical for tackling plant biodiversity losses and ensuring their sustainable conservation and use as raw materials for crop improvement for global food security.

The conservation and use of yam GR follows the main policy development that impact the conservation of plant genetic resources in genebanks and lies within the context of availability and distribution of germplasm. The policies arose from the adoption of various international agreements including the Convention on Biological Diversity (CBD), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), the International Plant Protection Convention (IPPC) and the World Trade Organization/Sanitary and Phytosanitary Agreement (WTO/SPS). In 2010, the CBD adopted the Nagoya Protocol on Access to Genetic Resources and Equitable Sharing of Benefits Arising from their Utilization, which has potential for impact upon germplasm exchange. Thus, the key principles genebank operations are the preservation of germplasm identity, maintenance of viability and genetic integrity, and the promotion of access. This includes associated information to facilitate use of the stored plant material in accordance with relevant national and international regulatory instruments. In general, it's about developing an efficient, effective, rational and transparent global system of *ex situ* conservation that provides optimal maintenance of germplasm and genetic integrity in gene banks, thereby ensuring access to, and use of, high quality germplasm of conserved plant genetic resources.

Untill now, the trend of biotechnology was to increase crop yield of clonal crops by propagation of elite/improved genotypes. In the context of climate change however, focus should be put on the maintenance of the adaptive potential of clonal crops by the understanding and the improvement of their mixed clonal/sexual reproductive system. This, of course, needs to take advantage of standardized farmer knowledge on sexual reproductive biology of clonal crops.

Vegetatively propagated crops like yam produce recalcitrant or intermediate heterogeneous seeds in few numbers and require a long life-cycle to generate planting materials. Consequently, they cannot be conserved as orthodox seeds and require other methods of conservation. Yam is conserved as live plants in field gene banks, as potted plants in enclosed structures or different plant parts are conserved into *in vitro* either in slow growth (medium-term) or cryopreservation (Long-term). DNA or pollen bank are also other conservation methods to maintain genes. The collection, conservation and duplication of yam has to be prioritized according to the repository size, resources and the material type (local landraces, introduced

accessions, improved and breeding material). In case of large collection, a core collection can be defined with high priority in terms of availability for evaluation and breeding.

In situ, on Farm/Field Conservation of Yam GR

The first conservation system for yam is the *in situ* conservation, especially for local and crop wild relatives. However, this was highly under-documented and inadequately managed. A small portion of it was maintained in gene banks although *in situ* conservation is an important potential contributor to the overall conservation effort (Brush 1991). The yam collections are badly represented in protected areas and the global priority genetic reserve locations for wild relatives of yam are located in West Africa. The relevance of *in situ* conservation is about the direct involvement of farmers/ smallholders, combining socio-cultural and ecological factors needed to understand characters and processes for the management of genetic diversity. For example, West African yams' genetic diversity management is a recombination between wild and cultivated forms which is an ongoing process that farmers manage (Hamon et al. 1995).

The procedures of yam field bank must be based on the maintenance of general principles like germplasm identity, purity and health, its reproductive capacity, its documentation availability and security. Yam is principally conserved vegetatively in field gene banks.

Agronomic parameters are important for yam field banking. Weed control is one of the most important factors. The effect of weed competition was observed to be responsible for the high yield loss, both the number and size (tuber weight), especially in drought environment (Wall and Friesen 1990). On white guinea yam (*Dioscorea rotundata*) field, chemical control gave better results in term of yield than hoeing on Congo grass (*Imperata cylindrica*) weed (Chikoye et al. 2006).

The major challenges of field conservation of yam are pests and diseases, mislabelling and duplication leading to germplasm losses. These obstacles have been major problems for international exchanges for diversification and global agriculture, especially for such root and tuber crops which are vegetatively propagated. This is due to the risk of spreading plant pathogens like virus, fungi, bacteria, phytoplasma and other diseases caused by microbes as well as insects and nematodes. However, many studies were carried out to explore the control of these clonal crop aggressors. The development of new and improved disease control method for viral diseases of vegetatively propagated staple food crops have to take into account the evolutionary response of the virus itself, through its biology and transmission (Van Den Bosch et al. 2007).

In vitro (tissue culture) Slow Growth Conservation and Cryopreservation

To support safer and longer conservation of yam genetic diversity for sustainable utilization, *in vitro* slow growth conservation and cryopreservation are the best methods. Biotechnological approaches will help to address future economical and environmental demands on yam (Pilatti et al. 2011). Conserving true seed in seed banks and cryobanks provides an additional safeguard against the risks (e.g., loss due to disease, climate change) of field conservation and permit the long-term conservation of a wider genetic base which offsets the labour and space intensive costs of conserving in the active growing state. Yam pollen storage at 0% relative humidity and –5ºC for up to one year (Akoroda 1983) and at –80°C for more than two years (Daniel et al. 2002) were reported. Pollen storage using the wet–freeze procedure was recommended for the conservation of haploid gene pool of yams in base collections (Daniel et al. 1999). However, yam seed conservation can only be applied to female plants while pollen conservation is only applicable to male plants and non-flowering genotypes can only be conserved vegetatively (Balogun 2009).

Slow growth conservation leads to the reduction of loss risks associated with the field banks, and constitute a viable alternative to complement and reduce the large size required for field banks. Yam gene banks around the world have *in vitro* tissue culture facilities as a complementary conservation system, giving the possibility to clean the germplasm from diseases and pest via meristem culture and/or thermo-treatment. This conservation method requires technical expertise, facilities and operating budget. They are generally more economical and less risky in a long-term perspective; as compared to field collections. Plant tissue culture is a powerful tool for safer and faster way to multiply large quantity of material for distribution, duplication in other gene banks 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/necroses are visible (Balogun 2009). The *in vitro* slow growth conservation of yam needs small quantities of material and allows longer duration between two regenerations or subcultures. The principle is to place the *in vitro* plantlets under slow growth conditions, through growth adaptation to cultural growth factors (light, temperature, culture medium, growth retardants). The *in vitro* conservation procedures details are available in IITA Manual (Dumet et al. 2007), also accessible at www.iita.org (IITA website).

Yam conservation is also done *in vitro* for safety duplication in some gene banks, using slow growth conditions. Apical and axillary buds or nodal cuttings are mainly used as initial explants for *in vitro* culture,

which increases the possibility to initiate pathogen-free yam plantlets. Yam conservation duration under *in vitro* slow growth conservation varies according to species/genotype. Most of *D. alata* and *D. rotundata* can be stored up to 2 years under 16–18°C, 12 hr light a day, as described in IITA yam *in vitro* processing manual (Dumet et al. 2007). Taylor (1996b) reported yam conservation duration of 9–12 months at 20°C and Zamora and Paet (1996) only one year at 25–28°C for different yam species.

Cryopreservation, almost systematically associated with *in vitro* conservation, is another conservation method for yam germplasm. It is about the plant material maintenance at ultra-low temperature (in liquid nitrogen at –196ºC) using cryogenic techniques. At such low temperature, plant cell 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 longer term perspective. Cryopreservation is the most reliable technique for long-term storage of plant genetic resources (Popov et al. 2005). It avoids the disadvantages of irreversible loss of totipotent competencies caused by *in vitro* ageing process (Benson 2008), time and labour consumption. Many studies confirmed that it is economically more competitive compared to other conservation systems (Harvengt et al. 2004, Reed et al. 2004, Keller et al. 2008). Cryopreservation helps to overcome many of the *in vitro* maintenance disadvantages such as labour-intensive subculturing, potential elimination of fungal and bacterial contaminants and somaclonal variation. It also ensures the safe long-term conservation of genetic resources. Thus, cryoconservation techniques have been increasingly used for Long Term Storage (LTS). In the last 25 years, several cryogenic techniques have been developed, especially those based on vitrification method (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, preculture-dehydration, and encapsulation/vitrification. Therefore, the main requirement for using cryopreservation method is that it should be simple, economical, reproducible and should allow relatively high regrowth rate (Leunufna and Keller 2003).

Various cryopreservation processes were described in the past for yam (Mandal et al. 1996, 2000, Malaurie et al. 1998, Leunufna and Keller 2005) with various levels of success depending on accessions and approach. To date, there is no generic process for cryopreservation of yam genetic resources.

Yam cryopreservation trials were already carried out using separate vitrification methods (Leunufna and Keller 2003, 2005, Gonzalez-Arnao et al. 2007) or encapsulation-dehydration (Malaurie et al. 1998). A comparative study between vitrification, encapsulation-dehydration and encapsulation-vitrification methods was done on yam apices by Mandal (2000).

Yam cryopreservation protocol setting is ongoing at IITA. High variability was observed in the ability of meristems to recover after exposure to liquid nitrogen, from one accession to another, within and across species (Dumet et al. 2012). However, the essence of the yam collection lies in its diversity. Somehow, the diversity of accession performance during the cryopreservation process echoes the diversity of the entire collection. The more diverse a collection, the more valuable it is, and the more likely adjustments will be needed to cryopreserve successfully the entire collection.

Propagation of Yam

In Africa, yam farmers still depend on the informal seed sources, including farm-savings from previous year (self supply), local markets and neighbours leading to use of poor quality seeds that accelerates build up of seed-borne diseases. This is because certified seeds are scarce. An efficient, cost-effective propagation system must therefore be developed (Asiedu et al. 1998, Quin 1998).

Traditional yam propagation has been done by field planting of whole tubers or large pieces ("sett") of between 200 to 500 grams. Setts may be pre-germinated in moist sawdust or coconut coir and more recently, carbonized rice husk. Thus, a large quantity of otherwise consumable tubers is reserved for planting yearly and this causes scarcity of propagules (Okoli et al. 1982), which alone, accounts for 50% of production costs (Nweke et al. 1991, Akoroda and Hahn 1995).

The "milking" technique (Okigbo and Ibe 1973) is also used, where tubers are harvested two-thirds into the growing season without destroying the root system. This provides early yam for home consumption and market. The parent plant then regenerates fresh small tubers from the corm at the base of the vine which are used as planting materials for the following season. This system therefore logically doubles the propagation ratio at the least relative to traditional method. These multiplication ratios are still very low (traditional: 1:6; Milking: 1:12), relative to other staple crops like cassava (1: 10) and some cereals (1:200) (Mbanaso et al. 2011). This relatively low multiplication rate reduces production and also delays the release of improved varieties. True seeds are also used in some cases when found on vines but not all genotypes flower. In addition, plants can be obtained from sexual seeds but the produced tubers are small compared to those from plants raised from tubers, due probably to small amount of stored food reserves in the seed (Okonkwo 1985).

The modified minisett technique (Ikeorgu et al. 2000, 2001, 2003, 2007, Kalu and Erhabor 1992), has reduced the production cost of yam (Okoli et al. 1982, Otoo et al. 1987) but the rate of adoption is still low (Kalu and Erhabor

1992). Increasing contact with extension workers, education, income and farm sizes of yam farmers were however suggested for a higher adoption (Nlerum 2009). In the vine rooting technique (Acha et al. 2004, Kikuno et al. 2007, Agele et al. 2010), about 20 cm long 3-node vines are rooted in carbonized rice husk, transplanted to the field to produce minitubers after 8 months. However, response varied among genotypes and percentage rooting and survival should be increased. To determine the propagation ratio of this technique, the number of nodes available per plant three months after planting should be determined. The differential response of genotypes may be a consequence of their being at different physiological stages at the time of vine collection since this was not pre-determined. Growth stages of yam, at which plant growth regulators were applied, were reported to affect rate of microtuber production *in vitro* (Balogun 2005). Use of histological techniques in determining growth phases of specific genotypes should be explored rather than the time after planting or sprouting since crop growth durations differ among genotypes. Multiplication rates are doubled using the partial sectioning technique (Nwosu 1975), but enormous manpower is needed for frequent examination and digging out to excise sprouted tuber sections for field planting. Also, the layering technique is unsuitable for farm use although applicable to some genotypes (Acha et al. 2004). Irrespective of the propagation technique, tuber dormancy, the programmed inability for growth in various types of plant meristematic apices, often in spite of suitable environmental conditions (Lang 1996) occurs in yam for about 5 months after harvesting and this limits out-of-season production (Craufurd et al. 2001).

Tissue culture techniques have been used for yam multiplication. Organogenesis from pre-formed meristems (Malaurie et al. 1995a, 1995b) have been reported in *D. zingiberensis* (Chen et al. 2003), shoot organogenesis from immature leaves (Kohmura et al. 1995) and roots (Twyford and Mantell 1996) of *D. opposita*, shoot (nodes) culture and microtuber formation in *D. composite, D. rotundata* and *D. alata* (Alizadeh et al. 1998, Balogun et al. 2006, John et al. 1993, Salazar and Hoyos 2007, Ovono 2007). Tuber pieces were reported not to produce *in vitro* plantlets in *D. rotundata, D. trifida* and *D. cayenensis* (Mitchell et al. 1995) while it did in *D. alata* (Fosto et al. 2013). Meristem culture combined with heat therapy has been successfully used to produce virus-tested plantlets, which are used in rapid multiplication of superior clones (Mantell et al. 1980, Ng 1984, 1992). This technique ensures that the viral inoculum is not passed on to subsequent generations. Gibberellin inhibitors were reported to induce multiple shoots from nodal explants in *D. oppositifolia* and *D. pentaphylla* (Poornima et al. 2007) and numerous axillary nodes in *D. alata* and *D. rotundata* (Bimbaun et al. 2002, Balogun 2005). Younger and more vigorous mother plants had higher rate of micropropagation *in vitro* (Mitchell et al. 1995a).

The use of synthetic seeds (Standardi and Piccioni 1998) for yam propagation is a possibility, although it will require conversion. This is because shoots and buds do not have root meristems and they must regenerate roots in order to be able to convert (Piccioni 1997). Synthetic seeds can be used to time production cycles in micropropagation laboratories if the development of the plant could be properly directed towards proliferation and rooting. In contrast, somatic embryos are bipolar structures and have been reported in *D. floribunda, D. composita, D. alata* and *D. bulbifera* (Ammirato 1978, 1982). Recently, (Padron et al. 2011) somatic embryos were induced from leaf tissues of *D. rotundata* in medium containing 2.4-D and incubated in darkness. However, low induction frequencies (<30%) were recorded and protocols will have to be optimized. In *D. alata* and *D. opposite*, embryogenic cell masses were induced from root explants in liquid MS supplemented with 2.4-D and cultured in light (Twyford and Mantell 1996, Nagasawa and Finer 1989). Germination of somatic embryos of *D. alata* increased in the presence of GA3 (Deng and Cornu 1992, Twyford and Mantell 1996). Plantlet recovery from somatic embryos of *D. rotundata* was enhanced at 4.5% sucrose but not affected by benzylaminopurine (Okezie et al. 1994, Pandro et al. 2011). These reports point to probable genotype-dependent protocol for yam embryogenesis.

Meristem and/or shoot tip culture combined with thermotherapy (Kartha and Gamborg 1975) cleaned cassava plantlets of leaf distortion symptoms. Cryotherapy (Wang et al. 2009) has also been shown to be capable of eliminating some viral diseases. However, this is yet to be integrated into the operational seed distribution system. It is also possible to increase multiplication rates using advanced tissue culture technology like temporary immersion systems.

For yam, low multiplication rates limit the use of *in vitro*-produced, virus-tested plantlets in conventional tissue cultures (about 1:4; Malaurie et al. 1995a,b, Medero et al. 1999, Chu and Ribeiro 2002, Borges et al. 2004, Ondo et al. 2007) in addition to losses during transplanting. In germplasm transfer where conditions of transit are unpredictable (Ng 1988), losses are encountered. Microtubers produced from *in vitro* plantlets were therefore suggested to be adequate for conservation (Balogun 2009) due to their hardiness, small size and easy establishment in the soil. In addition, they can be kept for a longer period due to microtuber dormancy (Ng 1988). Plant raised from microtubers produced more tubers on the field than plants from tuber crowns (Jova et al. 2011). This will not only facilitate international germplasm exchange, but also provide excellent means of storage. However, research is needed in the area of further increasing the size of microtubers, control of microtuber dormancy and applicability to more of the economically important genotypes.

The use of temporary immersion bioreactor systems (TIBs) will reduce culture losses by contamination from handling due to automation of the system while optimizing the rate of plantlet and microtuber production and sprouting (Jova et al. 2011, Watt 2012). In TIBs, growth is enhanced (Escalona 2006) since there is lack of continuous immersion in liquid medium, contamination is controlled while aeration is timed. In addition, microtubers from TIBs can be grown on the field and used in original seed production programmes. Due to enhancement of growth in TIBs, it can be explored for the production of medicinal secondary metabolites like yam steroidal diosgenins (Raju and Rao 2012, Tripathi and Tripathi 2003) as done for fenugreek (Sharareh 2011). Shoots grown in TIBs had enhanced growth and the leaves had higher photosynthetic pigment content than other techniques (Jova et al. 2011, 2012, Cabrera et al. 2011).

Photoautotrophic propagation (PAP) is another system that should be tested for yam. In PAP, explants are directed towards autotrophy while in culture by reducing or completely substituting sucrose (which directs towards heterotrophy) with carbon dioxide. Plantlet growth is enhanced in photoautotrophic more than heterotrophic conditions if environmental control is adequate (Hazarika et al. 2003, Xiao 2004, Afreen 2005). However, after about 4 weeks, plants may stop responding to carbon dioxide and growth is reduced due to slight closing of the stomata on the bottom of the leaves as plants sense high CO_2. Use of CO_2 enrichment for 2 weeks, then a week off intermittently will ensure higher yields as the plant continuously seeks as much CO_2 as possible (Andrew 2002). It may be worthwhile to investigate the performance of *in vitro* yam plantlets in a combination of TIBs and PAP. In potatoes, immense work has been done on PAP and photomixotrophic propagation (Mohamed and Alsadon 2010).

Aeroponics, being used in potato propagation (CIP 2008), is yet to be reported for other root and tuber crops like yam. In the case of potato, yield in aeroponics was 14–18 times greater than from the conventional methods (Badoni and Chauhan 2010, Muthoni et al. 2011, Chiipanthenga et al. 2012). Pumps are used to spray or produce a mist of nutrient solution around the root zone using micro-nozzles. Interplant root contact is minimal and this aids sanitation while the soilless technique avoids soil-associated plant pests. The system is a much higher aerated environment than traditional hydroponics and this reduces the risk of microbial contamination which limits productivity in tissue cultures. In addition, the technique has proven to be an efficient system for use of biofertilizer, e.g., growing vesicular arbuscular micorrhizal fungi inoculum without a physical substrate (Hung and Sylvia 1988) which has the potential of reducing the use of inorganic fertilizers. Irrespective to the propagation technique used, it is necessary to

determine the relative importance of clean materials, percentage survival after transplanting, cost, and multiplication ratio in deciding the technology to adopt.

Biotechnology for Yam Improvement

Smallhold farmers need access to innovative ways of coping with production constraints of yam and cassava. Selection and breeding for genotypes with high nutrient use efficiency, nutrient profile and adaptation to abiotic factors (drought, flood, etc.) in addition to adequate control of duration of yam tuber dormancy to achieve year-round production is indispensable for smallhold farmers to produce optimally.

The creation of genetic variations, followed by selection and identification of those that are desirable remain the basis of viable improvement programmes. Yam genotypes exhibit wide variations (Ng and Ng 1997) which provides a broad germplasm base for selection and breeding when conserved. Njualem et al. (2007) reported farmer/researcher participatory evaluation of 53 local landraces of yam in the Cameroon (Otoo 2003) while Oke et al. (2013) reported variations in pasting of starch from *D. alata* genotypes. In terms of duration of dormancy, some genotypes of *D. rotundata* from the drier area of the savannah keep for longer time (can be stored for 3–4 months and sometimes for longer periods (Coursey 1967).

Conventional yam hybridization (Lopez et al. 2012) breeding involves production of botanic seeds from bi-parental crosses or in poly-cross fields among genotypes. This is followed by seedling evaluation and selection in nurseries, and identification of superior genotypes in clonal trials. Consequently, populations developed for specific traits are improved over years, principally through recurrent selection. In collaboration with the National Agricultural Research Systems, this method has led to the release of 16 high-yielding, disease-resistant cultivars and good organoleptic attributes in Nigeria and Ghana in the last decade (Lopez et al. 2012). However, this method takes 6–9 years and is from intraspecific crosses. Production of haploid plants through anther culture of yams will shorten the breeding time by doubling haploids and early selection. There is a great challenge in inter-specific hybridization due to lack of synchronization of flowering and cross-compatibility, especially in crossing either of *D. rotundata* or *D. cayenensis* to *D. alata.* Flowering also varied with season and location (Hamadina et al. 2009). Flowering was induced in tissue culture of inflorescences of *Dioscorea zingberensis* C.H. Wright (Yang et al. 2009), which if developed for economically important genotypes will allow for location-independent, *in vitro* screening of flowering responses among genotypes. Biotechnology can overcome these limitations in the area of development of protocols for embryo rescue for interspecific crosses (Amazue 2009)

genetic transformation and protoplast fusion. Genetic transformation (Tor et al. 1993) using a biolistic particle gun to deliver DNA into intact yam cells of *D. alata* resulted in the recovery of the transformed cell. It is however necessary that protocols for successful regeneration of cultivated yams through somatic embryogenesis is developed for field evaluation of transformed plants.

In creating new genetic variations, mutation breeding was explored at the National Root Crops Research Institute and putative mutants were selected (Nwachukwu and Obi 2001). Exploiting the use of site-targeted mutagenesis (Kunkel 1985, Suprasanma et al. 2012) will speed up the mutation breeding process. Transposable elements (TEs) (McClintock 1950, Kidwell 1992, Schnable and Peterson 1988, McCarty et al. 2005) if found in yam may facilitate genetic modification for desirable traits. The TEs can be used to generate insertion mutations, map them and facilitate gene cloning and generation of transgenic plants. Although TEs (P elements) were used to modify genes in Drosophila by inserting into developing embryo *in vivo* (Rubin et al. 1982, Spradling et al. 1982), immature embryo/endosperm culture may also have a role to play in the genetic modification of yam using transposable elements. Somatic embryogenesis can be explored in generating somaclonal variants with desirable traits.

Identification of molecular markers that are linked to desirable traits will facilitate selection in breeding programmes. However, these require mapping populations. In yam, only two mapping populations were reported for each of *D. alata* and *D. rotundata* until 2003 (Mignouna et al. 2002a, 2002b, 2003). A suitable marker system is also being developed at the IITA for *D. alata* and *D. rotundata*. In 2011, Alieu and Asiedu reported 3 F1 full-sibs mapping populations in *D. rotundata* for virus disease, multiple tuber production, cooking quality or tuber texture and 4 in *D. alata* for anthracnose disease, cooking quality and oxidation. However, sizes of the mapping populations reduced due to plant death and tuber rotting. It was reported from the sequence repeat marker analysis that the parental crosses were successful and the progeny were true hybrids. Sequencing of the Dioscorea genome has been initiated (Bhattacharjee et al. 2012), conclusion of which should fast-track genetic improvement of yams by application of molecular techniques in marker-assisted selection and site-specific mutagenesis, among others.

In vitro screening will enhance selection for desirable traits such as tolerance to abiotic factors, disease resistance and production of secondary metabolites. In fenugreek, Rezaeian (2011) found higher diosgenin content in calli produced from leaf than root or shoot explants, such that improving callus production efficiency in medicinal yam (Gomez et al. 2004) for use

by pharmaceutical companies may be worthwhile. Yam grown in TIBs can be used to investigate cellular pathways and processes (Ivanov et al. 2012, Tripathy and Tripathy 2003) as in exo/endogenous control of tuber dormancy by stage-wise application of growth factor.

Conclusion

Yams are in urgent need of research for the optimization of protocols for sustainable conservation, formal seed systems, efficient micro/macro-propagation and genetic improvement. Biotechnological approaches of *in vitro* medium-term storage and cryopreservation (long-term conservation) have increased efficiency of conservation of yam genetic resources. However, it is necessary to increase the genotype inspecificity, duration of storage and percentage survival after recovery, especially for cryopreservation. In improving production and propagation rates of disease-free plantlets, many aspects of conventional tissue culture like meristem culture, shoot tip culture, direct organogenesis combined with thermo or cryotherapy has been reported for yam. It is necessary to develop protocols toward more automation and macropropagation systems, ranging from temporary immersion bioreactors through aeroponics to photoautotrophic propagation so as to reduce transplanting losses and boost yam seed system for economically important genotypes. Marker-assisted selection, embryo culture/rescue, genetic transformation, genome sequencing have been reported but need to be optimized and concluded for yam. Investigations into the presence of transposable elements, site targeted mutagenesis, somatic embryogenesis and haploid plant production will be necessary to fast track genetic improvement. More importantly, research should focus on protocol setting and adaptation for the conservation, propagation and improvement techniques with respect to recalcitrant genotypes of yam.

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