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Review

Status and prospects for improving yam seed systems using temporary immersion bioreactors

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Yam production is constrained by scarcity of clean seed, pests, diseases and low soil fertility in the informal seed system, which is still operational, causing up to 90% yield losses. Although meristem culture can be effective for producing healthy seed yam, its use is limited by slow rate of regeneration and propagation in conventional tissue cultures. In most crops tested, temporary immersion bioreactor systems (TIBs) increased propagation rates. To determine the potential of TIBs in improving the yam seed system, 23 databases were consulted and three returned a total of eight publications with only 2 for *Dioscorea rotundata-cayenensis*. Both plantlets and microtubers can be produced in TIBs, which will facilitate production of quality breeder, foundation and certified seeds and fast-track genetic improvement and the evolution of a formal from informal seed production system. Control of contamination, direct use of field explants, culture of micro-explants like immature embryos and anthers, increasing the size of microtubers produced and standardization for various economically important yam genotypes are knowledge gaps that require immediate research attention. No report has put a cost on yam TIBs, but it will be necessary to use cost-effective TIBs to encourage integration public-private partnerships into emerging formal seed system.

Key words: Tissue culture, healthy seed yam, temporary immersion bioreactors, *dioscorea* spp.

CLONAL PROPAGATION AND BIOREACTORS

Traditionally, the multiplication of genetically identical individuals by asexual methods, otherwise known as clonal propagation, is achieved by cuttings, grafting, layering and tuber portions, among others. The use of tuber portions has been applied to yam for ages, preserving traits of selected genotypes. Micropropagation and clonal propagation which utilize plant tissue culture techniques in a closed, sterile container however occur

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Abbreviations: **TIBs:** Temporary immersion bioreactor systems; **NACGRAB:** National Centre for Genetic Resources and Biotechnology; **YIIFSWA:** Yam Improvement for Income and Food Security in West Africa; **USAID:** United States Agency for International Development; **NAS:** National Academy of Science; **NSF-PEER:** National Science Foundation-Partnership for Enhanced Engagement in Research.

within the laboratory environment. These methods are now being applied industrially for numerous crops (Yam and Arditti, 2009). In the case of yam, reports on micropropagation include organogenesis from pre-formed meristems, immature leaves and nodal culture, and microtuber formation (Balogun and Gueye, 2013). However, yam meristem cultures took more than 1 year to regenerate in some cultures. In conventional micro-propagation, frequent sub-culturing also increases labour costs while small size of culture container (hence nutrients) and insufficient aeration (Ziv, 1991) results in fragile plantlets (Ziv et al., 1998) and sub-optimal propagation rates. The need to improve on these systems caused the emergence of the bioreactor technology.

Bioreactor technology is an advanced tissue culture - an enclosed sterile environment provided with inlets and outlets for air flow under pressure. In most crops tested, better growth performance due to better aeration was reported compared to continuous immersion in medium. Several bioreactor systems have been successfully applied for cultivation of differentiated plant *in vitro* systems (Steingroewer et al., 2013), including liquid-phase (stirred tank, airlift and connective flow bioreactors), gas-phase, hybrid bioreactors, and Tem-porary Immersion Bioreactor systems (TIBs). The TIBs' are uniquely able to provide lower level of shear stress and significantly reduce shoot hyperhydricity culminating in increased productivity. In TIBs, there is timed immersion of plant tissues in liquid medium to allow for culture aeration, which circumvents limitations associated with conventional tissue culture.

THE MAJOR CHALLENGE OF SEED YAM PRODUCTION

In spite of its status as a staple and source of livelihood in West Africa, (FAO, 2013), yam production is still sub-optimal. The major pre-requisites for enhanced yam productivity and storability are high quality seeds of improved genotypes. However, quality seed yam is scarce. This is a consequence of the slow rate of propa-gation (less than 1:10 compared to 1:200 in some cereals) (Mbanaso, 2011) which is also vegetative, encouraging a build-up of an array of fungal, nematode, bacterial and viral diseases and pests.

The situation is further complicated by lack of a formal seed system which has a functional regulatory structure, such that there is scarcity of certified and quality declared seeds. Yam production therefore revolves quite repeatedly, around the use of mixed, undelineated genotypes, pre-infected seed yam and farmlands, causing a build-up of an array of diseases (Winch et al., 1984). This leads to 50 to 90% yield reduction. Consequently, there is a significant demand for clean seed in a market driven seed system and 50 to 70% of production costs (Nweke et al., 1991; Agbaje et al., 2005; Ironkwe, 2005; Coyne, 2010) is spent on purchase of seed.

Other constraints include shrinking land area due to flooding and desertification, which impacts soil fertility, high cost of labour in the absence of mechanization, high post harvest losses, tuber dormancy which prevents off-season production and uncontrolled sprouting after dormancy break which further causes storage losses. However, the challenge of seed scarcity is central to these constraints due to inadequacy of improved genotypes specifically adapted to prevailing challenges, frequently renewed without losing its quality. Global annual production was therefore projected to reach a plateau, having decreased by 11.5% in 2007 (Manyong et al., 1996).

EXISTING SEED YAM PRODUCTION SYSTEM

In the current informal seed system, farmers reserve up to half of the year's harvest for future planting, obtain seeds from fellow farmers or purchase from the market, in decreasing order of preference. Traditional yam propagation is by planting 200 to 500 gram setts, which takes a large portion of the harvest. In the "milking" technique (Okigbo and Ibe, 1973; Okoli et al., 1982), tubers are harvested two-thirds into the growing season without destroying the root system, providing early ware yam for consumption. The parent plant regenerates new small tubers used as seed yam for the following season. This system therefore doubles the propagation ratio relative to other traditional methods although, multipli-cation ratio is still very low.

In the modified minisett technique (Kalu and Erhabor, 1992; Okoli and Akoroda, 1995; Ikeorgu and Igbokwe, 2003; Ikeorgu et al., 2007) use of 25-80 g minisett has reduced the production cost of seed yam (Okoli et al. 1982; Otoo et al., 1987; Oguntade et al., 2010) but rate of adoption is low (Kalu and Erhabor, 1992). More recently, rooting of 20 cm long 3-node vines (Acha et al., 2004; Kikuno et al., 2007; Agele et al., 2010) produced minitubers of 50 to 600 g after 8 months giving a 1:22 propagation ratio.

Multiplication rates are doubled in the partial sectioning technique (Nwosu, 1975), where planted tubers are dug out and sprouted sections excised for field planting but labour requirement is enormous. The layering of vines into soil for tuber production while on the mother plant is unpractical for farm use although up to 1:80 propagation ratio is possible (Acha et al., 2004). All of these macropropagation techniques are genotype-dependent, have no provision for cleaning infected seed yam and tuber dormancy remains a challenge. Sexual seeds are only useful in breeding but not multiplication as the product is different from the parents due to outcrossing and are therefore not true-to-type (Okonkwo, 1985). However, the informal seed system preserves abundant

Table 1. Formal versus informal seed systems.

Item	Informal	Formal
New technology generation	No	Yes
Technology transfer/dissemination	No	Yes
Wide diversity of adapted genetic resources	Yes	Obtainable
Infrastructure for assessing quality seed	No	Yes
Quality control at all levels of seed production	No	Yes
Public sector involvement	No	Yes
Private sector involvement	Yes	Yes
Official recognition of impacts	No	Yes

Source: Extracts from Larinde and Ilboudo (2006); Wekundah (2012)

diversity of landraces, including wild types.

THE CONCEPT OF FORMAL SEED SYSTEM IN YAM

In contrast to the informal system which is based on family heritage in terms of experience, the bases of the formal seed systems are scientific research, new variety selections, field/laboratory seed control and testing which constitute the technology-based aspect while the economic and legal aspects involve production/marketing and rules/regulations, respectively. The vision is that the first 2 aspects can be handled by the public or private sector or a partnership between the two, while the legal aspect is governmental and public sector-managed. Consequently, the formal seed system is all about following the rules: quality control and certification at all stages, the increase in quantity of breeder to foundation seeds and then certified seeds that can be distributed to farmers to ensure the genetic and physiological quality (includes disease and sprouting status) of the seeds. In Nigeria for example, the National Centre for Genetic Resources and Biotechnology (NACGRAB) is responsible for registration of new varieties, involving aspects of confirmation of genetic purity. To date, 19 researchers' varieties (improved) of yam have been officially released and registered by NACGRAB with no landrace varieties attributable to farmers' selections. The National Agricultural Seed Council (NASC) has the mandate for quality control and certification at all levels of breeder, foundation, certified seed production. In partnership with stakeholders, YIIFSWA is facilitating the development and dissemination of quality management protocols in addition to robust and cost-effective virus diagnostic tools which will fast-track establishment of the formal seed system. For yam, national standards are adopted but yet to be applied by NASC inspectors as the formal seed yam system is not yet in place.

FORMAL VERSUS INFORMAL SYSTEM

In as much as the formal seed system has many advantages, many of the quality control aspects are still not being applied for yam. Checking the sprouting ability status of seed yam is a challenge as even different tuber portions may have different sprouting abilities. Use of small whole tubers as seed will facilitate seed quality testing in addition to knowing what the standards are for field delineation of real genetic differences versus nutrient deficiency and disease symptoms (O'Sullivan, 2006, 2010). These overlaps have implications for high variations within yam somatic cells.

A formal seed system, involving adequate monitoring, is adequate for cross pollinated crops which produce seeds different from the parent at each generation. The vegetative propagation of yam is normally expected to produce uniform varieties as with self-pollinated crops for which genetic uniformity is high and informal seed system can thrive, which has helped yam over the years. However, variations are encountered within a yam variety. The genetic basis and rate of somaclonal variation in yam need to be determined. If yam is a transition between genetic uniformity and variation, it will be necessary to combine elements of the formal with informal seed systems. It should be noted that one of the consequences of the formal seed system which is market-driven, is a narrower genetic base resulting from need for genetic purity and uniformity while that of the informal is a wider genetic base due to community drive (Table 1). A good quality, locally adapted farmer-preferred variety is certainly better than an improved, non-adapted and over-selected variety (Otoo, 2003)'.

TISSUE CULTURE AND ITS ROLE IN EVOLUTION OF THE FORMAL SEED SYSTEM

The change from informal to formal seed system will require novel technologies, especially in terms of rapidity of production, certification of seeds as disease-free, propagation and distribution of 'CLEAN' breeder/-foundation/ certified seed yam in large quantities. Such technique should be applicable at most, if not all stages of development and propagation of disease tolerant or

resistant varieties that are adapted to targeted agro-ecologies.

Tissue culture has, and is still being explored for yam propagation. The basis of this is the potential of every plant cell to regenerate into the complete plant. In improving yam micropropagation rates, there are reports on many aspects of conventional tissue culture: organogenesis from pre-formed meristems (Malaurie et al., 1995a, 1995b) 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. composita D. rotundata and D. alata* (Alizadeh et al., 1998; Balogun et al; 2006; John et al., 1993; Salazar and Hoyos, 2007, Ovono et al., 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* (Fotso et al., 2013). In addition, aseptic conditions are observed and this cleans the plant of fungi, nematodes and bacteria. Meristem culture combined with heat/cold/chemo therapy is the only technique that is able to clean infected plants from viruses to date, followed by rapid multiplication of superior clones (Mantell et al., 1980; Ng, 1984, 1992; Sengupta et al., 1984, Mitchell et al., 1995). This technique ensures that the viral inoculum is not passed on to subsequent generations.

Conventional tissue culture employs manual introduction into culture vials. However, the slowness of yam propagation *in vivo* is also witnessed in *vitro* cultures and a 1:4 multiplication rate (Chu and Ribeiro, 2002; Borges et al., 2004; Ondo et al., 2007) is reported averagely for yam tissue cultures. Meristem cultures took more than 1 year to regenerate in some cultures. This low multiplication rates limit the use of *in vitro*-produced, virus-tested plantlets in conventional tissue cultures in addition to losses during acclimatization and trans-planting. A high level of contamination is encountered in the culture of tuber pieces, probably due to high load of endophytes in the explants.

There is also limitation of frequent sub-culturing which increases labour costs, limitation to size of culture container (hence nutrients), insufficient aeration, hyper-hydricity and vitrification, a stress condition in tissue-cultured plants, manifested mainly as abnormal leaf functioning (Ziv, 1991; Ziv et al., 1998). Protein- and photo-syntheses, gas exchange, cellulose and lignin synthesis and ethylene production are affected, resulting in fragile plantlets (Ziv et al., 1998) and sub-optimal propagation rates.

TEMPORARY IMMERSION BIOREACTOR SYSTEMS FOR YAM PROPAGATION

Among other factors, culture aeration combined with automation has been proposed to increase productivity and reduce cost in conventional tissue cultures due to significant reduction of contamination and cost of labour respectively; in addition to faster multiplication rate. This suggested a role for temporary immersion bioreactor systems (TIBs) (Cabrera et al., 2011; Watt, 2012).

A bioreactor is an enclosed, sterile environment which is provided with inlets and outlets for airflow under pressure and utilizes liquid medium (Watt, 2012). TIBs are types of bioreactor used for differentiated plant tissues. Different designs of TIBs exist, but most common are the twin flask types, having 2 containers, one for the medium and the other for the cultures (Adelberg and Simpson, 2002). Another type of TIBs is the recipient for automated temporary immersion (RITA) (Alvard et al., 1993) in which the upper container containing the plant is linked to the lower compartment containing the medium and internal pressure regulates the movement of medium up or down such that immersion of cultures can be timed. There is also the Bioreactor of Immersion by Bubbles (BIB) (Soccol et al., 2008) where nutrient and air is provided to cultures by bubbling. In all these cases, the cultures are immersed in the medium in a timed manner, in terms of frequency and duration of immersion to allow for aeration. TIBs require interplay of plant physiology, chemical and physical sciences since components like air compressor for air flow, silicon tubing connections, membrane filters and remote monitoring may be involved. In TIBs, growth is enhanced (Escalona, 2006) since there is lack of continuous immersion in liquid medium. Use of TIBs has been reported for potatoes, pineapple, apple, coffea, lemon grass, oil palm, eucalyptus, strawberry among others (Watt, 2012).

Among databases consulted (Table 2), Scirus, Springer and Google scholar returned a total of 8 reports on yam TIBs from Cuba, Japan, China and France. Four out of 8 reports were on *D. alata*, 1 on *D. fordii,* 1 on *D. opposita* and the last 2 on *D. cayanensis-rotundata*. Yam shoots grown in TIBs had enhanced growth and the leaves had higher photosynthetic pigment content than other techniques (Table 3, Jova et al., 2005, 2011, 2012; Cabrera et al., 2011), The exciting aspect of use of TIBs for yam propagation is that both plantlet and microtuber production are possible (Balogun, 2009), although sprouting was higher in bigger microtubers. This implies that large enough microtubers can be planted directly on the field as reported for *D. alata*. Since *D. rotundata* is the most economically important species in the West African yam belt, it is critical that attention be directed towards its propagation in TIBs and this system be incorporated into the seed system.

PRODUCTION OF CERTIFIED SEEDS

The most important thing in production of certified (breeder, foundation and commercial) seeds yam is that it is true-to-type as described by the breeder. The greatest challenge is that the existing yam diversity is a

Table 2. Databases with articles on yam propagation in temporary immersion bioreactors*

Database	Article
Scirus	2
Springer	1
Social Science research network	0
Worldcat	0
Proquest Dissertation and Thesis database	0
JSTOR	0
AJOL	0
SciDevNet	0
Ideas	0
PubScience	0
Royal Tropical Institute	0
ISI Highlycited.com	0
Gogglescholar	7
Current Agricultural Research Information	0
Agris:International information system for the Agricultural sciences and technology	0
USDA National Aricultural Library	0
CGIAR library	0
Agricola	0
Eidis	0
Findarticles.com	0
Highwire press	0
Directory of Open Access Journals	0
Ingentaconnect.com	0

Although the table shows a total of 10 publications, the 2 articles in Scirus were among the 7 found in Gogglescholar'

Table 3. Multiplication rate of yam in conventional tissue culture and temporary immersion bioreactor system.

Product	Genotype	Conventional gelled medium	TIBs	Author
Plantlet	*D. alata*	5.7	10.1	Salazar and Hoyos, 2007
Plantlet	*D. alata*	2.2	4.1	Jova et al., 2005
	D. alata	4.4	8.0	Yan et al., 2011
	D. rotundata-cayenensis	Not compared	8.5	Jova et al., 2008
	D. cayenensis-rotundata	Compared for stem length, no significant difference (6.6)	6.3	Polzin et al., 2013
	D. opposita	Not compared	Not reported	Akita and Ohta, 2002
	D. fordii	2.4	5.0	Yan et al., 2011
Microtuber production (%)	*D. alata*	Not reported	2.8	Jova et al., 2012
	D. alata	6	47	Jova et al., 2005
	D. fordii	8.3	73.8	Yan et al., 2011

component of the existing informal seed yam system and they are not certified. Breeders therefore need to incurporate characterization of their varieties for certification at some stage of variety development for the end product to be confirmed as being same as the initial stock.

The role of tissue culture in this study will be cleaning of breeders' seed before production of foundation seed using meristem culture combined with one or all of the

Plate 1. Left: Seed yam tubers from field-grown plants at Ilushi market in Nigeria (Courtesy: Aighewi, Personal communication); Right: Yam microtubers from conventional tissue culture (Balogun, 2005).

therapies. It will be worthwhile to investigate the possibility of meristem culture in TIBs to overcome the slowness encountered in conventional tissue cultures and this is yet to be reported. The cleaned breeder seeds can in turn be multiplied in TIBs to increase initial stock ofbreeder seed yams, whether as plantlets or small whole microtubers. The challenge here with *D. rotundata* will be to experimentally determine optimum immersion frequency and duration, nutrient/hormone requirement, age of mother plant and number of medium renewals that will be optimum for the two (plantlet or microtubers) products.

If higher quality plantlets are the products of TIBs, losses due to transplanting will be considerably reduced and they can be directly transplanted into macropropagation systems like aeroponics but there might be a waiting time during the dormancy of the minitubers produced from transplanted plantlets. In cases where microtuber production (Plate 1, right) is the target of TIBs, it is important to increase the size and weight of the microtubers to enhance sprouting when directly planted on the field.

It was reported that *in vitro* microtubers do not go into dormancy if immediately put into fresh *in vitro* medium (Ovono et al., 2009) with the advantage that it can be ploughed back into the *in vitro* system for multiple sprout production. However, sprouting ability varied with stage of physiological maturity and size (Balogun, 2009). The NSF-PEER is on-going to standardize the stage of physiological maturity and size of microtubers in photoautotrophic TIBs among farmer-preferred varieties to control their dormancy. The smaller size of the two products and smaller space used is not only cheaper, but also an advantage for easier handling in disease diagnostics and germplasm distribution rather than plants or tubers in a large field or screenhouse.

ENHANCING BREEDING FOR TARGET TRAITS THROUGH TIBS

Ample diversity and efficient selection sieves constitute the core of yam improvement. In spite of wide diversity existing among yam genotypes (Ng and Ng, 1997) 19 yam varieties have been released in Nigeria (Lopez et al., 2012) but those resistant to specific diseases are yet to be identified. This slow rate of variety release (compared to in maize) is due to conventional yam hybridization breeding process, which is from intra-specific crosses, takes six to nine years with only one generation produced per year. In addition, flowering varies with season and location (Hamadina et al., 2009) and is irregular in genotypes, making successful hybridization unpredictable.

There is still 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.* Other options for creating new variations include genetic transformation, somaclonal variation and mutagenesis while embryo rescue is useful in inter-specific hybridization. This is however constrained by lack of protocols for yam regeneration via somatic embryogenesis. It will be worthwhile to test for ability to regenerate in TIBs since growth is enhanced.

In exploiting mutation breeding for yams, *in vitro* regeneration of plantlets from adventitious buds will facili-

tate production of solid mutants and avoidance of unstable chimeras. Also, yam grown in automated systems can be used to investigate cellular pathways and processes (Jova et al., 2011; Ivanov et al; 2012) as in control of yam tuber dormancy.

Although most landraces are farmer-preferred varieties, a continuously changing agroclimate and new strains of pathogens is a threat to optimum performance of landraces in a specific environment. The challenge will be to introduce disease resistance or environmental adaptation into specific landraces so as to reduce the menace of yield losses due to pests and diseases in yams (Asala et al., 2012). Efficient selection for resistance to toxins from pathogens is possible *in vitro* within minimal space in a controlled environment, as opposed to larger field space with risk of environmental spills.

It is advisable that TIBs be set up in plant protection/-germplasm health units as this will facilitate an understanding of the mechanisms of resistance. This includes *in vitro* screening for resistance to obligate parasites like nematodes which require continuous supply of inoculum, efficient inoculation and diagnostic protocols. There is no nematode-resistant variety of white or water yam to date (Claudius-Cole, Personal communication). 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) as done for Fenugreek (Rezaeian, 2011).

PRODUCTION OF QUALITY DECLARED SEEDS

Existing farmers' seeds in the current informal seed system stand a chance of harbouring large amounts of disease inoculums. Migration from an informal to a formal seed system will require gradual replacement with quality-declared seeds. Since the starter materials remain the current farmers' planting materials, there must be assemblage of the landraces, identification of different types, disease diagnostics followed by rapid multiplication of disease-free ones and therapy of disease-infected materials. Such rapidity of multiplication can be provided in TIBs, followed by macropropagation in aeroponic systems, further propagation, certification and distribution which will involve a public-private partnership. The beauty of TIBs is that it can also be private-sector managed, facilitating evolution of the formal seed system.

CHALLENGES OF TIBS

The time required to produce the starter stock to feed into yam TIBs is a major challenge. In previous reports on yam culture in TIBs, plantlets already established *in vitro* were used as starter stocks. Although initial *in vitro* establishment takes time, this method ensures reduced or no contamination, and increased survival of plantlets. It will be worthwhile to develop protocols that will permit the use of direct field explants especially for shoot organogenesis. Control of contamination will be critical as done for Eucalyptus and other crops (Thomas, 2004; Watt, 2002). To achieve this, a Hazard Analysis and Critical Control Point Protocol (HACCPP) should be developed for yam culture in TIBs.

Culture of small plant parts in TIBs is also a challenge. This includes use for somatic embryogenesis, meristem, anther and immature embryo culture as it will require supports that are inert, non absorbent or fibrous. In resistance breeding, use of TIBs may be limited to toxin-producing pathogens or obligate parasites like viruses. In the case of viruses, protocols for inoculating *in vitro* calli or other materials with virus for screening will have to be developed followed by an efficient regeneration protocol. In terms of cost, cheaper sources of culture vessels and power need to be explored. The "glass jar temporary immersion bioreactor" and the "horizontal disposable temporary immersion bioreactor" (''Box-in-Bag'') were developed for purposes of cost reduction for coffea cultivation (Ducos et al., 2008). With TIBs at the heart of yam seed systems, it will be necessary to standardize the protocols for different economically important genotypes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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