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Standard Review

Microtubers in yam germplasm conservation and propagation: The status, the prospects and the constraints

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The conservation of yam genetic resources using field genebanks, *in vitro* plantlets, pollen and seed storage are constrained by high losses and space requirements, maintenance cost and an irregular flowering, respectively. Microtubers produced from *in vitro* plantlets are proposed for conservation and propagation, as they have a longer shelf-life due to dormancy, and are also hardier and less bulky than plantlets. A lot of work has been done on microtuber production, especially the use of temporary immersion systems in production of larger, multiple microtubers. However, there have been different degrees of success, and, very few reports on microtuber dormancy. Also, research findings on post-sprout management and efficiency of microtubers relative to other systems in terms of cost, ease of handling and savings on time are sparse. These research gaps limit the practical use of microtubers in conservation and propagation. Future research should be on dormancy control and post-sprout management. A microtuber to microtuber cycle for the conservation and propagation of yam germplasm is proposed in this review, and the invaluable potentials of microtubers in these regards is emphasised.

Key words: Yams, *Dioscorea* species, germplasm conservation, propagation, microtuberization.

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INTRODUCTION

The yams, family *Dioscoreaceae*, genus *Dioscorea*, are staples (Hahn 1995) in many countries of the tropics where it provides 200 dietary calories daily to 300 million people (Coursey, 1967; Nweke et al., 1991; FAO, 2000). Yam production is however constrained by abiotic factors, pests and diseases (Emehute et al., 1998) and scarcity of propagules (Nweke et al., 1991). Tuber dormancy also prevents year-round production and this hampers productivity. In addition, uncontrolled sprouting after dormancy break causes tremendous storage losses (Asiedu et al., 1998; FAO, 2000; Craufurd et al., 2001). Selection for desirable traits for breeding of improved varieties and development of an efficient, cost-effective propagation system is thus crucial for sustainable yam production (Asiedu et al., 1998; Quin, 1998). To provide a broad germplasm base for selection and breeding, conservation of yam genetic resources must continue (Quin, 1998).

Plant tissue culture techniques of meristem culture combined with heat therapy have been successfully used to produce high-yielding plantlets tested to be virus-free which are not only conserved in *in vitro* genebanks but also used in rapid multiplication of superior clones (Mantell et al., 1980; Ng, 1984, 1992). However, the stress of transportation causes low survival rates during transplanting and germplasm exchange despite the specialized handling (Ng, 1988). There is also the need for frequent subculturing when plantlets show signs of deterioration. Microtubers produced from pathogen-tested, *in vitro* plantlets have been proposed as an addi-tional means of germplasm conservation and propa-gation (Ammirato, 1984). This is because they are less vulnerable to transportation hazards during germplasm exchange, less bulky and can be kept for several months due to microtuber dormancy (Ng, 1988; Balogun et al., 2004). They can also be easily established in the soil, not requiring acclimatization and transplanting (Ng, 1988; Ng and Ng, 1997). A lot of work has been done on yam microtuberization in recent times. This review discusses the status and current trends in the production and utilization of microtubers for germplasm conservation and propagation.

Other options in yam conservation and propagation

Conservation

The rich diversity among yam collections (Ng and Ng, 1997) calls for germplasm conservation to prevent their genetic erosion and enhance the use of these genetic resources in breeding programmes (Ng and Ng, 1994; Acheampong, 1996). Field conservation requires considerable space, maintenance and time while annual losses from collections, as high as 10% have been reported in field genebanks due to pest and disease problems, poor sprouting, unsatisfactory storage of tubers, drought and poor handling (Okoli, 1991; Acheampong, 1996; Taylor, 1996a).

Other options for conserving the yam genepool have therefore been investigated. These include seed conservation for a considerable period at low temperature and low seed moisture content (Daniel et al., 1999), pollen storage at 0% relative humidity and –5^{o}C for about one year (Akoroda, 1983) and at –80°C for more than two years (Daniel et al., 2002). However, seed conservation can only be applied to female plants while pollen conservation is only applicable to male plants while non-flowering genotypes can only be conserved vegetatively.

In vitro methods of plantlet storage at reduced temperature to slow down their growth rates, thereby extending the viable storage period, is also used for several species of yam. This serves as a duplication and backup to the field genebank (Zamura and Paet, 1996). At the International Institute of Tropical Agriculture (IITA) for example (Ng and Ng, 1997), axillary buds and nodal cultures are most frequently used for the rapid clonal propagation of meristem-derived, pathogen-tested plantlets in the establishment of an *in vitro* gene-bank under slow growth. This is however for short to medium term conservation, after which the plantlets are subcultured when signs of deterioration are visible. With this method, plantlet storage period of *Dioscorea alata* and *Dioscorea rotundata* could be extended to 1 - 2 years by reduction of the incubation temperature from 28 – 30°C to 18 - 22°C (Ng and Ng, 1997). In some cases however, storage period was only 9 - 12 months at 20°C (Taylor 1996b) and one year at 25 - 28°C (Zamora and Paet, 1996). In addition, the need for subculturing when deterioration signs are visible increases labour costs for the maintenance of an *in vitro* genebank.

The use of artificial (synthetic) seeds (Rao et al., 1998) in storage and exchange of yam germplasm has also been reported (Hasan and Takagi, 1995). Synthetic seeds are artificially encapsulated somatic embryos, shoots, or other tissues that are able to grow into plantlets after sowing under *in vitro* or *ex vitro* conditions (Standardi and Piccioni, 1998). The efficiency of using these artificially encapsulated propagules lies in their small size and relative ease of handling.

The alginate coat which protects the micropropagules makes them more tolerable than plantlets to the stress of transit during yam germplasm conservation and exchange (Hasan and Takagi, 1995). However, viability of the synthetic seeds decreased as the sucrose concentration in both the matrix and polymerization medium increased (Hasan and Takagi, 1995) while high levels of sucrose were toxic to the explants (Kitto and Janick, 1985; Uragami, 1993). An optimum sucrose concentration of 0.1 - 0.3 M was found suitable for keeping the micropropagules viable for up to 2 weeks during inter-national

germplasm transfer before direct trans-planting to soil (Hasan and Takagi, 1995). To achieve long-term storage of desirable elite genotypes, the use of artificial seeds for cryopreservation via encapsulation dehydration or encapsulation vitrification (Pennycooke and Towil, 2001; Wang et al., 2002; Tessereau et al., 1995; Ng and Daniel 2000; Ng and Ng, 2000) was reported.

Cryopreservation is a process where micropropagules are preserved by cooling to low, sub-zero temperatures, such as 77K or −196°C. However, yam genotypes differ in their response to cryopreservation protocols (Leunufna and Keller, 2003). *Dioscorea bulbifera, Dioscorea oppositifolia* and *Dioscorea cayenensis* seemed to be more able to withstand the stress imposed during the cryopreservation procedure than *D. alata*. In terms of survival, *D. oppositifolia* exhibited the highest rate, but only one-third of the surviving explants developed further. *D. cayenensis* showed a lower (not significant) survival rate than D. *oppositifolia*, but most of the surviving explants grew further. *D. bulbifera* also showed a similar survival rate to that of *D. cayenensis* and *D. oppositifolia*, but very low numbers of surviving explants regrew.

The report of Reed et al. (2001) also indicated an inconsistence with respect to the reproducibility of cryopreservation protocols from one laboratory to the other. Kyesmu (1998) reported a shoot recovery of 47, 85 and 91% for three different cultivars of *D. alata*, respectively, using the vitrification method. Using encapsulation-dehydration, Mandal et al. (1996) obtained survival as high as 64% and a recovery of 21.8% for *D. alata* and 26% survival with no recovery for *D. bulbifera*.

Other *in vitro* methods for conservation include embryo, callus and suspension cultures although their use is limited by lack of successful regeneration protocols.

Propagation

Traditionally, yams are propagated by planting whole tubers or large pieces weighing 200 g or more (Okoli et al., 1982). A sizable portion of otherwise consumable tubers are therefore reserved for planting yearly, and this leads to scarcity of planting materials. Multiplication ratio for seed yam production in the field is 1:10 compared to 1:300 in cereals. Planting materials alone constitute about 50% of production costs (Nweke et al. 1991, Akoroda and Hahn, 1995). Most farmers propagate yams by “milking”. In this technique, tubers are harvested two-thirds into the growing season without destroying the root system. This provides early yam for home consumption and market. There is regeneration of fresh small tubers from the corm at the base of the vine and these are used as planting materials for the following season.

The major constraint of planting materials to yam production is being tackled by the development of more efficient propagation methods (Orkwor and Asadu 1998). These include partial sectioning technique (Nwosu, 1975) and minisett technique (Okoli et al., 1982). Although the latter has significantly increased propagation rates (Okoli et al. 1982), it has been associated with less uniform and poor rate of sprouting when applied to white yam (George, 1990; Sreekantan et al., 1995; Craufurd et al., 2001). Although multiplication rates are doubled using the partial sectioning technique, it requires considerable manpower for the repeated examining and digging out of tubers to excise sprouted sections for field planting (Nwosu, 1975). In the vine rooting technique, either tubers did not develop due to early senescence of rooted vines (Acha et al., 2005), or small tubers are produced when applied to *D. rotundata* relative to other species.

Also, the layering technique is unsuitable for farm use due to rigorous procedures involved (Acha et al., 2004).

Research is needed in aerial tuber production, which was proposed as an alternative means of seed tuber production (H. Shiwachi, personal communication), although they are seldom produced in *D. rotundata*, unless induced by stem girdling (Okonkwo, 1985). In addition, plants raised from sexual seeds and the tubers produced are small relative to plants raised from tubers probably due to small amount of stored food reserves in the seed (Okonkwo, 1985).

The synthetic seed technology is also useful for the propagation of vegetatively propagated plants (Kumar, 1998) whose true seeds are not used or readily available for multiplication (e.g. yam and potato). There is also the possibility of using synthetic seeds to time production cycles in micropropagation laboratories if the development of the plant could be properly directed towards proliferation and rooting. However, conversion is the most important aspect of the synthetic seed technology, and one of the factors that have limited its practical use (Standardi and Piccioni, 1998). In contrast to somatic embryos which are bipolar structures, shoots and buds do not have root meristems and they must regenerate roots in order to be able to convert (Bapat, 1993; Piccioni, 1997).

The microtuber option in yam conservation and propagation

The process of *in vitro* tuberization in yams

In vitro tuberization has been reported in *Dioscorea abyssinica* (Jean and Cappadocia, 1991); *Dioscorea floribunda* (Sengupta et al. 1984.); *D. alata* (Ammirato, 1976; Alhassan and Mantell, 1991); *D. bulbifera* (Forsyth and Van Staden, 1984; Mantell, 1987); *D. rotundata* and *D. cayenensis* (Ng and Mantell, 1996) and *Dioscorea opposita* (Mantell and Hugo 1986) with various degrees of success.

The origin of *in vitro* tubers is essentially similar to that in the development of aerial tubers produced on greenhouse-grown plants (Mantell, 1987). Microtubers develop from primary nodal complexes (Mantel, 1987). In the leaf axils of old nodes, there are two axillary buds and one shoot primordium, which also later develop into an axillary bud. A primary nodal complex (PNC), preceded by a meristematic PNC-initial is developed at the base of the

first-formed axillary bud. This PNC-initial has capacity for multiple bud production, roots and a tuberous storage organ (Wickham et al. 1982). The PNC-initial is the organ of renewed growth and the only true organ of vegetative propagation in *Dioscorea* species (Wickham et al., 1981, 1982). The morphogenetic expression of the PNC activity is also under hormonal control (Wickham et al., 1982).

Effects of cultural factors

Investigations on microtuberization have revealed the effect of a number of factors on the phenomenon. The Murashige and Skoog (1962) basal medium formulation (MS) inhibited microtuberization in *D. alata* and *D. bulbifera* (Mantell and Hugo, 1989) and *D. opposita* (Asahira and Yazawa, 1979). Microtubers were however produced on glycine-free MS medium in *D. alata* and *D. abyssinica* while half-strength MS medium enhanced microtuberization in *D. alata* (Chang and Hayashi 1995a; Chang et al. 1995b). Tuberization ('T') medium, specially designed for delivering reduced nitrogen (6% w/w total nitrogen present in full strength MS on a molar basis) to yam shoot cultures was optimum for microtuberization in *D. alata* (Mantell and Hugo, 1989). Microtuber formation was stimulated in *Dioscorea batatas* (Asahira and Nitsch, 1968) and *D. alata* (Mantell and Hugo, 1989; Jean and Cappadocia, 1991) in the absence of ammonium. It should be confirmed, however, whether this is due to the absence of NH_4^+ or reduction of total nitrogen content or high NO_3^-: NH_4^+ ratio, as observed in *D. opposita* (Asahira and Yazawa, 1979). Balogun et al. (2006) reported that optimum basal medium formulation for microtuberization varied with other factors like medium matrix, sucrose concentration, and light and temperature regimes.

Microtuberization is affected by plant growth regulators (Koda and Kikuta, 1991; Jean and Cappadocia, 1992; John et al., 1993; Kikuno et al., 2002a), the effect being greater when applied at culture initiation than at later stages (Balogun 2005). Naphthalene acetic acid (NAA) enhanced microtuber production in *D. rotundata* (Ng and Mantell, 1996). NAA was also superior to indole acetic acid in microtuber induction in *D. alata* (Chang and Hayashi, 1995). In *D. alata*, kinetin did not significantly affect MTZ (Ng and Mantell, 1996) but enhanced MTZ in *D. bulbifera* (Mantell and Hugo, 1989). In *D. rotundata*, kinetin induced the highest percentage microtuberization, followed by 2ip, while tuberization was poor in BAP. Also, supplementing the tuberization (T') medium with 1.0µM kinetin improved microtuber frequencies but not individual microtuber weights in *D. alata* (Alhassan and Mantell 1991). In *D. alata*, 50µM gibberrellic acid (GA_3) increased the tuber fresh weight (Ng and Mantell, 1996, Onjo et al., 2001), but was detrimental to shoot fresh weight and number of nodes per plantlet. GA_3 also induced fewer nodes at 5.0 µM in *D. rotundata* (Ng and Mantell 1996). In *D. alata* tuber weight increased with increasing concentration of abscisic acid at 8 h photoperiod (Jean and Cappadocia, 1992). Interaction of kinetin with abscisic acid reduced microtuber frequency but increased individual microtuber weights, suggesting a possible re-stimulation of tuberization by kinetin, earlier suppressed by abscisic acid (Alhassan and Mantell, 1991).GA_3 and JA-like substances might be related to initiation of tuber enlargement while ABA is not directly related (Kikuno et al., 2002b).

Tuberization in yam was reported to be controlled by jasmonic acid, a 12-carbon acid that has fragrant and plant growth-regulating properties, inhibiting growth and promotes senescence (Vick and Zimmerman, 1984). It was isolated from yam and potato leaves (Koda and Kikuta, 1991; Koda and Okazawa, 1988) and found to have strong tuber-inducing properties in both species. The threshold concentration of JA for induction of yam tuberization *in vitro* was found to be 10^{-7}M (Vick and Zimmerman, 1984). Kikuno et al. (2002b) reported that in *D. alata*, jasmonic acid synthesis is activated by short day length, while the peak of jasmonic acid content coincided with initiation of tuber enlargement and decreased significantly afterwards, although vigorous growth and enlargement of tuber continued. In addition, sensitivity to JA was greater in late maturing genotypes than earlier ones (Balogun, 2005). Uniconazole-p was inhibitory to MTZ (Balogun, 2005) regardless of the growth phase at which it was applied, probably due to its inhibitory effect on GA synthesis (Izumi et al., 1984). However, MTZ can be achieved without PGRs if dormancy control is not desired (Balogun et al., 2006).

Sucrose was found to be best for microtuberization in *D. rotundata* relative to fructose, although the reverse is the case for shoot multiplication. Sorbitol and galactose were however inhibitory to tuberization. Higher concentration (5%) of all carbon sources gave higher tuberization than lower (3%) concentration. 8% sucrose also enhanced MTZ in *D. rotundata* (Balogun, 2005; Balogun et al., 2006).

Short days stimulate yam tuberization depending on the growth stage at which it was administered and the earliness of varieties while long days inhibit it (Koda and Kikuta, 1991; Shiwachi et al., 2002). In *D. rotundata*, increase in day-length, (up to 16 h photoperiod) increased shoot and root weights, number of nodes and microtubers; but 24 hr photoperiod was detrimental. In *D. alata*, however, shorter day length was necessary to consistently produce more and larger microtubers (Ng and Mantell, 1996). At 8 h photoperiod, only basal nodes produced MTs in *D. alata*, but other nodes did at longer (16 and 24 h) photoperiods. The tubers were however larger at 8 h d^{-1} (Jean and Cappadocia 1991). Under 12 h photoperiod, MTZ was significantly higher than in complete darkness (Balogun, 2005).

Whether in liquid or agar-solidified medium, culture aeration was found to be beneficial to yam shoot development and microtuber production. This might be due to

the stimulation of photosynthesis *in vitro* by aeration (Ng and Mantell, 1996). Yam shoot growth and microtuber production in terms of weight and size was significantly enhanced in temporary immersion system relative to solid medium (Jova et al., 2005). It offers automation in culture systems, wherein temporary immer-sion of explants in medium allows all buds to be in con-tact with culture medium at the same time. This effect is not given by static culture systems. Explant growth is favoured because contact with media has a short duration with time to renew atmosphere (aeration) in flask. So, disadvantages of solid culture media are decreased and a high photosynthetic activity is obtained (Etienne and Berthouly, 2002). The technique can also be used for shoot multiplication during the planting season, when *in vitro* plants can be immediately acclimatized and transplanted. A similar observation on potato crop was previously carried out (Akita and Takayama, 1994).

Effects due to the cultured explant

Previous reports have shown that genotypes differ in their response to the above conditions and ability to tuberize. For example, *D. alata* produced microtubers more readily than *D. rotundata* (Balogun 2005). This confirms endogenous control of tuberization (Shiwachi et al. 2002). For propagation purposes therefore, microtuber production and dormancy control protocols specific to economically important genotypes will have to be developed. For conservation purposes however, it should be expected that not all genotypes of *D. rotundata* might be conserved using microtubers.

The source of explant affects MTZ frequencies ((Balogun et al., 2005). Screen house explants produced more MTs than *in vitro* explants. This may be due to better aeration and hence shoot vigour in the former than the latter growth environment (Balogun et al. 2004).

Tuber dormancy in yam conservation and propagation

The efficiency of any conservation and propagation system lies in the ability of regeneration of propagules as may be desired. The single most important factor that limits regeneration of yam propagules as needed is tuber dormancy. It is a physiological rest period in which there is no visible physiological or biochemical activity, the inability of growth in plant meristems in spite of suitable environmental conditions (Lang, 1996). It allows propagules to survive prolonged dry seasons, and hence, is ecologically significant (Craufurd et al., 2001). Thus, all techniques aimed at yam germplasm conservation and propagation, including microtuberization systems, need to be improved in the area of dormancy breaks so that yam sprouting can be achieved as and when desired (Craufurd et al. 2001). Dormancy break will allow easy regeneration of plantlets while prolonging the dormancy period will increase the viable shelf life during storage (Ng and Ng, 1997; Craufurd et al., 2001), as reported for potatoes (Keller and Schuler, 1996).

Ammirato (1982) reported the non-sprouting of yam microtubers, while Ng (1998) reported in studies on influence of carbon source on *in vitro* tuberization and growth of *D. rotundata*, that microtubers harvested from mannose, fructose and sucrose treatments did not sprout until eight months after harvest. Also, bigger MTs sprouted later than smaller ones although the frequency of sprouting was higher (Ammirato, 1982; Balogun, 2005).

Although GA_3 promoted the enlargement of microtubers in *D. alata* (Onjo et al., 2001), it also stimulated the thickening of tubers and hence extends the period of tuber dormancy (Onjo et al., 1999; Balogun, 2005; Giradin et al., 1998; Tschannen et al., 2003). Microtuber sprouting is enhanced by Jasmonic acid (Bazabakana et al., 1999, Balogun, 2005).

Yam tuber dormancy was reported to respond to plant growth regulators (Wickham et al., 1984).The production and dormancy of MTs vary with the growth phase of the plantlet at which specific Plant growth regulators are applied (Balogun, 2005). In a particular genotype, the phase of plant growth (that is, vine development, PNC formation, tuber initiation) whose length mostly affects the maturity period (e.g long versus short PNC formation phase) may determine the optimum PGR regime for dormancy control (Balogun 2005). Uniconazole-p inhibits MTZ but shortens the dormancy period of microtubers (Balogun 2005) as was reported *in vivo* (Park et al., 2003).

Microtubers versus synthetic seeds

Optimum protocol for cryopreservation and conversion of cryopreserved germplasm to plantlets differs among genotypes of yam ((Kyesmu, 1998; Kyesmu et al., 1997; Malaurie et al., 1998; Mandal, 2000; Mandal et al., 1996). In contrast, groups of genotypes can produce micro-tubers under similar protocols while all genotypes will eventually break dormancy under natural conditions. This will circumvent the rigorous procedures of conversion and recovery associated with cryopreservation.

The use of both microtubers (Ng, 1988) and encapsulated embryos (Hasan and Takagi, 1995) in germplasm exchange are not as vulnerable as plantlets to unfavourable conditions of transportation and this reduces germplasm losses. Both options produce virus-free, true-to-type materials while each is limited to those varieties amenable to them. This is because protocols for both procedures have not been optimized for all yam species (Jean and Cappadocia, 1991, Hasan and Takagi, 1995). Although conversion of synthetic seeds is comparable to dormancy break in microtubers, the former is artificial and while the latter is the natural physiology of the germplasm. The advantage of microtuber use in germplasm storage over synthetic seeds is that without any exoge-

Table 1. Previous reports on microtuberization in yams.

Author/Year	Species
Microtuber production	
Alhassan and Mantell, 1991	*D. alata*
Ammirato, 1976, 1982,1984	*D. alata, D. bulbifera*
Asahira and Yazawa, 1979	*D. opposita*
Asahira and Nitsch, 1968	*D. batatas*
Balogun et al., 2004, 2005	*D. alata, D. rotundata*
Bazabakana et al., 2003	*D. alata*
Chang et al., 1995	*D. alata*
Forsyth and Van Staden, 1984	*D. bulbifera*
John et al., 1993	*D. alata*
Jova et al., 2005	*D. alata*
Jean and Cappadocia, 1991, 1992.	*D. alata, D. abyssinica*
Kikuno, 2004, 2005	*D. alata, D. rotundata*
Mantell, 1987	*D. alata, D. bulbifera, D. rotundata*
Mantell and Hugo, 1986; 1989	*D. bulbifera, D. alata, D. opposita*
Ng and Mantell, 1996	*D. alata, D. cayenensis, D. rotundata*
Ng, 1988, 1998	*D. rotundata*
Onjo et al., 2001	*D. alata*
Sengupta et al., 1984	*D. floribunda*
Microtuber dormancy	
Ammirato, 1982	*D. alata, D. bulbifera*
Ng, 1998	*D. rotundata*
Bazabakana et al., 1999	*D. alata*
Balogun , 2005	*D. alata, D. rotundata*

nous influence, microtuber dormancy will break naturally after a minimum of 4 months without loss of viability (Balogun, 2005). The length of the dormancy period can also be controlled by exogenous application of plant growth regulators to extend or reduce the storage period. Cryopreservation of synthetic seeds will alsoex-tend their viability period although recovery and survival rates differ among genotypes and may be unpredictable

In contrast, high frequency of sprouting and survival were recorded in microtubers with exogenously extended dormancy period (Balogun, 2005). Use of microtubers could be more reliable than cryopreserved synthetic seeds if the dormancy period can be exogenously controlled for any desirable length of time. However, this is yet to be perfected and constitutes a research gap.

Implications of research findings and future trends

Although a lot of work has been done on microtuber production, there have been very few reports on yam microtuber dormancy (Table 1) while, only one report has proposed a protocol for yam conservation using MTs, and it is yet to be applied. In addition, more work has been done on *D. alata* than any other species (Figure 1). These reports may however not be exhaustive. The use of microtubers in germplasm conservation, propagation and exchange will be impossible without adequate protocols for microtuber production and dormancy control, as only this will allow for storage and regeneration as may be desired (Craufurd et al., 2001). Based on the available reports, a scheme for utilization of MTs in conservation is proposed here (Figure 2), although this may be far from optimum. Most of the research gaps hindering the use of MTs are related to dormancy and post-sprout studies. These include:

1. The genetic variation among yam collections in terms of ability to form MTs should be determined so as to know the scope of its applicability.
2. What is the optimum condition for storing MTs? This will involve investigations into the effects of light, temperature and humidity on microtuber dormancy.
3. What is the condition of plantlets regenerated from nodes excised from plantlets relative to those from MTs in terms of vigour?
4. What are the optimum conditions or protocols for raising seedlings from MTs?
5. How many generations of planting seedlings from microtubers will give tuber yield comparable to or bigger than those from field tubers?
6. What is the survival rate of plants from MTs relative to transplanted *in vitro* plantlets?

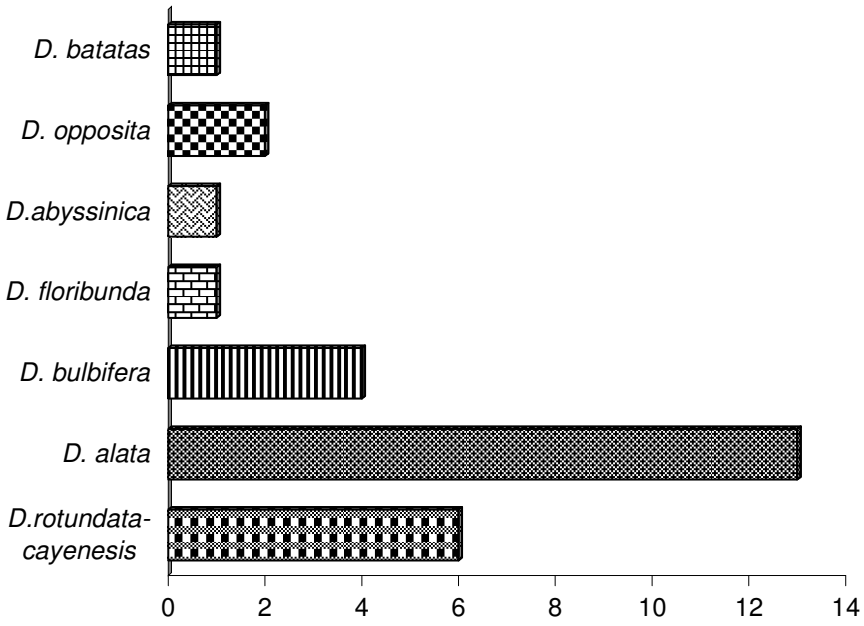

Figure 1. Number of authors who have worked on microtuberization in each species

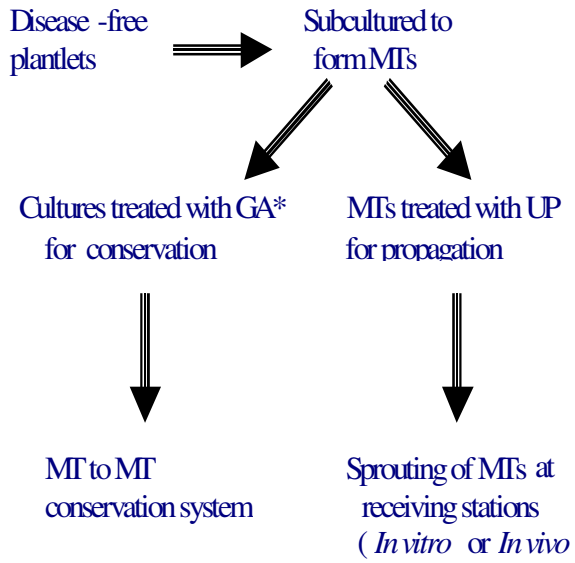

Figure 2. Proposed protocol for MT use in yam germplasm conservation and propagation.

7. What is the cost of conservation, propagation and exchange using MTs relative to other options? This will include investigations for possible economic benefits in the establishment of specialist producers who can produce healthy seed yams of desirable varieties to meet growers' needs.

Conclusion

The ability of yam plants to produce tubers *in vitro* has been established, and many factors which affect it are known. These range from genotype, micro-and macronutrients, light and temperature regime through plant growth regulators to sources of explants. However, investigations on the control of microtuber dormancy, post-sprout management and efficiency of microtubers relative to other systems in terms of cost, ease of handling and savings on time are sparse. Thus, for effective use of MTs in conservation and propagation of yam germplasm, future research should be on dormancy control and post-sprout management. Also, emphasis should be laid on the ultimate goal of a seed production system which is to produce good quality propagules that will yield optimally, good enough for reasonable econo-mic returns.

So far, research reports indicate a possibility of developping a MT to MT cycle for the conservation and propagation of yam germplasm. Specifically, a highly valuable alternative for the commercial production of microtubers as seed yam is offered by temporary immersion system which induces more tubers per plant and increases the size and weight of tubers. With the availability of reliable microtuber production and dormancy control systems, the germplasm propagation, conservation and exchange of *in vitro* propagated, pathogen-tested elite clones will be facilitated. However, different methods of conservation should still be combined for a better security of germplasm collections.

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