Macrothink Institute™

Journal of Applied Biotechnology
ISSN 2327-0640
2016, Vol. 4, No. 1

Propagation Methods of Yam (*Dioscorea Species*) with Special Attention to *In Vitro* Propagation

Obssi Dessalegn (Corresponding author)

College of Natural and Computational Science, Department of Biotechnology

Gondar University, Ethiopia

E-mail: dobssi@gmail.com

Received: December 27, 2015 Accepted: February 14, 2016 Published: February 15, 2016

Doi: 10.5296/jab.v4i1.9031 URL: http://dx.doi.org/10.5296/jab.v4i1.9031

Abstract

Yam is a monocotyledonous plant in the genus Dioscorea. It is a multi-species tuber crop cultivated in Africa, Asia and parts of South America. Yam is an important crop in South and Southwestern parts of Ethiopia. Many species of Dioscorea genus are economically important crops and many of them have been used in the pharmaceutical industry. Yam is propagated from seed tubers or sections of tuber and corms. Seed tubers are expensive, bulky to transport and the multiplication rate in the field is very low. Shortage of seed tubers for planting is one of the major constraints for yam production in Ethiopia. To overcome such problems and to increase production, different propagation methods have been implemented for many Dioscorea species. Convectional and In vitro propagation of Dioscorea species pave the way to meet the demand of this economically important plant. The protocols are designed to provide the optimal levels of mineral nutrients, environmental factors, vitamins and carbohydrates to achieve the high regeneration rate of the different species of Dioscorea in vitro. This review summarizes some of the important reports on different propagation technique of Dioscorea from the literature data.

Keywords: Dioscorea, *In vitro*, micropropagation, Yam, Minisett technique

1. Introduction

The genus *Dioscorea* is the type genus of the family *Dioscoreaceae* and is the largest genus within this family of about 644 species (Govaerts et al., 2007). All *Dioscorea* species are *Dioecious* twining climbers producing dry capsules, although occasionally both male and female flowers can be found on the same plant. All species of economic importance are tuberous (Lebot, 2009).

Yam is a traditional crop that has long been cultivated in Southern, Western and Southwestern parts of Ethiopia as staple or co-staple with enset (*Ensete ventricosum*), cereals, and other root and tuber crops (Westphal, 1975; Edwards, 1991; Mie´ge & Demissew, 1997; Wilkin, 1998; Gemeda, 2000). As the crop is adapted to dry season planting (mainly at the onset of the dry season in October) early harvests in May could fill a seasonal gap in food supply in Ethiopia (Tamiru et al., 2005; Tamiru, 2006; Tamiru, 2008). Their storage organs (underground and/or aerial tubers) are sources of proteins, fats, and vitamins for millions of people in Africa (Hahn et al., 1987; Lebot, 2009). In addition, *Dioscorea* is a well-known edible and traditional medicinal plant, since the genus is rich in steroidal saponins and as a source of biologically active compounds in pharmaceutical industries (Wang et al., 2006; Kole, 2011).

Yams are principally grown for food and have organoleptic qualities that make them the preferred carbohydrate food where they are grown. However, their storage organs (underground and/or aerial tubers) are also sources of proteins, fats, and vitamins for millions of people in West Africa (Kole, 2011). Chemical composition depends mainly on the species, the cultivar or wild form. Their protein, mineral and vitamin content are higher. When processed into flour, yams have a nutritional value comparable to cereals. The food value is composed of carbohydrates (starch, sugars, and fibers), proteins, minerals, vitamins and a negligible amount of lipids (Lebot, 2009). Nutritional qualities have been investigated for the major species, but there is less work on other *Dioscorea* species. In countries where yams are generally cultivated, wild yams are used as food in times of shortage or famine (Coursey, 1967). These wild species, although consumed only under famine conditions, also makes enormous contribution to human welfare. Apart from food, *Dioscorea* species are also used in pharmaceutical industries as sources of biologically active compounds or their precursors. A wide range of saponins and steroidal sapogenins (mostly diosgenin) have been extracted from various *Dioscorea* spp. with the aim of providing the pharmaceutical industry with compounds for oral contraceptives. It regulates the female reproductive system, particularly during menstrual distress and menopause, and is also used in treating infertility (Kole, 2011).

Yam is propagated from seed tubers or sections of tubers and corms. Seed tubers are expensive, accounting sometimes for about as much as 50% of total variable cost (Manyong, 2000); they are bulky to transport and have extended dormancy period. The multiplication ratio in the field is very low (less than 1:10) compared, for instance, to some cereals (1:300) (Balogun, 2009). Traditionally farmers obtain seed tubers by selecting small tubers (e.g. 200–500 g) from each harvest. Unfortunately these seed tubers are often produced by diseased plants by nematodes and Insects such as yam shoot beetle, which often interact with fungi (*Botryodiplodia*, *Fusarium*) and bacteria (*Erwinia* spp.) that damage tubers in the field and in storage (Aighewi et al., 2003a; Lebot, 2009). Diehl's (1982) survey report in Nigeria also showed shortage of planting material (owing to low reproductive rate) which may lead to future decline in yam production.

In Ethiopia there is shortage of seed tubers for planting, lack of formal seed supply system and specialization in the production of yam planting-materials (Tamiru et al., 2005; Tamiru et al., 2008). Farmers mostly rely on their own planting-materials saved from the previous

cropping season; some farmers partly meet their demand for seed tubers through purchases from local markets or exchanges with neighbors. This has led to a decrease in production (Tamiru et al., 2008) due to insufficient quantity and poor quality of planting material. In addition, productivity is hampered by pests and diseases and the limited availability and high cost of planting materials (Balogun et al., 2004). So, some farmers keep a reserve batch of seed yams (up to a third of the quantity planted) for replacement of seeds that do not germinate. Poor quality planting materials that germinate tend to carry disease and pest (viruses, fungi, nematodes and insects) from the storage barns to the field the next season resulting in low tuber yields, followed by poor shelf life (Ghosh et al., 1988; Asiedu & Sartie, 2010).

To overcome such problems and increase production, conventional methods such as partial sectioning, layering, vine rooting and minisett technique have been used to produce high amount of planting material (Okoli et al., 1982; Wilson, 1989). Minisett technique has significantly increased propagation rates, but it has been associated with less uniform and poor rate of sprouting when applied to white yam (Okoli et al., 1982). The partial sectioning requires considerable manpower for the repeated examining and digging out of tubers to excise sprouted sections for field planting. In case of vine rooting technique, either tubers did not develop due to early senescence of rooted vines (Acha et al., 2004), or small tubers are produced when applied to *D. rotundata* (Okoli et al., 1982). The layering technique is unsuitable for farm use due to rigorous procedures involved (Acha et al., 2004) and it is genotype specific (Acha et al., 2004; Shiwachi et al., 2005b).

Therefore, other methods of rapid propagation such as micropropagation have been developed (Balogun et al., 2004) including production of microtubers from plantlets *in vitro* (Aighewi et al., 2003b; Feng et al., 2007). Micropropagation of yam offers the distinct advantage of large scale multiplication of high quality, clonally propagated planting materials (Ng, 1988; Asha & Nair, 2007). It provides many advantages over conventional methods including: (1) it enables mass propagation of specific species, (2) it helps to produce pathogen-free planting material, (3) it enables clonal propagation of parental stock for hybrid seed production, and (4) it enables year-round nursery production (Hartmann et al., 2002).

This review summarizes some of the recent reports on the propagation of *Dioscorea* species with special focus on the significant achievements on *in vitro* propagation of yam.

2. Yam Cultivation

High yam yields depend on good planting material and husbandry and, in particular, timely weed control to permit establishment of a sufficient leaf area (Asiedu & Sartie, 2010). They also depend on adequate and near optimum temperatures (25-30° C). The most important constraints to production are the high labour requirements, the quality of the planting material and difficulties in mechanization (Lebot, 2009).

2.1 Soil Preparation for Yam Cultivation

Yams, being light-loving and shade-sensitive plants, require sites which are well exposed to solar radiation (Shiwachi et al., 2005a). If planted in traditional agroforestry systems, they

need to be established in the middle of the plot and to be staked in order to benefit from maximum sunlight (Lebot, 2009). Unlike cassava and sweet potato roots, which initially penetrate the soil and then expand, the yam tuber penetrates the soil while expanding (Asiedu and Sartie, 2010). It is therefore important that the soil is light, well drained and friable. Land preparation is the most important input and necessitates almost half of the total 1800 man-hours/ha in West Africa (Hahn et al., 1987).

2.2 Nutrient Requirement of Yam

Degradation of soil fertility is the major constraint identified by growers in yam production in West Africa (Asiedu & Sartie, 2010). Although farmers perceive the decline in soil fertility as their most important difficulty in improving yield and profit, they often lack suitable and practical solutions to correct the situation (Kang & Wilson, 1981; Lebot, 2009).

Responses to fertilizers are erratic and usually much less significant than the effects of sett size or staking (Okoli et al., 1982). No responses to N fertilization or even depressive effects have been reported. Kang and Wilson (1981) reported no significant effect of NPK fertilizer on tuber yield at all three locations where their experiments were conducted and noticed some depression in the yield of plants grown on flats. To complicate the situation, yams appear to depend on an effective mycorrhizal association to meet their P requirements. Apparently, yams respond well to N and K fertilizers, while their response to P is slight. This could be due to very efficient P uptake, possibly as a result of mycorrhizal contribution (Lebot, 2009).

2.3 Conventional Vegetative Propagation of Yam

Traditionally, yams are propagated by planting whole tubers or large pieces weighing 200 g or more (Okoli *et al*., 1982; Balogun, 2009). A sizable portion of otherwise consumable tubers are therefore reserved for planting yearly, and this leads to scarcity of planting materials. Most farmers propagate yams by “milking” (Balogun, 2009). 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 (Lebot, 2009).

The major constraint of planting materials to yam production is being tackled by the development of more efficient propagation methods. These include partial sectioning technique, vine rooting (Acha et al., 2004); the layering and minisett technique (Okoli et al., 1982) and these techniques do have their advantages and disadvantages (Table 1).

Table 1. Different types of yam propagation with their importance and limitation

Types	Advantages	Limitation	Reference
Minisett technique	Reduce the bulkiness of the planting material. Significantly increased propagation rates	Species-specific and requires considerable manpower for the repeated examining and digging out of tubers to excise sprouted sections for field planting.	Okoli et al., 1982
The layering technique	Some genotypes perform much better than others, i.e., it is genotype specific.	Technique is unsuitable for farm use due to rigorous procedures involved and it is genotype specific.	(Acha et al., 2004; Shiwachi et al., 2005b)
Vine cutting	Used for production of planting setts this not only accelerates propagation of selected clones but produces minitubers. These vine cuttings can be used to produce minitubers within 100–120 days	Either tubers did not develop due to early senescence of rooted vines, or small tubers are produced when applied to *D. rotundata* relative to other species	Okonmah 1980; Acha et al., 2004, Acha et al., 2005; Shiwachi et al., 2005b
Micropropagation	Offers very high multiplication rates and healthy propagules	Cost of propagation is high	(Ng, 1992).

2.4 Yam Tissue Culture

Tissue culture is the rapid method developed to address limitations of the traditional practices in yam propagation. Micropropagation in vitro following culture of apical meristems and nodal cuttings offers very high multiplication rates and healthy propagules (Ng, 1992). Other methods of rapid propagation developed at IITA include production of microtubers (Balogun et al., 2004) from plantlets *in vitro*, and the production of seed tubers using slips (sprouts) and peels (Aighewi et al., 2003b).

In vitro propagation protocols have been developed for many of the *Dioscorea* species. Multiplication of yam by *in vitro* growth of nodal segments is a practical way for rapid clonal multiplication and some international tissue culture companies like Vitrobio Valenda SL in

Spain are involved in commercial propagation of yam through the use of those protocols developed and there is also mass propagation in some African and Arabian countries (Omar & Aouine, 2007) but, in tropical countries, only a few agricultural research stations can afford to do it. All the protocol developed pass through different stages of *in vitro* propagation (Mantell *et al*., 1978). In 1974, Murashige outlined four stages that can be followed in tissue culture, each with specific nutritional and incubation conditions requirements. Such stages are shoot initiation, multiplication, rooting and acclimatization stages but before that the mother plant need to be grown on an appropriate condition.

2.4.1 Mother Plant Preparation

According to Omar and Aouine, 2007, mother plants were established from healthy seed yams (weighing 200 ± 5 g) which had broken dormancy, and were planted singly in 40 cm black polybags filled with sterilized top soil and kept in a greenhouse. Light watering with tap water was carried out twice daily until sprouts were produced. Young healthy sprouts were removed from mother plants after two months of growth, deleafed, and cut into smaller pieces (Mbanaso et al., 2007; Obssi et al., 2015).

In addition Behera and his co-workers had used the tubers of various yam genotypes were put in a greenhouse for germination to obtain the mothers plants and the explants were obtained from the established experimental site after 35 to 60 days after germination (Behera et al., 2008; Behera et al., 2009; Behera et al., 2010). And vines / top shoot cuttings having 5-8 nodes, excised from greenhouse grown, healthy plants of about 2 m height, raised from the tubers under uniform manorial conditions, served as source of explants (Adeniyi *et al*., 2008). On the other hand vines/shoot top cuttings, single nodal segments (1-2 cm) were excised and used as explants for *in* vitro experiments (Asha & Nair, 2007; Obssi et al., 2015).

2.4.2 Initiation of Aseptic Culture

Out of the various treatments tried, the treatment with $HgCl_2$ (0.1%) for 4 minutes followed by washing thrice with sterile water and then dipping in ethanol (70%) for 1 minute exhibited maximum establishment of aseptic as well as proliferating cultures, i.e., 78.67 and 76.00% respectively(Kharat et al., 2008; Ahanhanzo et al., 2010; Obssi et al., 2015). Mwirigi et al., (2010) reported that sequential sterilization that involved the use of bleach at concentration of 40% for 30 minutes followed by 20% for 20 minutes which gave the best results, 85% of the explants has survived.

According to many scholars the combination of plant growth regulators (auxins and cytokinin) had highest effect on initiation of aseptic culture (Adeniyi et al., 2008; Obssi et al., 2015). The highest shoot induction of 75% was obtained in the medium containing 0.10 µM NAA + 0.20 µM BAP and there was significant NAA x BAP interaction, indicating that the effectiveness of each of the phytohormone in inducing shoots and plantlets was influenced by the presence or absence of the other (Adeniyi et al., 2008).

2.4.3 Multiplication of Propagules *in vitro*

Different scholars have optimized a protocol for *in* vitro shoot multiplication for different

genus of *Dioscorea* species using different explant source (Table 2). According to those scholars different genus of *Dioscorea* species require different growth regulator formulation for optimal shoot multiplication (Table 3). A difference in the composition of the plant tissue culture media like carbon source and plant growth regulator concentrations also has resulted in a difference in the response. Sucrose levels greater than 20 g l^{-1} in culture media appeared to be a prerequisite for optimal *i n vitro* plantlet growth of *D. composita* micro plants (Alizadeh et al., 1998).

Nodal explants of tender stem cuttings of *D. nipponica* Makino were planted on basal media supplemented with hormone combinations at different concentrations. After 4–7 days in culture, the lateral buds displayed visible growth, and most of them grew into 35–50-mm-long shoots within 4 weeks. Multiple buds growing on a suitable initiation medium developed into plantlets, with part of the plantlet producing microtubers (Chen et al., 2007). The nodal segments of 15mm length produced higher percentage of shoot (77.33%) (Kharat et al., 2008; Ahanhanzo et al., 2010). Supplementing the media with 2.0 mg/l BAP (Benzyl amino purine), increased the rate of shoot induction and length of shoot but a further increase of BAP concentration up to 4.0 mg/l dropped the shoot number. A combination of cytokinin and auxin has also been reported to have best shooting. Chen *et al.,* (2007) reported highest shoot induction frequency with a growth regulator combination of 2.0 mg/l BAP and 1.0 mg/l NAA.

On the other hand Ahanhanzo et al., (2010) reported that BAP (0.5 mg l^{-1}) induced a significant increase ($p < 0.05$) in leaf number of varieties *Kounondakou* and *Gnon-boya* and a significant increase ($p < 0.01$) in the height of *vitro* plants for three varieties) and growth inhibitory effect of kinetin on shoot numbers of *D. oppositifolia* and *D. pentaphylla* micro plants was observed. But the promotive effects of kinetin (46.4µM) on plantlet growth for *D. bulbifera*, which increased the number of shoots per plantlet (Mahesh et al., 2010).

Belarmino and Gonzales indicated that no break in leaf growth was observed on the control medium (without cytokinin) but media with BAP presented a good plants aerial part development (Belarmino & Gonzales, 2008). Similarly Ahanhanzo et al., (2010) also reported, improvement of axillarys bud sprouting for all yam varieties studied and facilitated development of the stems and leaves of some varieties during the second week of culture. But the results of Yan et al. (2011) showed significantly higher shoot length, frequency of proliferation of *Dioscorea fordii* in MS basal medium supplemented with 1.0 mg l^{-1} BAP, 0.1 mg l^{-1} NAA, 30 g l^{-1} sucrose and 1.5 g l^{-1} AC (activated charcoal) in liquid culture)

In addition Ramierez-Magon et al. (2001) has reported that when *Spathiphyllum floribundam* was cultured on a media with BAP and IAA, there was increase in shoot multiplication from 1.8 shoots per cultured explant to average of 11.6 shoots per explant. Explants grown on a media with 0.5 mgl^{-1} BAP and 0.01mgl^{-1} NAA showed the highest rate of multiplication and survival as compared with explants in media with other growth regulators (Thankappan & Patell, 2011). Up to 9 shoots was observed from a single node (Thankappan & Patell, 2011). Of the combination tested by Behera et al. (2008) MS +BAP (2.0 mgl^{-1}) + NAA (0.5 mgl^{-1}) with ascorbic acid 100mgl^{-1}, elicited optimal response in which

an average of 6 ± 0.18 shoot lets with a mean shoot length of 5 ± 0.29 cm per explants was recorded. With the second best shoot multiplication 4.5 ± 0.12 was obtained on the medium MS + Kinetin (1.5mgl^{-1}) + NAA (0.5 mg^{-l}) + 100 mg^{-l} ascorbic acid with a mean shoot length of 4 ± 0.29 cm. In addition Behera *et al*. (2009) obtain average of 10.5 ± 0.51 shoot lets with a mean shoot length of 5.4 ± 0.24 cm per explants. The second best shoot multiplication 5.5 ± 0.43 was obtained in the medium MS + Kinetin (1.5mgl^{-1}) + BAP (1.0 mg^{-l}) + NAA (0.5 mg^{-l}) + 100 mg-l ascorbic acid with a mean shoot length of 4.2 ± 0.21 cm on MS+ Kinetin (2.0 mg^{-l}) +BAP (1.0 mgl^{-1}) + NAA (0.5 mgl^{-1}) with ascorbic acid 100 mgl^{-1}.

Table 2. In vitro multiplication of genus *Dioscorea* by using different explant

Plant Name	Explant source	References
***Dioscorea spp. (*Aw-004/00)**	Nodal segment	Obssi et al., 2015
D. floribunda	Nodal segment	Chaturvedi, 1975 Lakshmi sita et al., 1976 Uduebo, 1971
D. deltoidea	Axillary meristem	Furmanowa et al., 1984 Grewal et al., 1977
D. composita	Nodal segment	Ammirato, 1982 Datta et al., 1981
D. bulbifera	Axillary meristem	Uduebo, 1971
D. rotundata	Nodal segment	Mantell et al., 1978
D. opposita	tuber	Xu et al., 2009
D. zingiberensis	Nodal segment	Chen et al., 2003
D. oppositifolia , D. pentaphylla	Nodal segment	Poornima & Ravishankar, 2007
D. oppositifolia	Nodal segment	Behera et al., 2009
D. alata	Nodal segment	Borges et al., 2004

D. wightii	Nodal segment	Mahesh et al., 2010
D. zingiberensis	Stem, leaves, petioles	Shu et al., 2005
D.opposita	Stem segment	Nagasawa & Finer, 1989
D. zingiberensis	tuber	Heping et al., 2008
D. alata	Nodal segment	Wheatley et al., 2003
D. balcanica	tuber	Savikin- Fodulovic et al., 1998
D. zingiberensis	inflorescence	Huang et al., 2009
D. bulbifera	Nodal segment	Narula et al., 2007
D. alata	Nodal segment	Jova et al., 2011
D. deltoidea	Nodal segment	Mascarenhas et al., 1976.
D. floribunda	internode	Ammirato, 1978
D. alata	root	Twyford & Mantell, 1996
D. cayenensis-D. rotundata complex & D. praehensilis	Meristem tip	Malaurie et al., 1995

Behera et al. (2010) also reported that yam showed optimal response with an average of 9.5 ± 0.61 shootlets and with a mean shoot length of 6.7 ± 0.44 cm per explants on MS + Kinetin (2.0 mg^{-1}) + BAP (1.0 mg^{-1}) + NAA (0.5 mg^{-1}). The second best shoot multiplication (6.5 ± 0.42) was obtained in the medium MS + Kinetin (2.0 mg^{-1}) + BAP (0.5 mg^{-1}) + NAA (0.25 mg^{-1}) with a mean shoot length of 5.8 ± 0.63 cm. The frequency and rate of bud break and multiplication depends on the cytokinin type and its concentration either alone or in combination with an auxin (Poornima & Ravishankar, 2007).

Mwirigi et al. (2010) also reported that the best treatment for shoot multiplication was 0.5 mgl^{-1} BAP with a mean number of 1.1 shoots and 0.5 mgl^{-1} BAP + 0.02 mg/l NAA gave the best shoot formation with an average of 2.1 shoots followed by 1.0 mgl^{-1} BAP + 0.04 mgl^{-1} NAA with an average of 1.6. In general, the effectiveness of each phytohormone in inducing

plantlet regeneration was influenced by the presence or absence of the other (Table 3). But some scholars report that shoot multiplication was highest in medium cytokinin only which is most probably due to difference in variety and explant source (Table 2).

Table 3. Growth Regulators used for shoot multiplication by different workers

Plant name	Explant Source	Growth Regulators	References
***Dioscorea spp.* (Aw-004/00)**	Nodal segments	MS + 1.5 mgl-1 BAP + 0.15 mgl-1 NAA	Obssi et al., 2015
D. japonica	Shoot tips	LS + 0.44µM BA	Kadota & Niimi, 2004
D. zingiberensis	Nodal segment	MS + 4.4 µM BAP +1.1 µM NAA	Chen et al., 2003
D.oppositifolia ***D. pentaphylla***	Nodal segment	MS + 8.8 µM BAP+ 0.3% charcoal	Poornima & Ravishankar , 2005
D.oppositifolia	Nodal segment	MS+2mg/LKN+1.0mg/LBAP+0.5mg/LNAA+ 100mg/L ascorbic acid	Behera et al., 2009
D. opposita	Nodal segment	MS+ 1.0mg/L NAA+0.5-1.0mg/L BA	Shin et al., 2004
D. hispida	Nodal segment	MS+2.0mg/LBAP + 0.5mg/LNAA + 100mg/L ascorbic acid	Behera et al., 2008
D. bulbifera	Nodal segment	MS+0.5 µM/LNAA+5mg/L KN	Narula et al., 2007

2.4.4 Rooting of Propagules

According to different scholars the effectiveness of *in vitro* rooting of propagules of *Dioscorea* species depends on types of growth regulators, plant species and explant source (Table 4). The plantlets propagated with axillary segments could easily be rooted and transplanted, and were found suitable for the *ex vitro* rooting to produce mini tubers. The number of roots and leaves and the height of each young sprout could be determined after 5 weeks in culture (Ahanhanzo et al., 2010). Behera and his co-workers reported that NAA was

found more effective than IBA in induction of rooting as days required for rooting was only 6-8 as against 10 to15 in the case of IBA (Behera et al., 2008; Behera et al., 2009; Behera et al., 2010).

Behera et al. (2008) obtained highest rooting of *Dioscorea alata* L.cv. Hinjilicatu in 1/2 MS + 2.0 mg^{-1} NAA where about 90% cultures responded with an average number of 5.2 ± 0.28 roots per plantlet and an average root length 3.5 ± 0.12 cm. The same rooting success (90%) has been obtained when a different species; *Dioscorea oppositifolia* L. was cultured on the same media but the average root number and length has been found to be improved. Repeating the same experiment with *Dioscorea alata* L species also gave an improved rooting (92%) with an average roots number of 5.5 ± 0.48 per plantlet and an average root length 5.2 ± 0.26 cm (Behera et al., 2008). On the other hand Behera and his co-workers in 2009 were obtained 6.5 ± 0.30 roots per plantlet with an average root length of 4.5 ± 0.16 cm. And highest response (77%) was recorded at 2.0 mg^{-1} of IBA (Behera et al., 2010). However, Poornima and Ravishankar, (2007) obtained best rooting on a very less concentration of IBA, 0.5 mgl^{-1} NAA. The same concentration of IBA but in combination with 0.01mgl^{-1} NAA has been used to, induce profuse root of 12.5 to 14.5 cm in length in 8 to 10 weeks and there was no rooting on media free of auxin (basal media) (Thankappan & Patell, 2011).

Compared with the plantlet without microtubers, the plantlets with microtubers produced roots more easily with the highest rate being 100% (with 0.5 mgl^{-1} IBA and NAA supplements). Maximum rooting of the plantlets without microtubers (94.67%) was on a medium with 1.0 mgl^{-1} IBA and 0.5 mgl^{-1} NAA. Compared with the control, low concentrations of IBA could increase the rooting frequency in regenerated plantlets without microtubers, but 2.0 mgl^{-1} IBA was optimal only for plantlets without microtubers, and thus decreased the overall rooting frequency (Chen et al., 2007).

Table 4. Growth regulators, explant sources and species used for *in* vitro rooting

Plant Name	**Explant source**	**Growth Regulators**	**References**
***Dioscorea spp.* (Aw-004/00)**	Nodal segments	1/2 MS+2.0 mgl-l NAA + 0.5 mgl-l IBA	Obssi et al., 2015
D. hispida	Nodal segments	1/2 MS + 2.0 mg^{-1} NAA	Behera et al., 2008
		0.5 mgl^{-1} IBA + 0.01mgl^{-1} NAA	Thankappan & Patell, 2011
D. alata	Vine Nodes	1/2 MS + 2.0 mg^{-1} NAA	Behera et al.,

			2010
D. oppositifolia	Nodal segments	1/2 MS+2.0 mg^{-1} NAA	Behera et al., 2009
		1.0 mgl^{-1} IBA + 0.5 mgl^{-1} NAA & 2.0 mgl^{-1} IBA	Chen et al., 2007
D. esculenta	Nodal segments	0.1 mgl^{-1}, 2, 4-D + 80 mg l^{-1} + adenine sulphate	Kharat et al. 2008

2.4.5 *In* vitro Microtubers Induction

Differ scholars have developed different media supplement for *in vitro* microtubers induction *of* different *Dioscorea species* (Table 5). *In vitro* microtuber production has been studied as an alternative for safely propagating and distributing germplasm, as microtubers have been reported as less vulnerable to transport conditions and easier to establish in the soil. Culturing the plants on tuberization medium for 8 and 10 weeks, followed by MS medium, reported to have the highest induction of microtubers, the best frequency and yield and highest individual microtuber weights (Klu et al., 2005). The morphogenesis, growth and *in vitro* microtuber formation have been found to be controlled by external factors. The number of shoots and nodes is increased by the addition of jasmonic acid, which also induces an increase in microtuber numbers (Ovono et al., 2007). However, this technique is not being used on a routine basis and *in vitro* plantlets are still the most practical way of distributing germplasm internationally. The preferred medium for axillary shoot proliferation and tuberization of *D. fordii* was reported to be MS basal medium supplemented with 1.0 mg l^{-1} BAP, 0.1 mg l^{-1} NAA, 30 g l-1 sucrose and 1.5 g l-1 AC in liquid culture (Yan et al., 2011)

Table 5. Growth regulators used for tuber formation by different workers

Plant Name	Explant	Growth Regulators	References
D. opposita	Nodal segment	MS+30gm/Lsucrose+2.0mg/LKN+1.0mg/LBAP +0.5mg/LNAA	Behera et al., 2009
D. opposita	Nodal segment	MS+3%sucrose+8.9 µMBAP	Kohmura et al., 1995
D. bulbifera	Nodal segment	MS+2-8%sucrose+23.2-46.4 µM KN	Forsyth & van staden, 1984
D. composita	Nodal segment	MS+2.5 µM KN	Alizadeh et al., 1998

2.4.6 Acclimatization

Rooted plantlets grown *in vitro* need to be washed thoroughly to remove the adhering gel, transplanted to sterile poly pots (small plastic cups) containing pre-soaked vermiculite and maintained inside growth chamber set at temperature 28 °C and 70-80% relative humidity. After three weeks they should be transplanted to earthen pots containing mixture of soil + sand + manure in 1:1:1 ratio and should be kept under shade house for a period of three weeks for acclimatization. The potted plants need to be irrigated with Hoagland's solution every 3 days for period of 3 weeks. Survival rate of the plantlets could be recorded after 3 weeks (Behera et al., 2009).

Behera et al., (2010) reported that about 90% of the rooted plantlets have established in the greenhouse within 2-3 weeks of transfer. The plants grew well and attained a 6-8 cm height within 4 weeks of transfer. The acclimatized plants were established in the field condition and grew normally without morphological variation. According to Chen et al., (2007), the acclimated plantlets with 5-8 roots which showed obvious growth 1 week after being removed from culture flasks could be transferred to sterilized soil in the greenhouse. One month after transferring, the survival rate of the plantlets was found to be 91%, and the young plants grew vigorously in the greenhouse.

References

Acha, I. A., Shiwachi, H., Asiedu, R., & Akoroda, M. O. (2004). Effect of auxins on root development in yam (*Dioscorea rotundata*) vine. *Tropical Science, 44*, 80-84. http://dx.doi.org/10.1002/ts.140

Adeniyi O. J., Adetimirin, V. O., Ingelbrecht, I., & Asiedu, R. (2008). Shoot and plantlet regeneration from meristems of *Dioscorea rotundata* Poir and *Dioscorea alata* L. *African Journal of Biotechnology, 7*(8), 1003-1008

Ahanhanzo, C., Gandonou, C. B., Agbidinoukoun, A., Dansi, A. and Agbangla, C. (2010). Effect of two cytokinins in combination with acetic acid naphthalene on yams (*Dioscorea* spp.) genotypes 'response to *in vitro* morphogenesis. *African Journal of Biotechnology, 9*(51), 8837-8843.

Aighewi, B. A., Asiedu, R., & Akoroda, M. O. (2003a). Seed yam production from pre-sprouted mini-setts with varied thicknesses of storage parenchyma. *African Journal of Root Tuber Crops, 5*(2), 21-24.

Aighewi, B. A., Asiedu, R., & Akoroda, M. O. (2003b). Producing seed yams (*Dioscorea rotundata* Poir.) from young sprouts. *African Journal of Root Tuber Crops, 5*(2), 13-14.

Alizadeh, S., Mantell, S. H., & Viana, A. M. (1998). *In vitro* shoot culture and microtuber induction in the steroid yam *Dioscorea composita* Hemsl. *Plant Cell, Tissue and Organ Culture, 53*, 107-112. http://dx.doi.org/10.1023/A:1006036324474

Ammirato P. V. (1978). Somatic embryogenesis and plantlet development in suspension cultures of the medicinal yam, *Dioscorea floribunda*. *Am. J. Bot.*, *65*, 89-95.

Ammirato, P. V. (1982). Growth and morphogenesis in cultures of the monocot yam Dioscorea. In A. Fujiwara (Ed.), *Plant Tissue Culture* (pp. 169-170). Tokyo: Maruzen.

Asiedu, R., & Sartie, A. (2010). Crops that feed the World 1. Yams for income and food security. *Food Science, 2*, 305-315. http://dx.doi.org/10.1007/s12571-010-0085-0

Balogun, M. O. (2009). Microtubers in yam germplasm conservation and propagation: The status, the prospects and the constraints. *Biotechnology and Molecular Biology Reviews, 4*(1), 001-010.

Balogun, M. O., Ng, S. Y. C., Shiwachi, H., Ng, N., & Fawole, I. (2004). Comparative effects of explant sources and genotypes on microtuberization in yams (*Dioscorea spp*.). *Tropical Science, 44*, 196-200. http://dx.doi.org/10.1002/ts.168

Behera, K. K., Sahoo, S., & Prusti, A. (2008). Efficient *in vitro* micropropagation of greater yam (*Dioscorea alata* L.cv. Hinjilicatu) through nodal vine explants. *Indian Journal of Plant Physiol, 14*, 250-256.

Behera, K. K., Sahoo, S., & Prusti, A. (2010). Micropropagation of greater yam (*Dioscorea alata* L.) through vine nodes. *Journal of Root Crops, 36*(1), 27-32.

Behera, K. K., Sahoo, S., & Prusti, A. (2008). Effects of plant growth regulator on *in vitro* micropropagation of 'bitter yam' (*Dioscorea hispida*). *International journal of integrated biology, 4*(1), 50-54.

Behera, K. K., Sahoo, S., & Prusti, A. (2009). Regeneration of Plantlet of Water Yam (*Dioscorea oppositifolia* L.) through *In Vitro* Culture from Nodal Segments. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37*(1), 94-102.

Belarmino, M. M., & Gonzales, J. R R. (2008). Somatic embryogenesis and plant regeneration in purple food yam (*Dioscorea alata* L.) *Annals of Tropical Research, 30*(2), 22-33.

Borges, M., Ceiro, W., Meneses, S., Aguilera, N., Vazquez, J., Infante, Z., & Fonseca, M. (2004). Regeneration and multiplication of *Dioscorea alata* germplasm maintained *in vitro*. *Plant Cell Tiss. Org. Cult., 76*, 87-90. http://dx.doi.org/10.1023/A:1025804516226

Chaturvedi, H. C. (1975). Propagation of *Dioscorea floribunda in vitro* culture single node segment. *Curr. sci., 44*, 839-841.

Chen, F. Q., Fu, Y., Wang, D. L., Gao, X., & Wang, L. (2007). The effect of plant growth regulators and sucrose on micropropagation and microtuberization of *Dioscorea napponica* Makino. *J. Plant Growth Regul, 26*, 38-45. http://dx.doi.org/10.1007/s00344-005-0147-2

Chen, Y., Fan, J., Yi, F., Luo, Z., & Fu, Y. (2003). Rapid clonal propagation of *Dioscorea zingiberensis*. *Plant Cell Tissue Organ Cult, 73*, 75-80. http://dx.doi.org/10.1023/A:1022683824635

Chen, F. Q., Fu, Y., Wang, D. L., Gao, X., & Wang, L. (2007). The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of *Dioscorea*

nipponica Makino. *Plant Growth Regul*ation, *26*, 38-45. http://dx.doi.org/10.1007/s00344-005-0147-2

Chu, E. P., & Figueiredo Ribeiro, R. C. L. (2002). Growth and carbohydrate changes in shoot cultures of Dioscorea speciesas influenced by photoperiod, exogenous sucrose and cytokinin concentrations. *Plant Cell Tissue Organ Cult, 70*, 241-249. http://dx.doi.org/10.1023/A:1016565500201

Coursey, D. G. (1976), Yams. *Dioscorea* spp. (*Dioscoreaceae*) In E. D. Simmonds (Ed) *Evolution of crop plants* (pp. 70-74). Longman. London.

Coursey, D. G. (1967). Yams: an account of the nature, origins, cultivation and utilization of the useful members of *Dioscoreaceae* (pp. 230-233). Longmans, Greens and Co. Ltd., London, UK.

Datta, S. K., Datta, K., & Datta P. C. (1981). Propagation of yam, *Dioscorea composita* through tissue culture. In A. N. Rao, (Ed) Tissue Culture of Economically Important plants (pp. 90-93). Singapore: COSTED, ANBS.

Diehl, L. (1982). Smallholder farming systems with yam in the Southern Guinea Savannah of Nigeria. German Agency for Technical Cooperation (GTZ).

Edwards, S. B. (1991). Crops with wild relatives found in Ethiopia. In J. M. M. Engles, J. G. Hawkes, & M. Worede (Eds.), *Plant Genetic resources of Ethiopia* (pp. 42-47). Cambridge University Press, UK. http://dx.doi.org/10.1017/cbo9780511551543.004

Feng, F., Ye, C. H., Li, Y. Z., & Xu, W. F. (2007). Effects of growth regulators, carbon sources and photoperiod on in vitro formation and growth and development of microtubers of *Dioscorea fordii. Plant Physiology short Communication, 43*(6), 1045-1049.

Forsyth, C., & Van, S. J, (1982). An improved method of *in vitro* propagation of *Dioscorea bulbifera. Plant Cell Tissue Organ Cult., 1*, 275-281. http://dx.doi.org/10.1007/BF02318924

Forsyth C., & Van Staden, J. (1984). Tuberization of *Dioscorea bulbifera* stems nodes in culture. *J. Plant Physiol, 115*, 79-83. http://dx.doi.org/10.1016/S0176-1617(84)80053-X

Furmanowa M, Guzewska J., & Beldowska B, (1984). Organ regeneration in callus of *Dioscorea deltoidea* Wall. In F. J. Novak, L. Havel, J. Dolezel (Eds.), Int. Symp. *Plant Tissue and Cell Culture Application to Crop Improvement* (pp. 167-168). Prague: Czech Acad. Sci.

Gemeda, A. (2000). Root and tuber crop as compliments to sustainable livelihood of the farm family in West Ethiopia. In Agri Topia, Quarterly Newsletter of EARO, *15*. Addis Ababa, Ethiopia. pp, 2-8.

George, E. F., & Sherrington, P. D. (1984). Plant propagation by Tissue Culture. Eastern Press (p. 34). England.

George, E. F., Hall, M. A., & Klerk, G-J. D. (2008). *Plant propagation by tissue culture* (3rd edition, Volume 1). The background Published by Springer, The Netherlands.

Ghosh, S. P., Ramanujam, T., J. O., J. S., Moorthy, S. N., & Nair, R. G. (1988). Yams. In Tuber Crops (pp. 345-351). Published by Mohan Primlani for Oxford and IBH publishing Co. Pvt.Ltd, Naraina, New Delhi, India.

Govaerts, R., Wilkin, P., & Saunders, R. M. K. (2007) .World Checklist of Dioscoreaceae, Yams and their Allies. Kew Publishing, Royal Botanic Gardens, Kew, UK.

Grewal, S., Kaul, S., Sachdeva, V., & Atal C. K. (1977). Regeneration of plants of *Dioscorea deltoidea* Wall. by apical meristem cultures. *Indian. J. Expt. Biol., 15*, 201-213.

Hahn, S. K., Osiru, D. S. O., Akoroda, M. O., & Otoo, J. A. (1987). Yam production and its future prospects. *Outlook Agric, 16*, 105-110.

Hann, S. K. (1995). Yams. *Dioscorea* spp. (Dioscoreaceae). In J. Smartt, N. W. Simmonds (Eds.), *Evolution of crop plants*, U.K : Longman Scientific and Technical.

Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (2002). Plant propagation: Principles and practices, 7th ed. Prentice Hall. Pearson Education, Inc. New Jersey.

Heping, H., Shanlin, G., Lanlan, C., & Xiaoke, J. (2008). *In vitro* induction and identification of autotetraploids of *Dioscorea zingiberensis.* In vitro Cell. *Dev. Biol.-Plant., 44*, 448-455. http://dx.doi.org/10.1007/s11627-008-9177-3

Huang, X. L., Yang, B., Hu, C. G., & Yao J. L. (2009). *In vitro* induction of inflorescence in *Dioscorea zingiberensis. Plant Cell Tissue Organ Cult, 99*, 209-215. http://dx.doi.org/10.1007/s11240-009-9595-x

Jova, M. C., Kosky, R. G., & Cuellar E. E. (2011). Effect of liquid media culture systems on yam plant growth (*Dioscorea alata* L.'Pacala Duclos'). *Biotechnology. Agron. Soc. Environ., 15*, 515-521.

Kadota, M., & Niimi, Y. (2004). Improvement of micropropagation of Japanese yam using liquid and gelled medium culture. *Sci. Hort., 102*, 461-466. http://dx.doi.org/10.1016/j.scienta.2004.06.004

Kang, B. T., & Wilson, J. E. (1981). Effect of mound size and fertilizer on white guinea yam (*Dioscorea* rotundata) in Southern Nigeria. *Plant and Soil, 61*, 319-327. http://dx.doi.org/10.1007/BF02182013

Khanna, V. K. (2003). *Plant Tissue Culture Practice* (pp. 13-22). Kalyani Publishers. New Delhi, India.

Kharat, S. G., Bhave, S. G., Nadkarni, H. R., Sawant, S. S., & Bendale, V. W. (2008). Micropropagation of Lesser Yam [*Dioscorea esculenta* (Lour.) Burk]. *Journal of Root Crops, 34*(1), 65-69.

Kohmura, H., Araki, H., & Imoto, M. (1995). Micropropagation of 'yamatoimo' Chinese yam (*Dioscorea opposita* Thunb.) from immature leaves. *Plant Cell Tissue Organ Cult., 40*, 271-276. http://dx.doi.org/10.1007/BF00048133

Kole, C. (2011). Wild Crop Relatives: Genomic and Breeding Resources industrial crops. Springer-Verlag Berlin Heidelberg, pp. 71-96.

Lakshmisita, G., Bammi, R. K., & Randhawa, G. S. (1976). Clonal propagation of *Dioscorea floribunda* by tissue culture. *J. Hortic. S 1*, 551-554.

Lebot, V. (2009). Tropical root and tuber crops cassava, sweet potatoes, potatoes, yams and aroids. Crop Production Science in Horticulture Series, 17, MPG books group

Mahesh, R., Muthuchelian, K., Maridass, M., & Raju, G. (2010). *In vitro* propagation of wild yam, *Dioscorea wightii* through nodal cultures. *Int. J.of Bio. Technol., 1*, 111-113.

Malaurie, B., Pungu O., & Trouslot, M. F. (1995). Influence of meristem- tip size and location on morphological development *Dioscorea cayenensis* Lam., *Dioscorea rotundata* Poir. Complex and *Dioscorea praehensilis* Benth. *Plant Cell Tissue Organ Cult., 42*, 215-218. http://dx.doi.org/10.1007/BF00034240

Mantell, S. H., & Hugo, S. A. (1989). Effects of photoperiod, mineral medium strength, inorganic ammonium, sucrose and cytokinin on root, shoot and microtuber development in shoot cultures of *Dioscorea alata* L. and *D. bulbifera* L. Yams. *Plant Cell Tissue Organ Cult. 16*, 23-37. http://dx.doi.org/10.1007/BF00044069

Mantell, S. H. (1998). Microbes intimately associated with tissue and cell culture of tropical *Dioscorea* yams. *Plant Cell Tissue Organ Cult, 52*, 47-52. http://dx.doi.org/10.1023/A:1005999925907

Mantell S. H., Haque S. Q., & Whithall A. P. (1978). Clonal propagation of *Dioscorea alata* L. and *Dioscorea rotundata* Poir Yams by tissue culture. *J. Hortic. Sci., 51*, 95-98.

Mantell, S. H., Haque, S. Q., & Whitehall, A. P. (1978). Clonal multiplication of *Dioscorea alata* L. and *Dioscorea rotundata*, yams by tissue culture. *Journal of Horticultural Science, 53*, 95-98.

Manyong, V. M. (2000). Farmers' perceptions of the resource management constraints in yam-based systems. In Project 13: improvement of yam-based systems. Annual Report 1999. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp. 3-4.

Martine, J., & Cappadocia, M. (1991). In vitro tuberization in *Dioscorea alata* L. 'Brazo fuerte' and 'Florand *Dioscorea abyssinica* Hoch. *Plant Cell Tissue Organ Culture, 26*, 147-152.

Mascarenhas, A. F., Hendre, R. R., Nadgir, A. L., Ghugole, D. D., Godbole, D. A., & Prabhu, R. A.(1976). Development of plantlets from cultured tissue culture. In A. Fujiwara (Ed.), *Plant Tissue Culture.Tokyo.Maruzen*. (pp.719-720).

Mbanaso, E. N. A., Chukwu, L. I., & Opara, M. U. A. (2007). *In vitro* basal and nodal microtuberization in yam shoot cultures (*Discorea rotundata* poir, cv. Obiaoturugo) under nutritional stress conditions. *African Journal of Biotechnology, 6*(21), 2444-2446.

Mie´ge, J., & Demissew, S. (1997). *Dioscoreaceae*. In S. Edwards, S. Demissew, & I.

Hedberg (Eds.), *Flora of Ethiopia & Eritrea* (Vol 6, pp. 55-62), Hydrocharitaceae to Araceae. Addis Ababa, Ethiopia, and Uppsala, Sweden.

Murashige, T. (1974). Plant propagation trough tissue cultures. *Annual Review of Plant Physiology, 25*, 135-166. http://dx.doi.org/10.1146/annurev.pp.25.060174.001031

Mwirigi, P. N., Kahangi, E. M., Nyende, A. B., & Mamati, E. G. (2010). *In vitro* propagation of the Kenyan yam (*Dioscorea* spp.) *African journal of horticultural science, 3*, 112-122

Narula, A., Kumar, S., & Srivastava, P. S. (2007). Genetic fidelity of *in vitro* regenerants, encapsulation of shoot tips and high diosgenin content in *Dioscorea bulbifera* L., a potential source of diosgenin. *Biotechnol. Lett., 29*, 623-629. http://dx.doi.org/10.1007/s10529-006-9276-3

Ng, S. Y. C. (1988). In vitro tuberization in white yam (*Dioscorea rotundata*). *Plant Cell Tissue Organ Culture, 14*, 121-128. http://dx.doi.org/10.1007/BF00041185

Obssi, D., Kassahun, B., & Mulugeta, D. (2015). Effects of different combination of plant growth regulator on *in* vitro propagation of yam (*Dioscorea* species). *Journal of applied Biotechnology, 3*(2), 20-40. http://dx.doi.org/10.5296/jab.v3i2.7471

Omar, M. S., & Aouine, M. (2007). Commercial *in vitro* Mass Propagation of Plants: Current Status and Future, 94-99.

Poornima, G. N., & Ravishankar, R. V. (2007). *In vitro* propagation of wild yams, *Dioscorea oppositifolia* (Linn) and *Dioscorea pentaphylla* (Linn). *Afr. J. Biotechnol., 6*(20), 2348-2352.

Poornima, G. N., & Ravishankar, R. V. (2007). *In vitro* propagation of wild yams, Dioscorea oppositifolia (Linn) and Dioscorea pentaphylla (Linn). *African Journal of Biotechnology, 6*, 2348-2352.

Ramirez-Magon, R., Borodanenko, A., Barrera-Guerra, J. L., & Ochoa Alejo, N. (2001). Shoot number and shoot size as affected by growth regulators *in vitro* cultures of *Spathyphyllum floribundum* L. *Scin Horti., 89*, 227-236. http://dx.doi.org/10.1016/S0304-4238(00)00236-3

Razdan, M. K. (2003). Introduction to plant tissue culture (2nd ed., pp. 234-236) Science publisher, Inc., USA.

Saleil, V., Degras, L., & Jonard, R. (1990). Obtention de plantes indemmes de virus de la mosaique de l'igname americaine *Dioscorea trifida* L. *Agronomie, 10*, 605-615. http://dx.doi.org/10.1051/agro:19900801

Satour, M., Mitaine-Offer, A. C., & Lacaille-Dubois, M. A. (2007). The *Dioscorea* genus: A review of bioactive steroid saponins. *J. Nat. Med., 61*, 91-101. http://dx.doi.org/10.1007/s11418-006-0126-3

Savikin-Fodulovic, K., Grubisic, D., Culafic, L., Menkovic, N., & Ristic, M. (1998). Diosgenin and phytosterols content in five callus lines of *Dioscorea balcanica*. *Plant Sci., 135*, 63-67. http://dx.doi.org/10.1016/S0168-9452(98)00036-3

Schenk, R. U., & Hildebrandt, A. C. (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot., 50*, 199-204. http://dx.doi.org/10.1139/b72-026

Sengupta, J., Mitra, G. C., & Sharma, A. K. (1984). Organogenesis and tuberization in cultures of *Dioscorea floribunda. Plant Cell Tissue Organ Cult., 3*, 325-331. http://dx.doi.org/10.1007/BF00043084

Shin, J. H., Kim, S. K., Kwon, J. B., Lee, B. H., & Shon, J. K. (2004). Factors affecting the production of in vitro plants from the nodal pieces of Chinese yam (*Dioscorea opposite* Thunb). *J. Plant Biotech., 6*(2), 97-102.

Shiwachi, H., Ayankanmi, T., & Asiedu, R. (2005a). Effect of photoperiod on the development of inflorescences in white guinea yam (*Dioscorea rotundata*). *Tropical Science, 45*(3), 126-130. http://dx.doi.org/10.1002/ts.6

Shiwachi, H., Kikuno, H., & Asiedu, R. (2005b). Mini tuber production using yam (*Dioscorea rotundata*) vines. *Tropical Science, 45*(4), 163-169. http://dx.doi.org/10.1002/ts.22

Shu, Y., Ying, Y. C., & Lin H. H. (2005). Plant regeneration through somatic embryogenesis from callus cultures of *Dioscorea zingiberensis*. *Plant Cell Tissue Organ Cult., 80*, 157-161. http://dx.doi.org/10.1007/s11240-004-9543-8

Tamiru, M. (2006). Assessing diversity in yams (*Dioscorea* spp.) from Ethiopia based on morphology, AFLP markers and tuber quality, and farmers' management of landraces. PhD thesis, Georg-August-University Goettingen, Germany. Cuvillier Verlag, Goettingen, Germany, p. 155.

Tamiru, M., Becker, H. C., Maass, B. L. (2007). Genetic Diversity in yam germplasm (*Dioscorea spp*.) from Ethiopia and their relatedness to the main cultivated *Dioscorea species* assessed by AFLP Markers. *Crop Science* (accepted). http://dx.doi.org/10.2135/cropsci2006.11.0719

Tamiru, M., Heiko, C., Becker, Brigitte, L., & Maass, L. (2008). Diversity, distribution and management of yam landraces (Dioscorea spp.) In Southern Ethiopia. *Genetic Resource of Crop Evolution, 55*, 115-131. http://dx.doi.org/10.1007/s10722-007-9219-4

Tamiru, M., Maass, B. L., & Becker, H. C. (2005). Traditional management and use of yams (*Dioscorea* spp.) in Wolayita, Southern Ethiopia. Book of abstracts, p. 433.

Thankappan, S. S., & Patell, V. M. (2011). *In vitro* propagation studies and genetic fidelity assessment of endangered medicinal wild Yam- *Dioscorea prazeri. Plant Omic Journal, 4*(4), 177-189.

Tor, M., Twyford, C. T., Funes, I., Boccon-Gibod, J., Ainsworth, C. C., & Mantell, S. H. (1998). Isolation and culture of protoplasts from immature leaves and cell suspension of *Dioscorea* yams: Tools for transient gene expression studies. *Plant Cell Tissue Organ Cult, 53*, 113-125. http://dx.doi.org/10.1023/A:1006028406641

Twyford, C. T., & Mantell, S. H. (1996). Production of somatic embryos and plantlets from root cells of Greater Yam. *Plant Cell Tissue Organ Cult., 46*, 17-26. http://dx.doi.org/10.1007/BF00039692

Uduebo A. E, (1971). Effect of external supply of growth substances on axillary proliferation and development in *Dioscorea bulbifera*. *Ann. Bot., 35*, 159-163.

Wang, S. J., Gao, W. Y., Liu, H. Y., Chen, H. X., Yu, J. G., & Xiao, P. G. (2006). Studies on the physicochemical, morphological, thermal and crystalline properties of starches separated from different *Dioscorea opposita*. *Food Chemistry, 99*, 38-44. http://dx.doi.org/10.1016/j.foodchem.2005.07.007

Westphal, E. (1975). Agricultural systems in Ethiopia (p. 278). Center for Agriculture Publishing and Documentation, Wageningen, the Netherlands.

Wheatley, A. O., Ahmed, M. H., & Asemota, H. N. (2003). Development of salt adaptation *in vitro* greater yam (*Dioscorea alata*) plantlets. *In vitro* Cell. *Dev. Biol. Plant., 39*, 346-353. http://dx.doi.org/10.1079/IVP2002402

Wilkin, P. (1998). A morphometric study of *Dioscorea quartiniana* A. Rich (*Dioscoreaceae)*. *Kew Bulletin, 54*, 1-18. http://dx.doi.org/10.2307/4111020

Wilkin, P. (2001). *Dioscoreaceae* of South-Central Africa. *Kew Bull, 56*, 361-404. http://dx.doi.org/10.2307/4110963

Wilson, J. E. (1989). Rapid Multiplication of Yams (*Dioscorea spp*.). IRETA Publications, Western Samoa.

Xu, J., Yin, H., Wang, W., Mi, Q., & Liu, X. (2009). Effects of sodium nitroprusside on callus induction and shoot regeneration in micropropagated *Dioscorea opposita. Plant Growth Regul, 59*, 279-285. http://dx.doi.org/10.1007/s10725-009-9410-z

Yan, H., Yang, L., & Li, Y. (2011). Axillary shoot proliferation and tuberization of *Dioscorea fordii* Prain et Burk. *Plant Cell Tissue Organ Culture, 104*, 193-198. http://dx.doi.org/10.1007/s11240-010-9818-1

Copyright Disclaimer