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# Effect of Different Combinations of Plant Growth Regulators on *in vitro* Propagation of Yam (Dioscorea Species)

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**Abstract**

Seed tubers are expensive, bulky 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. No information is available on micropropagation of Dioscorea spp. in Ethiopia. To overcome such problem, the current study was, initiated to optimize type and concentration of plant growth regulators for shoot multiplication and rooting. Shoot multiplication and rooting in vitro were carried out using a completely randomized design with five replications. For shoot multiplication, shoots initiated from nodal cuttings were treated with BAP in combination with NAA. For rooting, the micro-shoots were transferred to ½ MS media containing NAA and IBA. Data on number of shoots per explant, roots per shoot and related growth parameters were recorded and statistically analyzed. The results showed that MS media supplemented with 1.5 mg/l BAP + 0.15 $mgl^{-1}$ NAA gave an average of 6.40 ± 0.28 shoots per explant with a mean shoot length of 2.0±0.11 cm. MS supplemented with BAP (1.0 mg $l^{-1}$) and NAA (0.15 mg $l^{-1}$) gave 5.40 ± 0.28 with a mean shoot length of 1.84 ± 0.20 cm. Plantlets

grown on half strength MS media supplemented with 2.0 mg $l^{-1}$ NAA and 0.5 mg $l^{-1}$ IBA produced 5.7 ± 0.27 roots per plantlet and an average root length of 4.63±0.23 cm. Thus MS + 1.5 $mgl^{-1}$ BAP + 0.15 mg $l^{-1}$ NAA and 1/2 MS +2.0 mg $l^{-1}$ NAA + 0.5 $mgl^{-1}$ IBA is the best hormone combination for shoot multiplication and in vitro rooting respectively.

**Keywords**: Dioscorea species, Micropropagation, Nodal cutting, Yam

### 1. Introduction

Yam belongs to the genus *Dioscorea* in the family Dioscoreaceae. The family is believed to be among the earliest angiosperms and probably originated in Southeast Asia (Coursey, 1976). It 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). About 600 species of yam have been recorded (Coursey, 1967; Govaerts et al., 2007), but the major cultivated ones are *D. alata, D. bulbifera, D. cayenensis, D. esculenta, D. opposita-japonica, D. nummularia, D. pentaphylla, D. rotundata* and *D. trifida* (Boussalem et al., 2006; Lebot, 2009).

The dominant region for yam production in the world is West Africa, where about 48 million tons (about 93% of the world's production) produced on 4 million hectares annually (Hahn, 1995; Wilkin, 2001; Mulaama, 2004; FAO, 2009). In Ethiopia, the total annual production of yam was estimated at about 277,000 metric tons from an area of about 68,000 ha, corresponding to a yield of about 4 tons per hectare (FAO, 2009). However, Gemeda (2000) reported that yam is more productive than the other tuber crops in the area, with an estimated yield of about 20 tons per hectare. Expanded production of yams in new areas or where it is produced in limited quantities in other parts of the tropics including Ethiopia would contribute significantly to food security, improved health and increased income (Westphal, 1975; Mie´ge & Demissew, 1997; Lebot, 2009; Asiedu & Sartie, 2010).

As the crop is adapted to dry season planting (mainly at the onset of the dry season in October) early harvests in May 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 sources of biologically active compounds in pharmaceutical industries (Wang et al., 2006; 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 are often those produced by diseased plants by nematodes and Insects such as yam shoot beetle, often interact with fungi (*Botryodiplodia*, *Fusarium*) and bacteria (*Erwinia* spp.) to 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, farmers often encounter shortages of yam planting material, especially following droughts and disease epidemics. 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 (Asiedu & Sartie, 2010; Ghosh et al., 1988).

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).

So, 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).

*In vitro* propagation has been implemented for many *Dioscorea* species, such as *D. rotundata* (Balogun et al., 2006), *D. nipponica* Makino. (Chen et al., 2007), *Dioscorea esculenta* (Lour.) Burk, (Kharat et al., 2008), *D. hispida (*Behera *et al.,* 2008*), Dioscorea alata* L.cv. Hatikhujia *(*Behera et al., 2010), wild yam (*Dioscorea wightii),* Mahesh et al., 2010, *Dioscorea fordii (*Yan et al., 2011) and they reported that the response of yam (*Dioscorea spp.*) towards *in vitr*o propagation depends on the presence and absence of auxin and cytokinin.

However, a protocol developed for one specific species of plant is not reproducible when applied to other genotypes even within the same species i.e. each genotype has its own

requirements in all stages of *in vitro* propagation (Omar & Aouine, 2007). Likewise the response of yam *in vitro* is genotype specific (Balogun et al., 2004; Ahanhanzo et al., 2010). Despite its importance especially for food security, no information is available on *vitro* propagation of yam variety Aw-004/00. The variety is believed to be widely distributed in Southern and Southwestern part of Ethiopia and it is the only released variety of yam in the country. This variety was released by Hawassa Agricultural Research Center in 2010. It is characterized by high yield (25 t/ha on farmers' field) and it is preferred by farmers (MoA, 2010). The current study was, therefore, initiated with the following objectives:

- To optimize concentration of BAP in combination with NAA for shoot multiplication
- To determine the optimum concentration and combination of NAA and IBA for rooting.

## 2. Materials and Methods

### *2.1 Planting Materials*

Yam variety Aw-004/00 (*Dioscorea spp.*) was used as a source of explant. The variety was obtained from Hawassa Agricultural Research Center.

### *2.2 Study Area Description*

These experiments were carried out in the tissue culture laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), during 2011-2012. The study area is located 363 km south west of Addis Ababa at 7°46' N latitude and 36°0'E longitudes. The mean annual rainfall is 1529.5 mm and the mean annual max/min temperature is 26.2/11.3 °C (IAR, 1997).

### *2.3 Mother Plant and Explants Preparation*

The seedlings of Aw-004/00 variety were established and grown in the greenhouse and they were grown until they form 15 or more nodal segments per plantlet. The growing nodal segments were cut from the seedling and prepared by removing extra leaf sheaths and then taken to laboratory for surface sterilization.

Explants (5-10 cm nodal segments) were washed under running tap water and then treated with 0.3% a fungicide (Kocide) solution for 20-30 minutes. Then, the explants were treated with mercuric chloride (0.1% $HgCl_2$ for 5 minutes) solution followed by 70% ethanol for 1 min. The explants were then thoroughly rinsed three times with sterile distilled water and the explants were reduced to one node size (about 1-2 cm) and placed on MS media containing 1 mg/l BAP under aseptic condition in laminar flow cabinet, which has been used as initiation media for yam (Mwirigi et al., 2010; Chen et al., 2007). The explants started initiation within 4-8 days after culturing.

### *2.4 Culture Medium and Condition*

Murashige and Skoog (MS) (1962) basic media was used for all experiments (Appendix 3). Surface sterilized explants were cultured in culture jar containing 30-40 ml media supplemented with $1mgl^{-1}$ BAP and one explant per jar was used for shoot initiation. Then the initiated explants were transferred to glass jars with a capacity of 350 ml (560 × 355 mm) containing 50-60 ml media for shoot multiplication. Similarly ½ MS media were prepared and 30-40 ml media were poured into pre sterilized glass culture jars with a capacity of 120 ml for rooting.

All the aseptic activities such as surface disinfection of explants, preparation and inoculation of explants and subsequent sub-culturing were carried out in the laminar air flow cabinet. The working table of laminar air flow cabinet and spirit lamp were sterilized by swabbing with 70% ethanol. All the required materials like media, spirit lamp, lighter and glass ware were kept under the clean laminar air flow hood. The cultures were maintained at 16 hour photoperiod, temperature of 25 ± 2 °C, humidity of 60-70% and light intensity of 1500-2000 lux (20-27 µmol $m^{-2}$ $s^{-1}$ ) provided by florescent light.

*2.5 Treatments and Experimental Design*

All the experiments were laid down in a completely randomized design (CRD) with five replications (five jars each with three explants , a total of 15 explants for multiplication and each with two shoots, a total of 10 shoots per treatment for rooting were used).

2.5.1 Experiment 1: Effects of BAP and NAA Combination on Shoot Multiplication

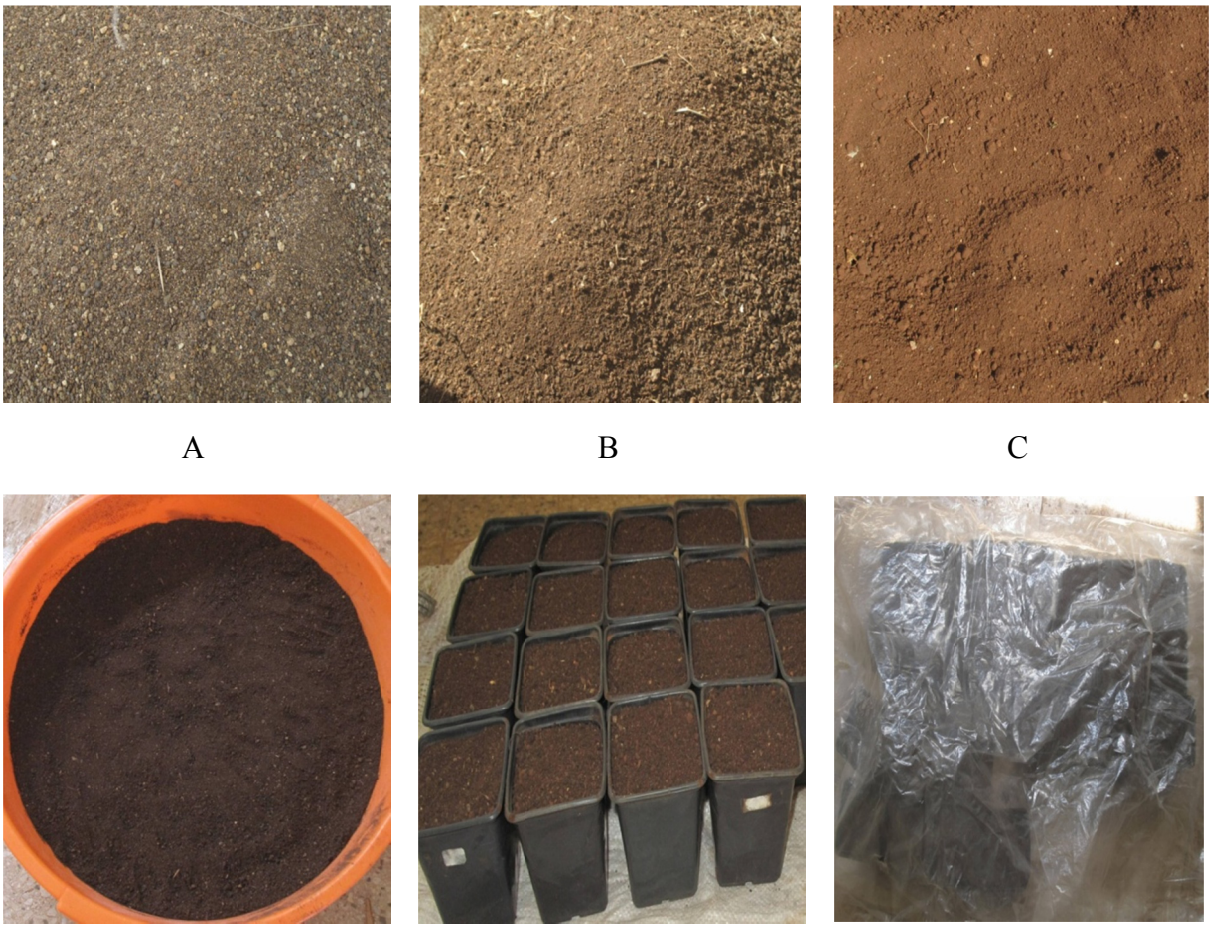
In this experiment, MS medium containing 3% sucrose, 0.8% agar, and different levels of BAP and NAA was used to test the effects of different plant growth regulators on shoot multiplication. Four concentrations of BAP (0.5, 1, 1.5 and 2 mg $l^{-1}$) and NAA (0, 0.15, 0.25, and 0.5 $mgl^{-1}$which is similarly NAA concentration used by Kharat et al. (2008)) in 4 × 4 factorial combinations were used. Basal medium (without plant growth regulators) was included as a control.

2.5.2 Experiment 2: Effect of NAA and IBA Combinations on *in vitro* Rooting

Under this experiment, the effect of auxin types and concentrations on rooting of *in vitro* shoots were studied. Accordingly, ½ MS medium supplemented with five concentrations of IBA (0, 0.5, 1, 1.5, 2 $mgl^{-1}$) and five concentrations of NAA (0, 0.5, 1, 1.5, 2 $mgl^{-1}$) in 5x5 factorial combinations were tested.

2.5.3 Acclimatization

Plantlets with three to four leaves were transplanted to plastic pots filled with sterilized nursery medium in equal ratio of manure, forest soil and sand (Figure 1) after agar is completely removed from the roots by washing under tap water and then covered with polyethylene plastic sheet in the greenhouse. The plants were sprayed three times with water every (morning, midday and night) for a period of three weeks. After four weeks, percent of acclimatized seedlings or plantlets were recorded.



A B C

D E F

Figure 1. Soil medium preparation for acclimatization. Sand (A). Dried and decomposed manure (B). Forest soil (C). Sterilized 1:1:1 combination of sand: manure: soil (D&E). Plantlets covered by plastics soon after their transfer on to the soil (F)

*2.6 Data Collected*

Data were collected on growth parameters of *in vitro* generated plantlets after 40-45 days of culturing on multiplication media and four weeks after culturing on rooting media. Number of nodes, number of shoots, and number of leaves were counted from five jars each containing three explants, a total of 15 explants. Number of roots and root length were counted from five jars each with 2 shoot, from a total of 10 shoots per treatment. The average number of nodes/explant, average number of shoots/explant, mean shoot length, average number of leaf/explant, average number of roots/shoot and mean of root length were computed from the collected data.

*2.7 Data Analysis*

The collected data were analyzed using SAS (version 9.2*)* software (SAS Institute Inc. 2008). The data were subjected to analysis of variance (ANOVA) and mean separation was done using procedure of REGWQ for significant means at a probability of 5%.

## 3. Results and Discussion

The present study was conducted to investigate optimal concentrations and combinations of plant growth regulators in the medium for micropropagation in yam via nodal culture. The results obtained are presented and discussed as follows:

### *3.1 Effects of BAP and NAA Combination on Shoot Multiplication*

Analysis of variance showed that BAP and NAA combinations were highly significantly different ($p< 0.0001$) for shoot multiplication (Appendix 1). The response of nodal explants cultured on different shoot multiplication media is presented in Table 1. The results showed that culture medium devoid of plant growth regulators (control) failed to stimulate any shoot multiplication in the cultured explants within a period of five weeks. MS media supplemented with growth regulators (BAP in combination with NAA) produced better results in terms of shoots /explant, average shoot length and average number of nodes per explant.

Among the combination tested, 1.5 $mgl^{-1}$ BAP + 0.15 $mgl^{-1}$ NAA resulted in the highest response in which an average of 6.40 ± 0.28 shoots per explant with a mean shoot length of 2.0 ± 0.11 cm was obtained (Table 1; Figure 2A). The second best shoot multiplication (5.40 ± 0.28 shoots per explants and mean shoot length of 1.84 ± 0.20 cm) was obtained on MS medium supplemented with BAP (1.0 $mgl^{-1}$) + NAA (0.15 $mgl^{-1}$) (Figure 2B). MS medium supplemented with BAP (1.5 $mgl^{-1}$) + NAA (0.25 $mgl^{-1}$) resulted in 4.33±0.34 shoots/explant with a shoot length of 4.0±0.12 where number of shoots per explant is significantly lower than that of the first two combinations of plant growth regulators whereas the shoot length is significantly improved (Table 1, Figure 3A).

The dependency of cultured nodal explants of *Dioscorea spp.* on the presence or absence of cytokinin and auxin for shoot multiplication has already been established (Adeniyi et al., 2008; Mwirigi et al., 2010). This has also been recently reported in the case of micropropagation of other yams like *D. composite* (Alizadeh et al., 1998), *D. oppositifolia* (Behera et al., 2009), *Dioscorea alata* (Behera et al., 2010). In this experiment, on MS medium supplemented with 1.5 $mgl^{-1}$ BAP alone, 4.33 shoots per explant was obtained. When 0.15 $mgl^{-1}$ NAA was added in the presence of 1.5 $mgl^{-1}$ BAP, a significant improvement in the number of shoots per explant (6.4,) was recorded showing the existence of interaction effect between BAP and NAA. This result confirms the reports of Chen et al. 2007 and Adeniyi et al. (2008) where media supplemented with BAP and NAA combination induced best shoot multiplication, indicating that the effectiveness of each of the phytohormone in inducing shoots multiplication was influenced by the presence or absence of the other.

Table 1. Shoot multiplication in nodal explants of *Dioscorea spp*. cultured on MS medium supplemented with various concentrations of BAP and NAA

| Treatments ($mg^{-1}$) | | Mean No of shoots/explant ±S.D. | Mean shoot length (cm) ±S.D. | Mean No of nodes/explant ±S.D. | Mean No of leaf/explant ±S.D. |
|---|---|---|---|---|---|
| BAP | NAA | | | | |
| 0 | 0 | $0.00^{h}$±0.0 | $0.00^{i}$±0.00 | $0.00^{h}$±0.00 | $0.00^{h}$±0.00 |
| 0.5 | 0 | $2.60^{gf}$±0.28 | $1.07^{fg}$±0.08 | $5.2^{ef}$±0.56 | $1.16(14.2)^{cd}$±0.13 |
| 0.5 | 0.15 | $4.53^{c}$±0.38 | $1.42^{e}$±0.16 | $6^{d}$±0.53 | $1.16(13.87)^{cd}$±0.11 |
| 0.5 | 0.25 | $2.20^{g}$±0.30 | $1.61^{de}$±0.12 | $6.20^{d}$±0.56 | $1.26(17.13)^{abc}$±0.01 |
| 0.5 | 0.5 | $0.00^{h}$±0.0 | $0.00^{i}$±0.00 | $0.00^{h}$±0.00 | $(0.00)^{h}$±0.00 |
| 1 | 0 | $3.60^{d}$±0.30 | $1.45^{e}$±0.19 | $6.00^{d}$±0.53 | $1.18(7.87)^{bcd}$±0.03 |
| 1 | 0.15 | $5.40^{b}$±0.28 | $1.84^{cd}$±0.20 | $7.00^{c}$±0.23 | $1.2(10.2)^{abcd}$±0.08 |
| 1 | 0.25 | $2.67^{efg}$±0.24 | $2.6^{b}$±0.15 | $7.53^{cb}$±0.30 | $1.3(11.6)^{ab}$±0.03 |
| 1 | 0.5 | $3.47^{d}$±0.3 | $1.06^{fg}$±0.18 | $3.40^{f}$±0.28 | $0.91(7.67)^{g}$±0.06 |
| 1.5 | 0 | $4.33^{c}$±0.24 | $1.75^{cd}$±0.12 | $7.07^{c}$±0.43 | $1.2(14.94)^{abcd}$±0.02 |
| 1.5 | 0.15 | $6.40^{a}$±0.28 | $2.0^{c}$±0.11 | $7.8^{b}$±0.18 | $1.26(17.27)^{abc}$±0.03 |
| 1.5 | 0.25 | $4.33^{c}$±0.34 | $4.0^{a}$±0.12 | $9.6^{a}$±0.28 | $1.32\ (19.67)^{a}$±0.01 |
| 1.5 | 0.5 | $3.20^{de}$±0.30 | $1.37^{e}$±0.13 | $4.6^{f}$±0.28 | $0.91\ (7.13)^{g}$±0.05 |
| 2 | 0 | $3.27^{d}$±0.28 | $0.77^{h}$±0.13 | $3.8^{g}$±0.38 | $0.95\ (14)^{fg}$±0.03 |
| 2 | 0.15 | $4.13^{c}$±0.38 | $1.104^{f}$±0.21 | $5.87^{de}$±0.38 | $1.05(14.93)^{ef}$±0.05 |
| 2 | 0.25 | $3.53^{d}$±0.38 | $1.64^{de}$±0.12 | $6^{d}$±0.53 | $1.1\ (18.67)^{de}$±0.05 |
| 2 | 0.5 | $3.07^{def}$±0.36 | $0.8^{gh}$±0.22 | $3.67^{g}$±0.24 | $0.94\ (7.13)^{g}$±0.05 |
| CV% | | 8.3 | 9.7 | 6.9 | 5.4 |

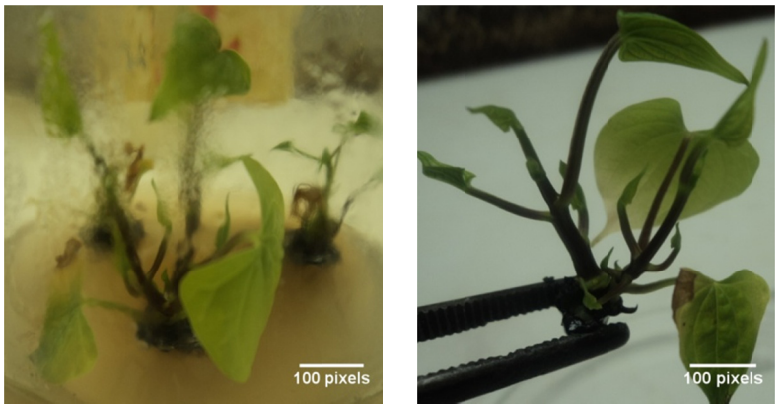
(a-i means having the same letters in a column were not significantly different at $P < 0.05$ level. S.D.-Standard deviation of mean, means in the brackets were the actual data while the one outside of the brackets were transformed data using log transformation)

It has been also reported that when Kenyan yam was cultured on media with BAP supplement alone, a limited proliferation of explants with an average of 1.1 shoots per cultured explant was observed while addition of 0.02 $mgl^{-1}$ NAA produced an average of 2.1 shoots per explant (Mwirigi et al., 2010).

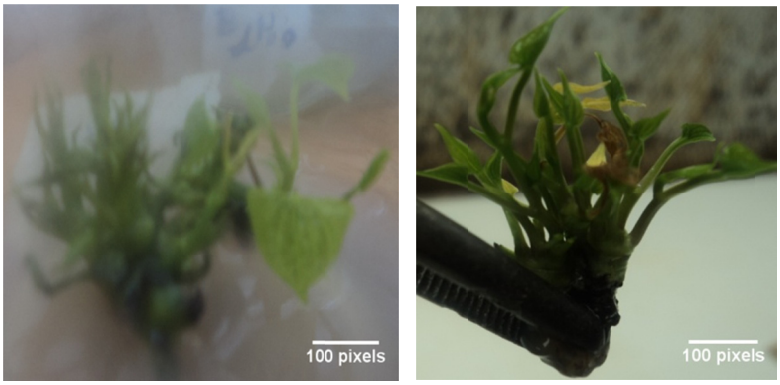
A B

Figure 2. Shoot multiplication. Shoot multiplied on BAP 1.5mgl$^{-1}$ & NAA 0.15(A). Medium supplemented with 1 mgl$^{-1}$ BAP & 0.15 mgl $^{-1}$ NAA (B)

In this study only single cytokinin was found to be used for shoot multiplication but some authors suggested that the combination of two cytokinins with auxin were needed for producing higher number of multiple shoots. Behera and his coworkers in 2009 obtain an average of 10.5 ± 0.51 shoots on MS+ Kinetin (2.0 mgl$^{-1}$) +BAP (1.0 mgl$^{-1}$) + NAA (0.5 mgl$^{-1}$) with ascorbic acid 100mgl$^{-1}$ followed by MS + Kinetin (1.5 mgl$^{-1}$) + BAP (1.0 mgl$^{-1}$) + NAA (0.5 mgl$^{-1}$) + 100 mgl$^{-1}$ ascorbic acid where 5.5 ± 0.43 shootlets were obtained. In 2010 the same authors reported 6.5 ± 0.42 shootlets on Kinetin (1.5 mgl$^{-1}$) + BAP (1.0 mgl$^{-1}$) + NAA (0.5 mgl$^{-1}$) + 100 mg l$^{-1}$ ascorbic acid with different yam species which is completely different from present study in terms of methodology but similar outcome with best result of the present study.




A

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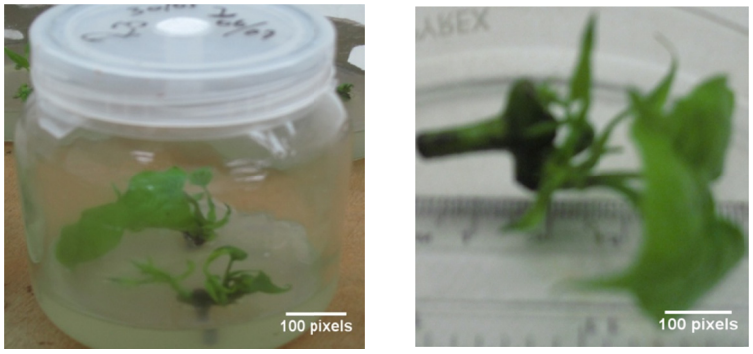


C

Figure 3. Shoot multiplication when it is inside and out of the jar. MS medium + 1.5 $mgl^{-1}$ BAP + 0.25 $mgl^{-1}$ NAA (A). MS + 1.5 $mgl^{-1}$ BAP + 0.15 $mgl^{-1}$ NAA ( B). MS + 1 $mgl^{-1}$ BAP + 0.15 $mgl^{-1}$ NAA (C)

In other reports a combination of single cytokinin with auxin was needed for better production of multiple shoots. Behera et al. (2008) reported that BAP (2.0 $mgl^{-1}$) + NAA (0.5 $mgl^{-1}$) with 100 $mgl^{-1}$ ascorbic acid, producing an average of 6 ± 0.18 shoot lets per explants. They also reported that the second best shoot multiplication (4.5 ± 0.12) on a medium supplemented with BAP (1.5 $mg1^{-1}$) + NAA (0.5 $mgl^{-l}$) + 100 $mg^{-l}$ ascorbic. Similarly

Thankappan and Patell (2011) reported highest rate of multiplication on media with 0.5 $mgl^{-1}$ BAP and 0.01$mgl^{-1}$ NAA which was the only hormone combination they have used. Yan et al. (2011) obtained 5.5 shoots per explant on 1.0 mg $l^{-1}$ BAP + 0.1 mg $l^{-1}$ NAA + 30 g $l^{-1}$ sucrose in liquid culture which is in agreement with the second best result of this study.

*3.2 Effect of NAA and IBA Combination on Rooting*

Analysis of variance showed that there was highly significant difference between treatments ($p< 0.0001$) for rooting (Appendix 2). There was also highly significant interaction between NAA and IBA. The rooting responses of shoots on different media, including mean number of roots/shoot and mean root length over a period of four weeks were significantly different (Table 2). There was no rooting in case of shoots planted on auxin free (basal) medium (Figure 4 C; Table 2). Similarly, at lower level of NAA (0.5 $mgl^{-1}$) there was hardly any rooting in the cultured shoots during the four weeks of observation period. However as shown in Table 2 higher concentration of NAA (1.5 and 2.0 $mgl^{-1}$) and IBA at some concentration tested responded well.

Rooting was best on cultures which had combinations of 1/2 MS+2.0 $mgl^{-1}$ NAA + 0.5 $mgl^{-1}$ IBA where an average number of 5.7±0.27 roots per plantlet and an average root length of 4.63 ± 0.23 cm was recorded (Table 2, Figure 4A). The second highest rooting was recorded on 1/2 MS + NAA (2 $mgl^{-1}$) + IBA (0 $mgl^{-1}$) with an average of 4.8 ± 0.27 roots per plantlets and an average root length of 3.52 ± 0.20 (Figure 4B). As shown in Table.2 NAA*IBA was more effective than their separate effects on rooting.

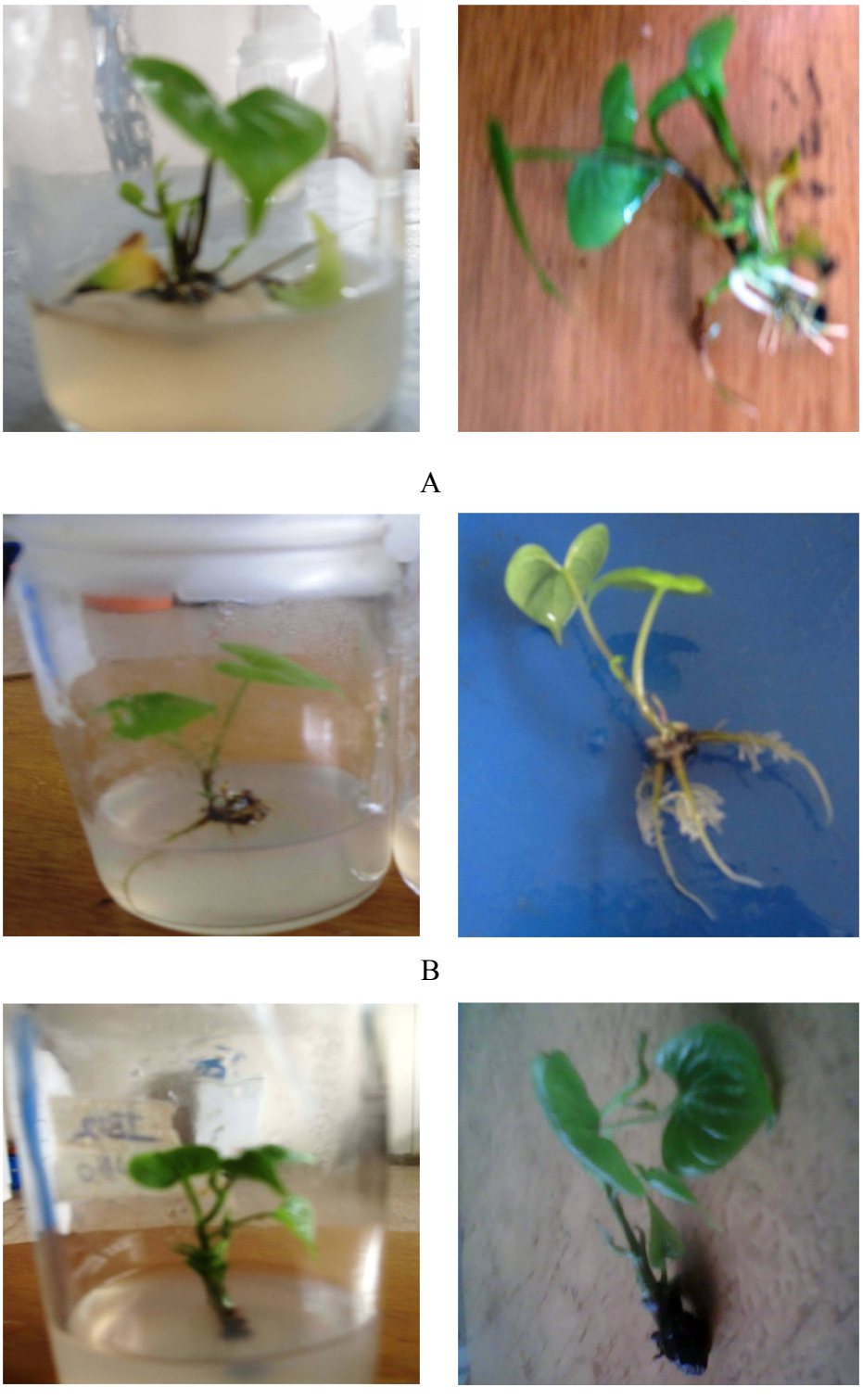

Figure 4. *In vitro* developed roots when it is in and outside of the jar. 1/2 MS medium supplemented with 2mgl$^{-1}$ NAA + 0.5 mgl$^{-1}$ IBA (A). Half MS + 2 mgl$^{-1}$ NAA + 0 mgl$^{-1}$ IBA (B). Control (i.e. NAA &IBA at zero level) (C)

Table 2. Influence of different combination of NAA and IBA on rooting of *in vitro* generated shoot lets of *Dioscorea* spp. (Aw- 004/00)

| Plant growth regulators ($mg^{-l}$) | | Mean numbers of root /shoot ±S.D | Mean root length (cm) ±S.D |
|---|---|---|---|
| NAA | IBA | | |
| 0 | 0 | $0.00^{l}$ ±0.00 | $0.00^{o}$±0.00 |
| 0 | 0.5 | $0.45(1.8)^{hij}$ ±0.04 | $0.84^{n}$±0.09 |
| 0 | 1 | $0.45(1.8)^{hij}$ ±0.04 | $0.92^{nm}$±0.04 |
| 0 | 1.5 | $0.57(2.7)^{efg}$ ±0.03 | $1.09^{klm}$ ±0.03 |
| 0 | 2 | $0.5(2.2)^{ghi}$ ±0.04 | $1.18^{kl}$ ±0.03 |
| 0.5 | 0 | $0.23(0.7)^{k}$ ±0.07 | $0.75^{n}$ ±0.03 |
| 0.5 | 0.5 | $0.43(1.7)^{ij}$ ±0.04 | $1.05^{lm}$±0.09 |
| 0.5 | 1 | $0.52(2.3)^{fgh}$ ±0.04 | $1.28^{jk}$ ±0.05 |
| 0.5 | 1.5 | $0.53(2.4)^{fg}$ ±0.03 | $1.42^{ij}$ ±0.04 |
| 0.5 | 2 | $0.43(1.7)^{ij}$ ±0.04 | $1.29^{jk}$ ±0.02 |
| 1 | 0 | $0.38(1.4)^{j}$±0.04 | $1.64^{gh}$ ±0.16 |
| 1 | 0.5 | $0.5(2.2)^{ghi}$ ±0.04 | $1.74^{fg}$ ±0.16 |
| 1 | 1 | $0.56(2.6)^{efg}$ ±0.03 | $1.52^{hi}$ ±0.05 |
| 1 | 1.5 | $0.58(2.8)^{efg}$ ±0.03 | $1.60^{ghi}$ ±0.10 |
| 1 | 2 | $0.5(2.2)^{ghi}$±0.04 | $1.91^{ef}$ ±0.06 |
| 1.5 | 0 | $0.68(3.8)^{cd}$±0.03 | $1.98^{e}$ ±0.06 |
| 1.5 | 0.5 | $0.72(4.3)^{bc}$ ±0.02 | $2.90^{c}$ ±0.11 |
| 1.5 | 1 | $0.63(3.3)^{de}$ ±0.03 | $2.40^{d}$ ±0.06 |
| 1.5 | 1.5 | $0.59(2.9)^{ef}$ ±0.03 | $2.12^{e}$ ±0.07 |
| 1.5 | 2 | $0.57(2.7)^{efg}$ ±0.03 | $1.74^{fg}$ ±0.12 |
| 2 | 0 | $0.76(4.8)^{ab}$ ±0.02 | $3.52^{b}$ ±0.20 |
| 2 | 0.5 | $0.82(5.7)^{a}$ ±0.02 | $4.63^{a}$ ±0.23 |
| 2 | 1 | $0.72(4.3)^{bc}$ ±0.02 | $2.90^{c}$ ±0.16 |
| 2 | 1.5 | $0.52(2.3)^{fgh}$ ±0.04 | $1.96^{e}$ ±0.08 |
| 2 | 2 | $0.28(0.9)^{k}$ ±0.06 | $1.47^{hij}$ ±0.06 |
| CV% | 6.97 | | 5.8 |

(a-o, means having the same letter in a column were not significantly different ($p < 0.05$). S.D.-Standard deviation of mean, means in the brackets is actual values while the one outside are transformed values using log transformation).

Production of plantlets with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil (Ohyam, 1970). In the present study best rooting was observed on half strength MS with NAA (2.0 $mgl^{-1}$) and IBA (0.5 $mgl^{-1}$). In this study the combinations of NAA and IBA were used but some authors suggested that auxin NAA or IBA alone is needed to profuse rooting on *Dioscorea hispida* (Behera et al., 2008), *Dioscorea oppositifolia l.* (Behera et al., 2009), *Dioscorea alata* (Behera et al., 2010) and reported that NAA is more effective than IBA.

On the other hand some authors reported that combination of auxin NAA and IBA was needed for better production of roots in *Dioscorea prazeri* (Thankappan & Patell, 2011), *Dioscorea nipponica* (Chen et al., 2007) who obtained best rooting on a medium containing 1.0 $mgl^{-1}$ IBA and 0.5 $mgl^{-1}$ NAA and 0.5 $mgl^{-1}$ IBA and 0.01 $mgl^{-1}$ NAA respectively. In addition Behera et al. (2008) reported 5.2 ± 0.28 roots per plantlet and an average root length of 3.5 ± 0.12 cm for *D. hispida.* Behera et al. (2009) reported 6.5 ± 0.30 roots per plantlet and an average root length 4.5 ± 0.16 cm. Similarly Behera et al. (2010) obtained 5.5 ± 0.48 roots per plantlet and an average root length 5.2 ± 0.26 cm on ½ MS + 2 $mgl^{-1}$ NAA without IBA, which is the second best in the present study.

*3.3 Acclimatization*

About 86% of the rooted plantlets exhibited establishment in the greenhouse within 3-4 weeks after being transferred to the sterilized soil medium (1:1:1 sand, forest soil and dried and decomposed manure, Figure 5). This result is almost similar with that of Behera et al., 2008; Behera et al., 2009 and Behera et al., 2010 who reported about 90% of the rooted plantlets established in the greenhouse within 2-3 weeks of transfer on similar medium. Chen et al. (2007) also reported 91%, survival rate of the plantlets one month after acclimatization and the young plants grew vigorously in the greenhouse.

Figure 5. Plantlets after four weeks of acclimatization in the greenhouse

## 4. Conclusion

From MS Medium supplemented with 1.5 mg/l BAP + 0.15 NAA 6.40 ± 0.28 shoot lets with a mean shoot length of 2.0 ± 0.11 cm per explant was recorded and 5.40±0.28 shoots with a mean shoot length of 1.84 ± 0.20 cm from 1 mg/l BAP + 0.15 NAA. There was no any multiplication on basal medium.

On half strength MS medium supplemented with 2 mg/l NAA + 0.5 IBA 5.7 ± 0.27 roots per plantlet and an average root length 4.63 ± 0.23 cm was recorded followed by 2 mg/l NAA + 0 mg/l IBA that is almost similar to 1.5 mg/l NAA + 0.5 mg/l IBA where 4.8 ± 0.27 roots per plantlets and an average root length of 3.52 ± 0.20 were recorded. There is no any rooting on basal medium (medium free of plant growth regulators). In addition NAA was better than IBA for *in vitro* rooting of this variety. The rooted plantlets were transferred to sterilized soil for further hardening with a 1:1:1 mixture of sand, forest soil and dried and decomposed manure for further hardening. After four weeks of acclimatization 86% survival was recorded.

Generally A-w 004/00 gave 6.4 shoots per explant within 4-5 week of culturing. Thus, the optimized protocol is useful for *in vitro* propagation of this specific variety of yam planting material. MS media supplemented with 1.5 mg/l BAP + 0.15 NAA for shoot multiplication and ½ MS supplemented with 2 mg/l NAA + 0.5 IBA for rooting were the best growth regulator combinations for micropropagation of this specific variety of yam and these media combination can be recommended for further use.

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## Appendices

Appendix 1. Mean squares for shoot growth parameters as affected by combination of BAP and NAA

| Source of variation | DF | Number of shoots/explant | Number of nodes/explant | Number of leaves/shoot | Average shoot length |
|---|---|---|---|---|---|
| BAP | 3 | 17.14*** | 33.85*** | 0.32605706*** | 7.1*** |
| NAA | 3 | 25.64*** | 75.65*** | 1.24430662*** | 9.8*** |
| BAP* NAA | 9 | 2.76*** | 4.58*** | 0.29455963*** | 0.89*** |
| CV (%) | | 8.3 | 6.9 | 5.4 | 9.7 |

***= highly significant at P< 0.0001, P = probability value, MS= mean square, DF= degree of freedom, CV = Coefficient of variation, BAP= 6-benzylaminopurine, NAA= α-naphthalene acetic acid.

Appendix 2. Mean squares for root growth parameters as affected by combination of IBA and NAA

| Source of variation | DF | Number of root / shoot | Average root length |
|---|---|---|---|
| NAA | 4 | 0.31*** | 17.38*** |
| IBA | 4 | 0.16*** | 2.09*** |
| NAA*IBA | 16 | 0.12*** | 2.09*** |
| CV (%) | | 6.97 | 5.8 |

***= highly significant at P< 0.0001, P = probability value, MS= mean square, DF= degree of freedom, CV = Coefficient of variation, IBA= indole-3-butyric acid, NAA= α-naphthalene acetic acid.