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Effect of Plant Growth Regulators on in Vitro Propagation of Yam Landraces (Dioscorea Species) Using Nodal Segments

Ibrahim Hussein* Berihu Mengs[1] Temesgen Matiwos[2]
1.Jimma University College of Agriculture and Veterinary Medicine, Horticulture and Plant Science Department, P. O. Box 307, Jimma, Ethiopia
2.Jimma Agricultural Research Center, P. O. Box 192, Jimma, Ethiopia

Abstract
Conventional propagation of yam (Dioscorea spp.) is limited due to low propagation rates. However, *In vitro* propagation is the best alternative to overcome such limitations of conventional propagation. For both shoot multiplication and rooting experiments, nodal segments were cultured on hormone free MS medium with combination of 30 g/l sucrose and 8 g/l agar. For shoot multiplication the initiated shoots of both landraces were cultured on MS media supplemented with 0.0, 0.5, 1.0, 2.0 and 2.5 mg/l benzyl amino purine (BAP). For root induction, shoot lets were cultured on MS media supplemented with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l indol -3-butyric acid (IBA). Finally, for acclimatization, *in vitro* multiplied plantlets were transferred to greenhouse for hardening off. The results showed that the interaction effects of both landraces with BAP and IBA concentrations was significantly influenced *in vitro* yam shoot multiplication and rooting respectively. MS media fortified with 1.5 mg/l BAP gave the highest shoot number (6.79 ± 0.09) with a shoot length of 7.47± 0.47 cm in 75/02. The maximum shoot number (7.23 ± 0.21) with a maximum shoot length7.68 ± 0.24cm was obtained on MS media supplemented with 1.5 mg/l BAP for 6/02. On rooting, MS media with 1 mg/l and 0.5 mg/l IBA gave maximum rooting percentage (54.33% and 68%) for75/02 and 6/02 landraces, respectively. The maximum root number (10.03 ± 0.49) and root length10.76± 0.16 cm were recorded on MS media with 1.5 mg/l IBA for 75/02. The maximum root number (10.58 ± 0.26) with highest root length (10.42 ± 0.32) cm was obtained on MS media with 2.00 and 1.00 mg/l IBA respectively for landrace 6/02. In vitro raised plantlets were acclimatized and recorded 86% and 90% of survival rate in75/02 and 6/02, respectively, on soil medium with combination ratio of 1:2:1 top soil, sand soil and compost, respectively.
Keywords: BAP, IBA, Micro-propagation, *in vitro* culture Nodes, Dioscorea species Yam

Introduction

Yam (*Dioscorea species*) is a monocotyledonous tuber forming tropical vine, which belongs to the order *Liliflorae*, family *Dioscoreaceae*, and genus *Dioscorea* (Course, 1976). It is a drought tolerant tuber cropwhich iscapable of producing a good yield under water scarce conditions (Asiedu and Sartie, 2010). Of the 600 known *Dioscsorea species,* only 10 are consistently cultivated for food consumption (Coursey, 1967). The major cultivated *D. species* include: *D. alata, D. bulbifera, D, cayenensis, D. esculanta, D. rotundata, D. trifida*and *D. pentaphylla* (Lebot, 2009).Among these, *D. rotundata* is the most preferred and cultivated one, accounting for a large proportion of yam production in West Africa, which produces 93% of the world's yam (FAO, 2013).

Globally, *Dioscorea* is grown in an area of about7.76 million hectares with production of 68.13 million tons; average yield being 29.68 tons/ha in 2014 (FAOSTAT, 2017). The total area under yam cultivation in Ethiopia is 0.05 million hectares with production of 1.45 million tons; average yield being 8.78 tons/ha in 2014 (FAOSTAT, 2017). According to the FAOSTAT (2017), Ethiopia considered the fifth country in production (1,191,809 tones) following Benin (3,177,265 tones). The major yam growing continents of the world are Asia, South America and West Africa (Coursey, 1967). Southern, Western and Southwestern parts of Ethiopia are regions where yams have been cultivated as staple or co-staple with enset (*Ensetventricosum*), cereals, and other root and tuber crops (Westphal *et al*., 2000). Gemeda (2000) opined that yam is more productive than the other tuber crops in Western part of Ethiopia; due to its ability of relative tolerance to drought and termite damage, it can provide about 20 tons per hectare of tuber yield.

Tubers are the main economic importance of all the *D. species* (Lebot, 2009). It is a valuable source of carbohydrates, fibers, negligible amounts of fats and it can be processed into various staple intermediate and end-product forms (Jaleel *et al*., 2007). Lebot, (2009) and Kole, (2011) reported that yam storage organs (underground and/or aerial tubers) are source of proteins and vitamins. In addition, it is considered as a medicinal herb, since the genus is rich in steroidal saponins that are used as a source of biologically active compounds in pharmaceutical industries (Wang *et al*., 2006; Poornima and Ravishankar, 2007). Zuluaga *et al*., (2007) reported that the edible underground or aerial tubers of some species of *Dioscorea* are sources of important chemical called diosgenin which is commercially used to synthesis sex hormones and corticosteroids that widely used for anti-inflammatory, androgenic and contraceptive drugs.

The main problem in *Dioscorea* species cultivation for commercial purpose is the use of traditional methods of propagation via tuber cuttings as a seed (Forsyth and Staden, 1981). The seed tubers which are used as

planting materials are expensive and counts about 50% of total variable cost (Manyong, 2000). According to Balogun *et al*., (2004) seed tubers have limitations like having very low multiplication ratio and difficulties to transport due to its bulkiness with extended dormancy period. On the other hand, Balogun (2009) indicated that the multiplication ratio of these tuber seeds in the field is less than 1:10comparedto some cereals (1:300). Tamiru *et al*., (2008);Balogun *et al.,* (2006) reported that other possible conditions responsible to the decrease in the production of yam such as pests and diseases, limited availability and high cost of planting materials.

Conventional methods like vine cutting and partial sectioning have been used to solve such problems and increase production with high amount of planting materials (Okoli *et al*., 1982). The minisett technique produces a reasonable amount of yam tubers but it is not highly adopted by the farmers because of its skilled manpower requirement (Ondo *et al*., 2010).Vine cuttings of *D. species* can result tuberization and primary nodal complex formation. But complexity, intensive care requirement and low tuber yield were limitations of this method (Aighewi *et al*., 2003a). Layering technique is specific to some genotypes (Acha *et al*., 2004; Shiwachi *et al*., 2005). Generally, almost all the traditional methods of yam propagations have their own limitations and they practiced due to lack of the improved method (Marfo *et al*., 1998).

To solve these problems other methods of rapid propagation like *in vitro* propagation method must be developed (Vaillant *et al*., 2005). It provides large scale multiplication of high quality planting materials (Yam and Arditti, 2009). It offers many advantages over conventional methods like mass propagation, produces pathogen free planting materials, enables clonal propagation and enables year round nursery production (Obsi*et al*., 2015).It can minimize the vulnerability of the plant to diseases those transmitted at the time of tuber cutting to propagate (Alizadeh *et al*., 1998).

So far protocols for the *in vitro* propagation of different *D. species* were conducted by different researchers including *D. alata* (Borges *et al*., 2004; Vaillant *et al*., 2005), *D. oppositifolia (*Behera *et al*., 2009), *D. zingiberensis* (Chen *et al*., 2003; Huang *et al*., 2009; Yuan *et al*., 2005), *D. nipponica* (Chen *et al*., 2007), *D. polystachya*, *D. sansibarensis* and *D. japonica* (Islam *et al*., 2008), and (Xu *et al*., 2009). Furthermore, *in vitro* propagation methods through direct shoot organogenesis were reported for *D. rotundata, D. cayenensis,* and *D. alata* (Adeniyi *et al*., 2008; Anike *et al*., 2012). In addition, micro-propagation for *D. remotiflora* (*Kunth*) (Yan *et al*., 2011) and *D. hispida* (Susmita and Shukla, 2014) was also conducted. Further, *in vitro* regeneration for *D. wightii* (Mahesh *et al*., 2010), *D. alata L.* (Das *et al*., 2013) and (Obsi *et al*., 2015) for AW/04 *D. species* has been reported.

However, an efficient *in vitro* protocol for micro-propagation through nodal segment is not available for Ethiopian yam genotypes 75/02 and 6/02.Therefore, there is a need to develop a protocol for *in vitro* propagation these genotypes.

General objective

- To optimize suitable protocol for the *in vitro* mass propagation of two yam (*Dioscorea*) landraces using nodal segments

Specific objectives

- ✓ To determine the optimum concentration of BAP for the *in vitro* shoot multiplication of two yam landraces.
- ✓ To determine the optimum concentration of IBA for the *in vitro* rooting of two yam landraces.

Material and Methods

Plant Materials

The two (75/02 and 6/02) yam landraces (*Dioscorea spp.*) were collected and used to conduct this experimental study. The number75/02 and 6/02 were an accession numbers used to differentiate one genotype from other. Their mother plants were obtained from the experimental field of Jimma Agricultural Research Center (JARC). They belong to the different *Dioscorea* species namely, landrace 75/02 to *D. alata,* and 6/02to *D. rotundata.* Both 75/02 and 6/02 landraces were selected because of their tolerance to drought and they provide a reasonable yield at this condition (Gemeda, 2000). In addition, landrace 6/02 has a medicinal value.

The tuber cuttings of both landraces were collected from JARC experimental field. Tuber of these two yam landraces were cut and prepared as a seed of yam with a number of buds, and planted in black poly bags filled with sterilized top soil and kept in greenhouse of JARC where the study was carried out. The tuber sets in black poly bags were watered twice day (i.e. morning and night) and allowed to grow for three months until bud sprout was produced which actively growing nodal segments were collected and prepared as source of an explants.

Stock Solution and Media Preparation

Murashige and Skoog, 1962(MS) media supplemented with various plant growth regulators was used (Appendix Table 1). Stock solutions of the macro salts, micro salts, vitamins, iron source and plant growth regulators (1mg:

1ml) were prepared and stored at +4^{o}C in refrigerator for immediate use. Plant growth regulators; IBA was dissolved using a drop of ethanol and BAP by 1N NaOH before making up the final volume with distilled water. Three anti-oxidants ascorbic acid (100 mg/l), citric acid (100 mg/l) and polyvinylpyrrolidinone (PVP) (150 mg/l) were used according to Susmita and Shukla, (2014) recommendation. The dissolved solution was poured into labeled volumetric flask to be fully dissolved and finally stored in refrigerator for later

The culture medium was prepared from their respective stock solutions, and the appropriate amount of sucrose (3% w/v), Myo-inositol (0.1%w/v), plant growth regulators BAP and IBA were added to the medium at required concentrations. The mixture was stirred using magnetic stirrer and the volume was adjusted using double distilled water (DDW).Then, the pH was adjusted in all cases to 5.78 (Islam *et al*., 2008) using 1M NaOH and 1M HCl. Finally, (0.7% w/v) agar was added and heated to melt throughout the experiment. Before autoclaving, the media was dispensed into sterilized culture jars and steam sterilized using an autoclave at a temperature of 121^{o}C with a pressure of 0.15 Kpa for 20 minutes and transferred to the culture room and stored until for later use.

Explants Preparation and Sterilization

Nodal segments were used as a source of explants and collected from actively growing shoots 2-3 months old. The lengths of nodal segments were trimmed to 1 cm after removal of leaf and removing of the petiole. These nodal segments were washed by largo soap three times under running tap water. After the explants were washed by tap water, they were rinsed in 30 gm/l of Copper Sulphate which has an antibiotic effect for 30 minutes and washed with sterilized distilled water (SDW).

Besides that, the vine nodal segments were treated with combination of 5gm/l of ascorbic acid, acetic acid and polyvinylpyrrolidone (PVP) and put on gyratory shaker for about two hours in order to minimize phenolics and browning of the explants and flashed with sterilized distilled water three times. Then the nodal segments were disinfected with 70% ethanol alcohol for 30 seconds in laminar flow chamber and followed by 25% (v/v) of active chlorine house bleach for 10 minutes. Finally, explants were washed thoroughly 3-4 times with sterilized distilled water.

Treatments and Experimental Designs

All the experiments were conducted in a completely randomized design (CRD) with factorial arrangement. Each experiment was replicated three times (each jars with five explants) for both shoot multiplication and rooting.

Experiment 1: Effect of BAP on shoot multiplication

The sterilized explants were cultured on basal MS medium supplemented with 1.0 mg/l BAP for shoot initiation from nodal segments explants. The initiated explants were cultured on MS medium (Appendix Table 1) that supplemented with different concentrations of BAP. These five concentrations of BAP 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l were used to investigate its effect on shoot multiplication. Basal medium without plant growth regulator was included as a control. Data like mean of shoot length, shoot number, leaf number and node number were recorded after 45 days of culture.

Experiment 2: Effect of IBA on rooting of micro-propagated plantlets

At rooting, *in vitro* raised plantlets were cultured on MS media with different levels of IBA (Yan *et al*., 2011). Accordingly, about seven levels of auxin were involved particularly IBA with concentrations of 0, 0.5, 1, 1.5, 2, 2.5 and 3 mg/l. The data like rooting percentage, mean of root number and root length were collected after 45 days of culture.

Acclimatization

For acclimatization, plantlets with well-developed root and leaf systems were washed with tap water to remove adhering media and agar attached on the roots of plantlets. Rooted micro-propagates were transplanted to plastic pots filled with compost, sand and top soil (1:2:1) respectively. The plastic pots were placed in greenhouse where pot trays were covered with polythene to maintain humidity at 70-80 %. Gradually, the *in vitro* raised plantlets were acclimatized to outdoor conditions and kept in green house until plantation in the field.

Data Analysis

SAS software (SAS, 2008 version 9.3) was used for data analysis of variance and Least Significance Difference (LSD) was used for mean separation at 1% probability level.

Result and Discussion

Effects of BAP on Shoot Multiplication

Analysis of variance revealed that the interaction effects of genotypes and BAP concentrations were highly

significant ($p< 0.01$) for number of shoots/explant, shoot length and average number of leaves/shoot (Appendix 2). On the other hand, the interaction effect of BAP and genotypes were significant ($p < 0.05$) for the number of nodes. On MS media devoid of BAP, young shoots were developed from the primary shoots and showed poor shoot elongation in both landraces after being cultured for a month. This might be due to the non-optimal concentrations of the indigenous cytokinin they contained and/or to their lower effect on shoot morphogenesis with poor multiplication of shoot (Sylvestre and Engelmann, 2014).

MS media which supplemented with 1.5 mg/l BAP gave the highest shoots/explants (6.79 ± 0.09)with (7.47 ± 0.47) cm average shoot length and 4.62 ± 0.12 leaves/ shoot for landrace 75/02(Table1; Fig.1).Similarly, MS media fortified with 1.5 mg/l BAP was produced maximum shoots/explants (7.23 ± 0.21) with maximum shoot length 7.68 ± 0.24 cm and highest leaves/shoot (5.31 ± 0.32)in landrace 6/02 (Table1; Fig.1). This multiplication rate difference might be due to genotypic difference, which affects the frequency of shoot organogenesis and also endogenous cytokinin and auxin concentration differences (George *et al*., 2008; Viet, 2009; Jahangir *et al*., 2014).The obtained different results were maybe there are differences in uptake of cytokinins in both landraces, recognition by the cells, or mechanisms of action of the cytokinin compounds. The performance of each cultivar is expected to be different in *in vitro* culture as a field response regarding shoot number and shoot length (Ogero*et al*., 2012).

Both 75/02 and 6/02, gave the highest mean number of shoot with mean shoot length and leaf numbers on MS media supplemented with 1.5 mg/l BAP. Therefore, MS media with 1.5 mg/l BAP could be taken as the optimum concentration for these landraces, because it provides the maximum value for the shoot number, shoot length and leaf number. This current finding was in line with the finding of Poornima and Ravishankar, (2007); Fay, (1992) and Chu *et al*., (2002). However, this finding is contradictory with the report of Manoharan *et al*., (2016) and Mwirigi *et al*., (2010) because they noticed that MS media with 0.4 mg/l and 0.5 mg/l BAP respectively was the optimum concentration to shoot proliferation. The contradiction was might be occurred due to the difference in source and age of explants, type of media and genotype.

Increasing 0.5 to 1.00 mg/l BAP showed a significant increase from 5.98 ± 0.21 to 6.71 ± 0.09 shoots/explant, from 4.28 ± 0.28 to 5.21 ± 0.79 cm shoot length and from3.47± 0.28 to 4.50 ± 0.1429 leaves/shoot in 75/02. Landrace 6/02 showed similar trend of increasing for but with different value. This indicates that both landraces needs further increase of BAP to get the optimum concentration for shoot proliferation.MS media with 1mg/l BAP gave the second highest mean shoot number (6.71 ± 0.09) with shoot length (5.21± 0.79 cm) and highest leaf number(4.50 ± 0.14) for landrace 75/02. The landrace 6/02 showed the second highest shoot number 7.10± 0.31 with shoot length 6.07 ± 0.19 cm and maximum leaf number 5.10 ± 0.49 on MS media supplemented with 1 mg/l BAP. This finding is harmonious with the report of Susmita and Shukla, (2014); Borges *et al*., (2004); Belarmino and Gonzales, (2008).

On the other hand, minimum shoot number 4.86± 0.13with minimum shoot length 2.35 cm on MS media with 2.5 and 2 mg/l BAP respectively and minimum leaf number 2.12 ± 0.15of landrace 75/02 was recorded on MS media with 2.5 mg/l BAP (maximum concentration). Similar trends i.e. increasing of BAP with decreasing of shoot proliferation was observed in landrace 6/02.

In both landraces increasing of BAP above 1.5 mg/l, resulted shoots with inseparable and stunted features, which are generally unusable due to high dosage of hormone that disorders the metabolism of the shoot. This shows that higher concentration of cytokinin led to inhibition of the metabolic activity which inhibits cell division and multiplication. Whereas, low concentration of cytokinin promotes shoot multiplication and elongation (Gopitha *et al*., 2010). This finding is contradictory with finding of Ammirato, (1982) and Ezeibekwe *et al*., (2009) because they reported MS media greater than 2.00 mg/l BAP results best shoot proliferation performance and differences were might be raised due to difference in MS media composition and genotype.

On further indication of comparison regarding the shoot length, the longest values for the two yam landraces was obtained on MS media with 1.50 mg/l BAP and yam landrace with 6/02 was performed impressively. Generally, 6/02 yam landrace showed impressive growth than 75/02 this might be due to difference of endogenous BAP concentration. Butin both landraces BAP at higher concentrations not only reduced the number of shoots but also resulted in stunted growth of the shoots due to inhibition of metabolic activities. Both yam landraces were recorded increasing leaf number, number and length of shoot with simultaneous increase of BAP until 1.50 mg/l BAP concentration. Particularly, BAP with 1.50 mg/l was the favorable concentration of cytokinin type for the parameters like leaf number, shoot number and shoot length. Therefore, MS media with 1.50 mg/l BAP identified as optimum and suitable concentration for shoot proliferation for both landraces of yam.

Despite the aim of this study, callus formation was observed on the shoots base in both yam landraces with increasing of BAP, this was observed might be due to the rapid division of cells and the synthesis of auxins at young shoot organs (Skoog and Miller, 1957).This report of induction of callus with increase of BAP concentration was in line with findings of Behera and co-workers in 2008 on *D. hispida*. Nevertheless, in some shoots, rooting observed but in very negligible amount, this makes the current study similar with the findings of

(Das *et al*., 2013). Induction of shoot in all treatments cultured on MS media supplemented with different concentration of BAP in present study was similar with the report of (Mahesh *et al*., 2010). The current result is in conformity with the finding of Sowa *et al*., (2002) who reported that the effectiveness of low concentration of BAP to result in the rapid shoot multiplication due to the activation of tRNA cytokinins resulting in rapid proliferation of shoot primordial.

Generally, either increasing or decreasing of the BAP concentration from 1.50 mg/l leads to decline for the growth parameters like leaf number, shoot height and shoot number. Findings indicated that excessively high or low concentrations of BAP could result in formation of fewer shoots and shorter shoots, or no shoot at all as well as callus might be induced which is in line with report of (Chen *et al*., 2007). But the findings of decreasing shoot proliferation at lowest levels of BAP contradicts report of Thankappan and Abraham, (2012) at which they opined best shooting media was MS with 0.45 mg/l BAP. This is might be difference in composition of their MS media used and genotype involved to carry out their experimental study.

On the other hand, this experimental study clarifies the significance of node number when it interacts with cytokinin particularly BAP. Accordingly, the maximum number of nodes (2.15 ± 0.10) and (2.10 ± 0.15 and 2.10 ± 0.07) obtained in 75/02 and 6/02 respectively, on MS media supplemented with 1.5 mg/l BAP (Table 1) this implies that MS media with 1.5mg/l BAP was an optimum concentration. The second highest number of nodes 1.94 ± 0.05 was obtained on MS media with 1.00 mg/l BAP 75/02 this indicates that the requirement of further MS media greater than 1mg/l BAP this finding is similar with finding of (Susmita and Shukla, 2014).Moreover, for both yam landrace 75/02 and 6/02 lowest node number (1.48 ± 0.08) and (1.52 ± 0.13) obtained on MS media with 2.50 mg/l BAP this is might be the metabolic inhibition of PGRs at high concentration which results in reduction of shoot elongation.

Table 1 Effect of BAP on shoot multiplication of two yam landraces

Landraces	BAP Conc. Mg/l	Shoot Number (Mean ± SD)	Leaf Number (Mean ± SD)	Node Number (Mean ± SD)	Shoot Height (Mean ± SD)
75/02	0.00	5.23 ± 0.24^{f}	2.54 ± 0.27^{e}	1.60± 0.03^{d}	2.45 ± 0.19^{e}
	0.50	5.98 ± 0.21^{de}	3.47± 0.28^{cd}	1.78 ± 0.14^{c}	4.28 ± 0.28^{d}
	1.00	6.71 ± 0.09^{c}	4.50 ± 0.14^{b}	1.94 ± 0.05^{b}	5.21 ± 0.79^{c}
	1.50	6.79 ± 0.09^{bc}	4.62 ± 0.12^{b}	2.15 ± 0.10^{a}	7.47^{a} ± 0.47^{a}
	2.00	4.99 ± 0.33^{fg}	2.27 ± 0.38^{e}	1.78 ± 0.02^{c}	2.35 ± 0.01^{e}
	2.50	4.86± 0.13^{g}	2.12 ± 0.15^{e}	1.48 ± 0.08^{d}	3.89 ± 0.06^{d}
6/02	0.00	5.71± 0.24^{e}	3.13± 0.32^{d}	1.57 ± 0.03^{d}	2.91 ± 0.85^{e}
	0.50	6.23± 0.15^{d}	3.80± 0.20^{c}	1.77 ± 0.06^{c}	4.13 ± 0.20^{d}
	1.00	7.10± 0.31^{ab}	5.10 ± 0.49^{a}	2.10 ± 0.07^{a}	6.07 ± 0.19^{b}
	1.50	7.23± 0.21^{a}	5.31 ± 0.32^{a}	2.10 ± 0.15^{a}	7.68± 0.24^{a}
	2.00	6.67± 0.09^{c}	4.44 ± 0.14^{b}	2.03 ± 0.03^{ab}	5.21 ± 0.34^{c}
	2.50	6.15 ± 0.19^{d}	3.71 ± 0.25^{c}	1.52 ± 0.13^{d}	3.63 ± 0.12^{d}
	CV	**3.38**	**7.36**	**4.76**	**8.77**
	LSD (0.05)	**0.35**	**0.47**	**0.15**	**0.68**

Note: BAP=Benzyl Amino Purine. LSDS= least significant difference, Means with the same letter in the same column are not significantly different at 0.01 probability level, CV= Coefficient of Variation.

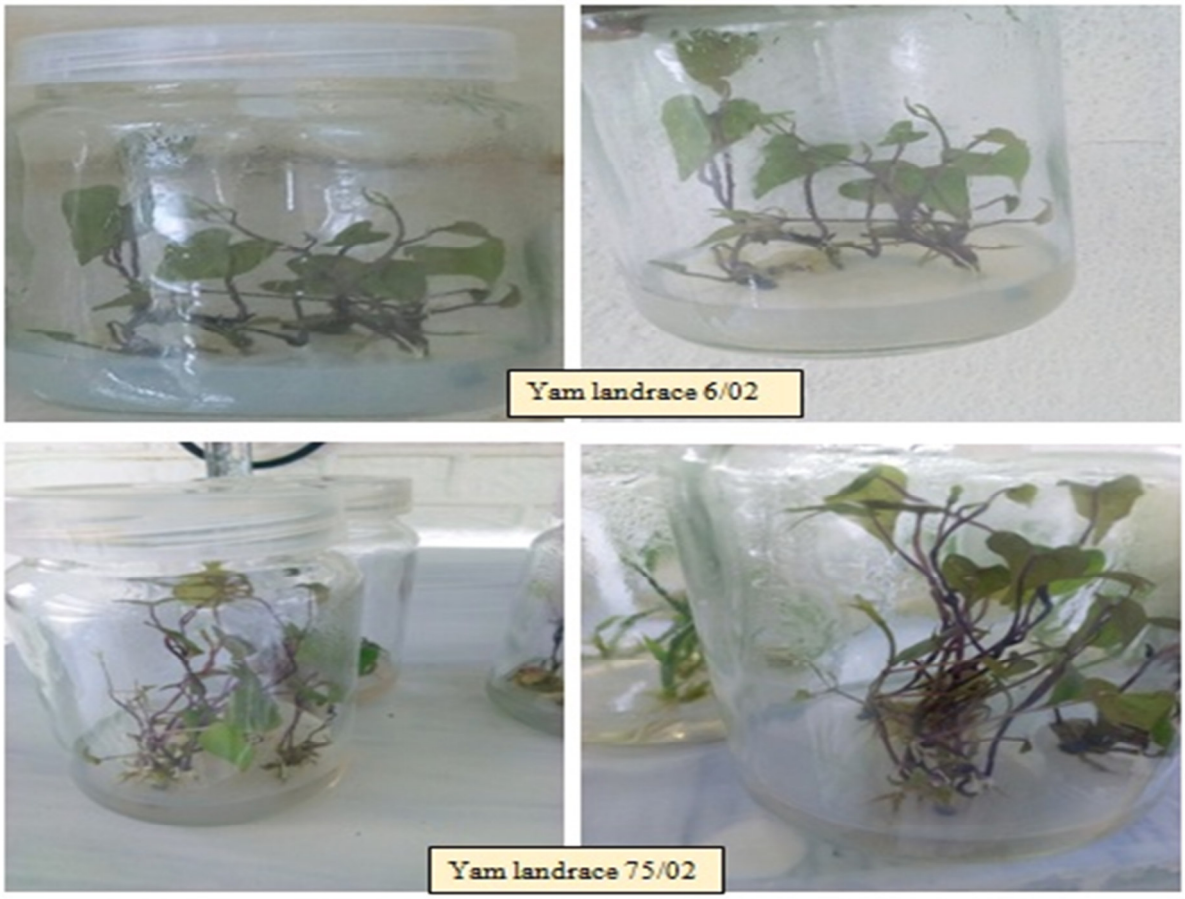

Figure 1: *In vitro* shoot multiplication for both 75/02 and 6/02 yam landraces on MS and after 45 days of culture.

Effect of IBA on Rooting

Analysis of variance revealed that the interaction effects of two genotypes and IBA were highly significant ($p< 0.01$) for rooting percentage, number of root/shoot and average root length (Appendix Table 3). On MS media without IBA showed not well elongated and minimum root number with minimum root length totally with poor root growth. This might be due to deficiency of indigenous IBA composition in each landraces (Ramakrishnan *et al*., 2013.Obtaining of root on control was might be due to ability of yam to propagate through vegetative means and although it is possible that there were endogenous auxin concentration in the explanted organ (Benmahioul *et al.,* 2012).

MS media supplemented with 1 and 2.50 mg/l IBA gave the highest (54.33 ± 0.08) and the lowest 32.67% rooted shoots for landrace 75/02 (Table 2). Whereas, MS media supplemented with 0.50 and 2.50 mg/l IBA was produced a maximum of 68.00% and minimum 34.33% of rooted shoots for landrace 6/02. This result indicates that each genotype responded differently due to their different endogenous auxin amounts. Each genotype requires different concentrations based on the amount of their endogenous auxin concentration (Ramakrishnan *et al*., 2013).By increasing the concentration of IBA from 1.5mg/l to2.5mg/l, percentage of rooted shoots decreased continuously from 46.33% to 32.67 % in 75/02, and discontinuously decreased from 49.00% to 34.33%in 6/02.The variation in rooting percentage of in these two landraces was raised due to genotypic variation which results variation in indigenous IBA concentration.

In landrace 75/02, root induction percentage increased from 43.00 % at 0.5mg/l to 54.33% at 1.0mg/l IBA, but the roots were not well grown, and they were extremely stunted at 0.5mg/l than 1.0mg/l. This indicates that low concentration of IBA promotes root induction and elongation than extremely higher concentration that inhibited rooting in both landraces. This observation disagrees with finding of Forsyth and Stadan, (1981) regarding on their report of finding impressive rooting relies on MS media with 5 mg/l IBA. This contradiction might be occurred due to genotype variation and the stage of mother plants which used as a source of explants.

In landrace 6/02, highest (68.00 ± 0.03) and minimum (36.00 ± 0.03) rooting percentage was obtained on MS media with 0.5 and 3.00 mg/l IBA respectively and this implies effectiveness of auxin at low concentration might be due to its efficiency at low concentration for metabolic activities to induce root. This finding was

contradictory with (Antonio *et al*., 2012) because of their suggestion at getting highest (96.9%) rooting percentage on MS media without PGR or 1.68 mg/l IBA the difference of finding was might be difference in source of variation.

The relationship between IBA and rooting percentage showed inverse relation with percentage of rooting i.e. increasing the concentration of IBA leads to decreasing of rooting percentage due to its inhibitory effect on metabolic activities (Mahesh *et al*., 2010). This finding contradicts with Poornima and Ravishankar, (2007) because they clarified better effects on rooting percentage and number of roots per segment on *D. oppositifolia and D. pentaphylla* obtained with increased concentration of IBA and variation was raised due to variation in genotype. In this finding, the effect of IBA on both landraces has no similar trend i.e. the concentration of IBA at which maximum rooting recorded was different for both. This clarifies the endogenous IBA variation between the landraces. Actually, the landrace providing maximum rooting percentage at minimum concentration of exogenously added IBA emphasizes presence of high concentration of endogenous IBA (Benmahioul *et al.,* 2012). Whereas, landrace with maximum rooting percentage on highly concentrated MS media with exogenous IBA implies low endogenous concentration of IBA. This finding clearly indicates landrace 6/02 is richer in endogenous IBA than 75/02.

Landrace75/02 gave the highest (10.03 ± 0.49) roots/shoot with 10.76± 0.16cm average root length on MS medium with 1.5mg/l IBA (Table 2, Fig.2 and 3). In 6/02 maximum 10.58 ± 0.26 roots/shoot with 10.42± 0.32 cm average root length were observed on MS media fortified with 2.0 and 1.0 mg/l IBA respectively (Table 2, Fig.2 and 3). This indicates that rooting was highly influenced by the concentrations of IBA used. Hence, appropriate amounts of auxin in the rooting medium are crucial for root induction.

Similarly, studies have shown that addition of 1.00 or 2.00 mg/l IBA to the medium induced fastest rooting and had a higher average number of roots per segment in *D. zingiberensis* (Chen *et al*., 2003). Maximum rooting was obtained on MS media with 2.00 mg/l IBA which was different from the finding of Behera *et al*., (2010), they obtained less root performance on MS media with 2 mg/l IBA. This difference was raised because of difference in genotype. Increasing of IBA from 1.5 mg/l was resulted in decreasing of roots per shoot in both landraces this indicates that inhibition of rooting at higher concentrations of auxin. The minimum root number (5.65 ±0.18) and shortest root length (5.54 ±0.14 cm) in 75/02 was recorded on MS media without IBA (control) and this is might be insufficiency of IBA to give profuse rooting. The second highest average mean root length 9.35 ± 0.57 and 8.59 ± 0.83 cm was observed on MS media 1.00 and 1.50 mg/l IBA in 75/02 and 6/02 respectively this implies further increasing of IBA. This finding is not similar with Antonio *et al*., (2012) because their finding elaborates best root performance could be resulted on MS media supplemented with 4.0 mg/l IBA because of genotype variation. As the concentration of IBA increased, number of root and roots length reduced significantly in both yam landraces. This indicates rooting was highly influenced by the concentrations of IBA (Chen *et al*., 2007; Poornima and Ravishankar, 2007).

Table 2: Effect of IBA on rooting of two yam landraces

Landraces	**IBA conc. (mg/l)**	**Rooting (%)**	**Root Height (Mean ± SD)**	**Root Number (Mean ± SD)**
75/02	0.00	38.33 ± 0.02^{de}	5.54 ±0.14 ^{e}	5.65±0.18^{f}
	0.50	43.00 ± 0.03^{cd}	8.64 ± 0.53^{bc}	8.14 ± 0.53^{c}
	1.00	54.33 ± 0.08^{b}	9.35 ± 0.57 ^{b}	7.60 ± 0.30^{de}
	1.50	46.33 ± 0.01^{c}	10.76± 0.16 ^{a}	10.03 ± 0.49^{b}
	2.00	43.00 ± 0.03^{cd}	7.11± 0.38 ^{d}	8.40 ± 0.44^{c}
	2.50	32.67 ± 0.06^{e}	7.76 ± 0.57^{cd}	7.23 ± 0.31^{e}
	3.00	37.00 ± 0.02^{de}	7.04± 0.21^{d}	7.09 ± 0.11^{e}
6/02	0.00	37.00 ± 0.03^{de}	5.94 ± 0.37^{e}	5.50 ± 0.21^{f}
	0.50	68.00 ± 0.03^{a}	7.61± 0.83^{d}	8.17 ± 0.41^{c}
	1.00	55.00 ± 0.05^{b}	10.42± 0.32^{a}	8.06 ± 0.38^{cd}
	1.50	49.00 ± 0.05^{bc}	8.59 ± 0.83^{bc}	8.09 ± 0.17^{cd}
	2.00	45.67 ± 0.01^{c}	7.79 ± 0.33^{cd}	10.58 ± 0.26^{a}
	2.50	34.33 ± 0.05^{e}	7.28 ± 0.34^{d}	7.44 ± 0.21^{e}
	3.00	36.00 ± 0.03^{e}	7.33 ± 0.93^{d}	7.32 ± 0.34^{e}
CV (%)		**8.98**	**6.63**	**3.88**
LSD (0.05)		**0.66**	**0.21**	**0.12**

Note: IBA=Indol-3-Butyric acid, LSD= least significant difference. Means with the same letter in the same column are not significantly different at 0.01 probability level, CV= Coefficient of Variation.

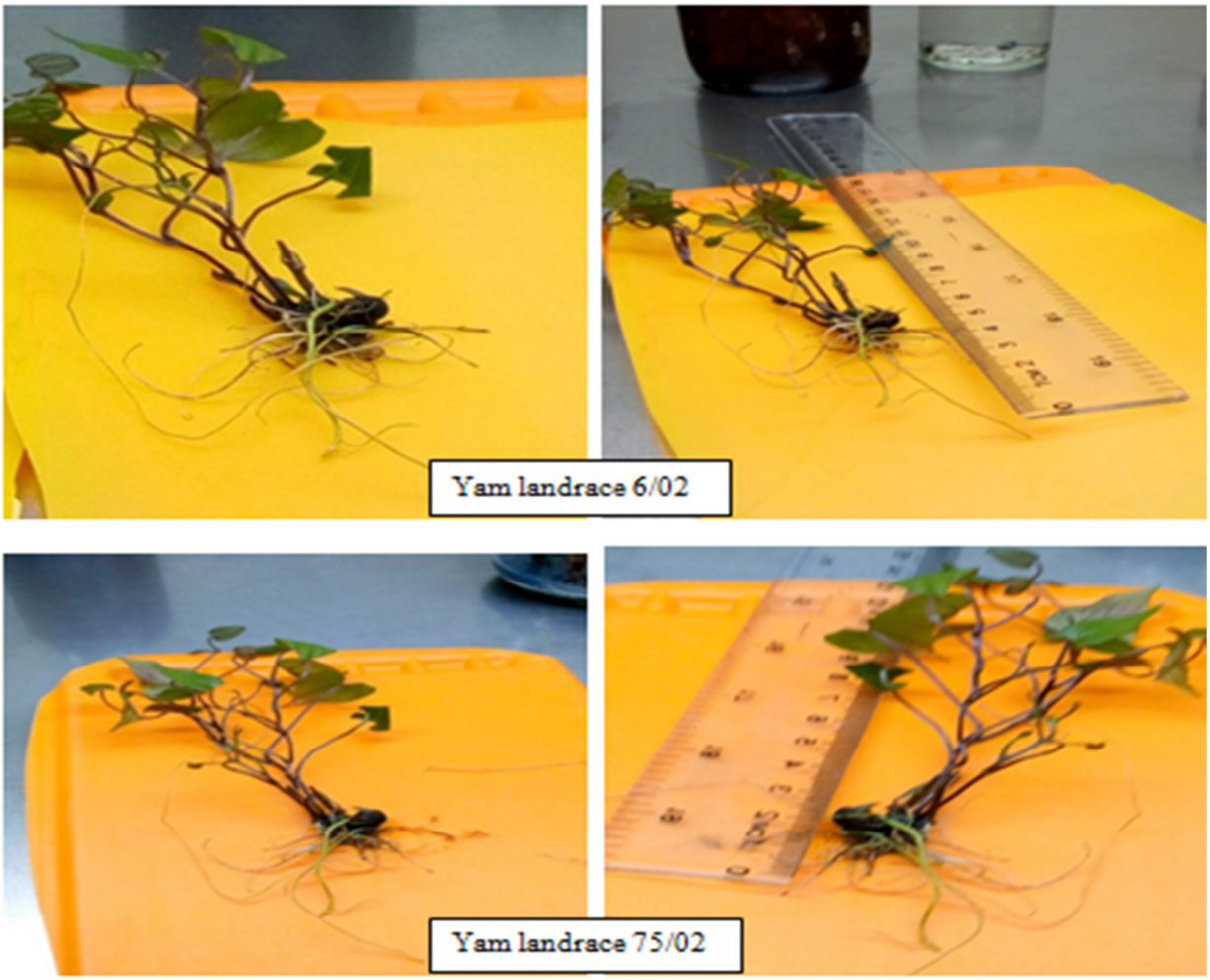

Figure 2: *In vitro* raised shootlets for rooting parameters after 45 days of culture on MS media of both 6/02 and 75/02 landraces of yam

Acclimatization of in vitro Raised Plantlets

The *in vitro* rooted plantlets were hardened in the greenhouse. After one month of acclimatization, 86% and 90% of plantlets were survived and successfully established from *in vitro* experiments of 75/02 and 6/02 yam landraces, respectively (Fig. 4). Data was recorded in the greenhouse within 3-4 weeks after transferred to the sterilized soil medium (1:2:1 sand, top soil and compost). The difference in survival rate of the two landraces was might be due to differences in genotype which affects the adaptation ability to new environment. However, in comparison landrace 6/02 performed well in *ex vitro* establishment than 75/02. The present finding of 90% survival rate in 6/02 is similar with finding of Behera *et al*., (2008); Behera *et al*., (2009) and Behera *et al*., (2010) who reported 90% survival rate of different *Dioscorea species* in *ex vitro* establishment.

On other hand, landrace 75/02 exhibited about 86% of survival rate at *ex vitro* condition and this finding was similar with finding of Obsi *et al*., (2015) and almost similar survival rate (80%) was obtained by Kadota and Niimi, (2003) when micro-propagated plants of *D. japonica* were transferred to pots containing (1:1) vermiculite and soil (v/v) mixture under greenhouse.

Figure 4: Acclimatized plantlets of two yam landraces after 45 days in greenhouse

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