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# Effect of Plant Hormones for Micro Propagation on Improved and Landrace Yam Varieties (*Dioscorea species*) Via Nodal Culture

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**Abstract:** Yam (*Dioscorea species*) is a monocotyledonous tuber forming tropical vine which belongs to the order *Liliflorae*, family *Dioscoreaceae*, and genus *Dioscorea.* Conventional propagation of yam (Dioscorea spp.) is limited due to low propagation rates and highly infected by pests. However, *In vitro* clonal propagation is the best alternative to mass multiplication over conventional propagation. Moreover, shoot multiplication and rooting induction experiments were incubated on hormone free MS medium within 30 g/l sugar and 7 g/l agar. For shoot multiplication the initiated shoots of both landraces were cultured on MS media supplemented with 0, 1, 2, 3, 4 and 5 mg/l benzyl amino purine (BAP). For root induction, shoot lets were cultured on MS media supplemented with 0, 1, 2, 3, 4, 5 and 6 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 induction respectively. The maximum shoot number (7.23±0.21) with a maximum shoot length 7.68±0.24cm and 6.79±0.09 shoot number with 7.47±0.47 cm were obtained on MS media supplemented with 3 mg/l BAP for 11/02 and Koffea cultivars respectively. The maximum root number 10.03±0.49 and root length 10.76±0.16 cm and 10.58±0.26 root number with 10.42±0.32 cm were recorded on MS media with 3 mg/l IBA for Koffea and 11/02 cultivar respectively. *In vitro* raised plantlets were acclimatized and recorded 86% and 90% of survival rate in Koffea and 11/02, respectively, on soil medium with combination ratio of 2:1:1 top soil, sand soil and compost, respectively.

**Keywords:** Plant Hormones, Micro-Propagation, *in vitro* Nodal Culture, *Dioscorea species* and Yam

## 1. Introduction

Yam (*Dioscorea species*) is a monocotyledonous rhizome forming tropical vine, which belongs to the order *Liliflorae*, family *Dioscoreaceae*, and genus *Dioscorea* [22]. This root crop is drought tolerant rhizome crop which is capable of producing a good yield under water scarcity [7]. Of the 600 known *Dioscsorea species,* only 10 are consistently cultivated for food consumption [21]. The major refined *D. species* include: *D. alata, D. bulbifera, D, cayenensis, D. esculanta, D. rotundata, D. trifida*and *D. pentaphylla* [36]. In the middle of these, *D. rotundata* has the greatest preferred and nurtured one, accounting for a huge percentage of yam invention in West Africa, which produces 93% of the world's yam.

Worldwide, *Dioscorea* is grownup in a space of about 7.76 million hectares with fabrication of 68.13 million tons; average yield being 29.68 tons/ha in 2014. Ethiopia considered the fifth country in production (1,191,809 tones) following Benin (3,177,265 tones). The main yam rising landmasses of the world are Asia, South America and West Africa [36]. Southern, Western and Southwestern parts of Ethiopia are regions where yams have been cultivated as staple or co-staple with enset (*Enset ventricosum*), cereals, and other root and tuber crops [58]. Gemeda [27] opined that yam is extra fruitful than the other tuber crops at Western part of Ethiopia, due to its ability of relative tolerance to drought and termite damage and it can provide about 20 tons per hectare of tuber yield.

Rhizomes are the main economic position of all the *D. species* [36]. It is a respected basis of starches, fibers, negligible fats and it could be treated into various staple intermediate and end-product forms [33]. Lebot, [36] and Kole, [35] reported that yam packing organs (underground and aerial corms) are spring of proteins and vitamins. In addition to that, this is reflected as a medicinal herb, since the genus is ironic in steroidal saponins that are used as a source of biologically active amalgams in pharmaceutical industries [57];[46]. Zuluaga *et al*., [63] reported that the edible underground or aerial corms of some species of *Dioscorea* are causes of significant chemical know as diosgenin that is commercially used to production sex hormones and corticosteroids that widely used for anti-toxicity, androgenic and contraceptive medications.

The core difficult in *Dioscorea* species farming for viable purpose is the use of outdated methods of propagation via tuber cuttings as a seed [26]. The seed tubers which are used as planting materials are expensive and counts about 50% of total variable cost [40]. According to Balogun *et al*., [11] seed tubers have limitations like having very low multiplication rate and difficulties to transport due to its bulkiness with extended dormancy period. On the other hand, Balogun [11] indicated that the multiplication rate of these tuber seeds in the field is less than 1:10 compared to some cereals (1:300). Tamiru *et al*., [54] and Balogun *et al.,* [11] reported that other possible conditions responsible to the decrease in the production of yam such as pests, diseases, inadequate availability and great cost of planting supplies.

Conventional methods like vine cutting and partial sectioning have been used to solve such problems and increase production with high amount of planting materials [44]. 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 [45]. 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 [8]. Layering technique is specific to some genotypes [2, 49]. Generally, almost all the traditional methods of yam propagations have their own limitations and they practiced due to lack of the improved method [39].

To solve these problems other methods of rapid propagation like *in vitro* propagation method must be developed [56]. It provides clonal multiplication of high quality planting materials [61]. It deals many rewards over straight methods like mass proliferation, produces diseases free planting resources, enables clonal propagation and enables year round nursery production [42]. It can reduce the vulnerability of the plant to contaminations those spread at the time of corm cutting clonal multiply [1]. So far protocols for the *in vitro* clonal propagation of various *D. species* were conducted by different researchers including *D. alata* [17]and [56], *D. oppositifolia* [13], *D. zingiberensis* [18, 30, 59], *D. nipponica* [19], *D. polystachya*, *D. sansibarensis* and *D. japonica* [31] and [60]. Furthermore, *in vitro* propagation methods through direct shoot regeneration were informed for *D. rotundata, D. cayenensis,* and *D. alata* [3, 5]. In addition, micro-propagation for *D. remotiflora* (*Kunth*) [62] and *D. hispida* [52] was also conducted. Further, *in vitro* regeneration for *D. wightii* [37], *D. alata L.* [23] and [42] for AW/04 *D. species* has been reported. However, an efficient *in vitro* protocol optimization for clonal propagation through nodal culture is not available for Ethiopian yam genotypes Koffea and 11/02. Therefore, we will be developing a protocol for *in vitro* clonal proliferation yam cultivars.

General objective

To optimize a protocol for *in vitro* clonal multiplication of yam (*Dioscorea*) cultivars via nodal cultures.

Specific objectives

1. To regulate the best concentration of BAP for the *in vitro* shoot proliferation of yam cultivars.
2. To govern the optimum concentration of IBA for the *in vitro* root induction of two yam cultivars.
3. To acclimatized the seedling in the greenhouse condition.

# 2. Material and Methods

### *2.1. Plant Materials*

The two (Koffea and 11/02) yam cultivars (*Dioscorea spp.*) were collected and used to conduct this experimental study. The number Koffea and 11/02 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, variety of Koffea to *D. alata,* and 11/02 to *D. rotundata.* Both cv Koffea and 11/02 landraces were selected because of their tolerance to drought and they provide a reasonable yield at this condition [27]. Moreover, landrace 11/02 has a medicinal value.

The tuber cuttings of both cultivars were collected from JARC experimental field. Tuber of these two yam cultivars were cut and prepared as a seed of yam with a number of buds and planted in poly bags and kept in greenhouse of JARC was carried out. The tuber sets in poly bags were watered once day and allowed to grow for three months until bud sprout was produced which actively growing nodal segments were collected and prepared as source of explants.

### *2.2. Stock Solution and Media Preparation*

Murashige and Skoog, 1962 (MS) media supplemented with various plant growth regulators were used. Stock solutions of the macro salts, micro salts, vitamins, and plant growth regulators (1mg: 1ml) were prepared as a stock solution and stored at +4°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. In addition to that, three anti-oxidants ascorbic acid (100 mg/l), citric acid (100 mg/l) and poly vinyl pyrrolidinone (PVP) (150 mg/l) were used according to Susmita and Shukla, [52] 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 sugar (3% w/v), Myo-inositol (0.1%w/v), plant growth regulators BAP, KN, IBA and NAA were added to the medium at required concentrations. The mixture was stirred using magnetic stirrer and the volume was adjusted using distilled water. Then, the pH was adjusted in all cases to 5.8 (Islam *et al*., 2008) using 1M NaOH and 1M HCl. Finally, (0.7% w/v) agar was added and heated to melt in the micro wave oven. Before autoclaving, the media was dispensed into sterilized culture jars and autoclave at a temperature of 121°C with a pressure of 0.15 Kpa for 20 minutes and transferred to the media storage room and stored for 72 hours.

### *2.3. Explants Preparation and Sterilization*

Nodal segments were used as a source of explants and collected from actively growing shoots two months old explants. 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 6 g/L of Copper Sulphate for 30 minutes [47] and rinsed with sterilized water for three times.

Thus, nodal segments were treated with combination of 5gm/l of ascorbic acid, acetic acid and poly vinyl pyrrolidone (PVP) and put on gyratory shaker for about two hours in order to minimize browning of the explants and flashed with sterilized water three times. Hence the nodal segments were disinfected with 70% ethanol alcohol for one minutes in laminar air flow hood and followed by 30% (v/v) of 5% active chlorine house bleach for 15 minutes. Finally, explants were incubated at 25±2°C, 16/8h photoperiod and light intensity of 40 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps for a month.

### *2.4. Treatments and Experimental Designs*

All the experiments were conducted in a completely randomized design (CRD) with factorial arrangement. Each experiment was replicated three times for both shoot multiplication, rooting and hardening off:

Experiment 1: Effect of BAP for 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 that supplemented with different concentrations of BAP. These five concentrations of BAP 0, 1, 2, 3, 4 and 5 mg/l were used to investigate its effect on shoot multiplication. Growth parameter like mean of shoot length, shoot number, leaf number and node number were recorded after 45 days of culture.

Experiment 2: Effect of IBA for rooting induction.

For *in vitro* rooting induction of plantlets were cultured on ½ MS media with different levels of IBA and NAA [62]. Accordingly, about seven levels of auxin were involved particularly IBA with concentrations of 0, 1, 2, 3, 4, 5 and 6 mg/l. The growth parameter like rooting percentage, mean of root number and root length were collected after 45 days of culture.

### *2.5. 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 (2:1:1) respectively. The plastic pots were placed in greenhouse where pot trays were covered with polythene to maintain humidity at 65-80%. Gradually, the *in vitro* raised plantlets were acclimatized to outdoor conditions and kept in green house until plantation in the field.

### *2.6. Data Analysis*

Data were analyzed using the analysis of variance procedure in the SAS statistical software (version 9.3) and Least Significance Difference (LSD) was used for mean separation. For all the data analysis, probability level of less than 5% ($P < 0.05$) was considered for statistical significance.

## 3. Results and Discussion

### *3.1. Effects of BAP for Shoot Proliferation*

Analysis of variance shown that the interaction effects of genotypes and BAP concentrations have been extremely significant change at ($p< 0.05$) for number of shoots/explant, shoot length and average number of leaves/shoot (Table 1; figure 1). 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 [53].

MS media which supplemented with 3 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 cv. Koffea (Table 1; Figure 1). Similarly, MS media fortified with 3 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 11/02 (Table 1; Figure 1). This exponentiation rate production might be due to genotypic changes, which have emotional impact to the frequency of tuber organogenesis and also endogenous cytokinin and auxin concentration variances [28] and [32]. 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 presentation of each cultivar is estimated to be unlikely *in vitro* culture as a field response concerning shoot number and shoot length [43].

***Table 1.*** *Effect of BAP for shoot multiplication of two yam cultivars.*

| Landraces | BAP Conc. mg/l | Shoot Number (Mean ± SD) | Leaf Number (Mean ± SD) | Node Number (Mean ± SD) | Shoot Height (cm) (Mean ± SD) |
|---|---|---|---|---|---|
| Koffea | 0 | 5.23±0.24$^{f}$ | 2.54±0.27$^{e}$ | 1.60±0.03$^{d}$ | 2.45±0.19$^{e}$ |
| | 1 | 5.98±0.21$^{de}$ | 3.47±0.28$^{cd}$ | 1.78±0.14$^{c}$ | 4.28±0.28$^{d}$ |
| | 2 | 6.71±0.09$^{c}$ | 4.50±0.14$^{b}$ | 1.94±0.05$^{b}$ | 5.21±0.79$^{c}$ |
| | 3 | 6.79±0.09$^{bc}$ | 4.62±0.12$^{b}$ | 2.15±0.10$^{a}$ | 7.47$^{a}$±0.47$^{a}$ |
| | 4 | 4.99±0.33$^{fg}$ | 2.27±0.38$^{e}$ | 1.78±0.02$^{c}$ | 2.35±0.01$^{e}$ |
| | 5 | 4.86±0.13$^{g}$ | 2.12±0.15$^{e}$ | 1.48±0.08$^{d}$ | 3.89±0.06$^{d}$ |
| 11/02 | 0 | 5.71±0.24$^{e}$ | 3.13±0.32$^{d}$ | 1.57±0.03$^{d}$ | 2.91±0.85$^{e}$ |
| | 1 | 6.23±0.15$^{d}$ | 3.80±0.20$^{c}$ | 1.77±0.06$^{c}$ | 4.13±0.20$^{d}$ |
| | 2 | 7.10±0.31$^{ab}$ | 5.10±0.49$^{a}$ | 2.10±0.07$^{a}$ | 6.07±0.19$^{b}$ |
| | 3 | 7.23±0.21$^{a}$ | 5.31±0.32$^{a}$ | 2.10±0.15$^{a}$ | 7.68±0.24$^{a}$ |
| | 4 | 6.67±0.09$^{c}$ | 4.44±0.14$^{b}$ | 2.03±0.03$^{ab}$ | 5.21±0.34$^{c}$ |
| | 5 | 6.15±0.19$^{d}$ | 3.71±0.25$^{c}$ | 1.52±0.13$^{d}$ | 3.63±0.12$^{d}$ |
| CV% | | 5.38 | 7.96 | 6.76 | 8.17 |
| LSD (0.05) | | 0.34 | 0.46 | 0.16 | 0.68 |

Note: BAP=Benzyl Amino Purine. LSD=least significant difference, Means with the same letter in the same column are not significantly different at 0.05 probability level, CV=Coefficient of Variation.


***Figure 1.*** *In vitro shoot multiplication for both koffea and 11/02 yam landrace on MS medium containing 3 mg/l BAP after 45 days of culture.*

Cultivars of cv. Koffea and 11/02, gave the highest mean number of shoot with mean shoot length and leaf numbers on MS media supplemented per 3 mg/l BAP. Hence, full strength MS basal media using 3 mg/l BAP could be full as the best concentration for these landraces, because it makes available to maximum value for shoot number, shoot length and leaf number. This current finding was in line with the finding of Poornima and Ravishankar, [46]; Fay, [25] and Chu *et al*., [20]. However, this finding is contradictory with the report of [38] because they noticed that MS media with 4 mg/l and 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.

Growing 1 to 2 mg/l BAP revealed a important 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 from 3.47±0.28 to 4.50±0.1429 leaves/shoot in cv. Koffea. Landrace 11/02 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 85/02. The landrace 11/02 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 2 mg/l BAP. This finding is harmonious with the report of [52]; Borges *et al*., [17]; Belarmino and Gonzales [15].

Fortunately, slightest shoot number 4.86±0.13 with lowest shoot length 2.35 cm on MS media with 4 mg/l BAP respectively and smallest leaf number 1.89±0.15 of landrace cv. Koffea was recorded on MS media with 5 mg/l BAP. Similar trends i.e. increasing of BAP with decreasing of shoot proliferation was observed in landrace 11/02.

In both cultivars increasing of BAP above 3 mg/l, resulted shoots with close and undersized features that have been usually unfeasible due to high amount of hormone that syndromes the metabolism of the shoot. This shown that advanced concentration of cytokinin led to inhibition of the metabolic activity which hinders plant growth. Howevere, small concentration of cytokinin promotes shoot prolifaration and root elongation [29]. This finding is contradictory with finding of Ammirato, [4] and Ezeibekwe *et al*., [24] because they reported MS media greater than 4 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 3 mg/l BAP and yam landrace with 11/02 was performed impressively. Generally, 11/02 yam landrace showed impressive growth than cv. Koffea this might be due to difference of endogenous BAP concentration. However, in both cultivars 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 cultivars were recorded increasing leaf number, number and length of shoot with simultaneous increase of BAP until 3 mg/l BAP concentration. Particularly, BAP with 3 mg/l was the favorable concentration of cytokinin type for the parameters like leaf number, shoot number and shoot length. Therefore, MS media with 3 mg/l BAP identified as optimum and suitable concentration for shoot proliferation for both landraces of yam.

Despite of 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 [50]. 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 [23]. 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 [37]. The current result is in conformity with the finding of Sowa *et al*., [51] 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 [19]. But the findings of decreasing shoot proliferation at lowest levels of BAP contradicts report of Thankappan and Abraham, [55] 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 cv. Koffea and 11/02 respectively, on MS media supplemented with 3 mg/l BAP (Table 1) this implies that MS media with 3 mg/l BAP was an optimum concentration. The second highest number of nodes 1.94±0.05 was obtained on MS media with 2 mg/l BAP cv. Koffea this indicates that the requirement of further MS media greater than 2mg/l BAP this finding is similar with finding of [Susmita and Shukla, 2014]. Moreover, for both yam landrace cv. Koffea and 11/02 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.

### *3.2. Effect of IBA for Root Inductions*

Analysis of variance revealed that the interaction effects of two genotypes and IBA were highly significant ($p< 0.05$) for rooting percentage, number of root/shoot and average root length (Table 2; figure 2). 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 [48]. 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 [16].

MS media supplemented with 1 and 4 mg/l IBA gave the highest (54.33±0.08) and the lowest 32.67% rooted shoots for cv. koffea (Table 2). Whereas, MS media supplemented with 1 and 5 mg/l IBA was produced a maximum of 68.00% and minimum 34.33% of rooted shoots for landrace 11/02. These outcomes point out that each genotype recalcitrant due to different endogenous auxin amounts. Every single genotype needs different concentrations based on the quantity of endogenous auxin concentration [48]. By increasing the concentration of IBA from 3mg/l to 5mg/l, proportion of rooted shoots reduced continuously from 46.33% to 32.67% in koffea, and discontinuously decreased from 49.00% to 34.33%in 11/02. The variation in rooting percentage of in these two landraces was raised due to genotypic variation which results variation in indigenous IBA concentration.

In koffea, root induction and elongation proportion increased from 43.00% at 0.5mg/l to 54.33% at 2mg/l IBA, nevertheless the roots were not well grownup, and they were extremely short at 1mg/l than 2mg/l. This shows that little concentration of IBA encourages root initiation and elongation than extremely upper concentration that inhibited rooting in both landraces. This observation disagrees with finding of Forsyth and Stadan, [26] 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 11/02, highest (68.00±0.03) and minimum (36.00±0.03) rooting percentage was obtained on MS media with 1 and 6mg/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 [6] 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 [37]. This finding contradicts with Poornima and Ravishankar, [46] 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 cultivars. This clarifies the endogenous IBA variation between the landraces. Moreover, the landrace providing maximum rooting percentage at minimum concentration of exogenously added IBA emphasizes presence of high concentration of endogenous IBA [16]. 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 11/02 is richer in endogenous IBA than koffea.

Cultivar koffea gave the highest (10.03±0.49) roots/shoot with 10.76±0.16cm average root length on MS medium with 3mg/l IBA (Table 2, Figures 2 and 3). In 11/02 maximum 10.58±0.26 roots/shoot with 10.42±0.32 cm average root length were observed on MS media fortified with 2 and 1 mg/l IBA respectively (Table 2; Figure 2). 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.

Therefore, these studies have been raveled that addition of 1 or 2 mg/l IBA to the medium induced fastest rooting and had a higher average number of roots per segment in *D. zingiberensis* [18]. Maximum rooting was obtained on MS media with 2 mg/l IBA which was different from the finding of Behera *et al*., [14], 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 3 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 short root length (5.54±0.14 cm) in 85/02 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 2 and 3 mg/l IBA in koffea and 11/02 respectively this implies further increasing of IBA. This finding is not similar with Antonio *et al*., [6] because their finding elaborates best root performance could be resulted on MS media supplemented with 4 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 [19, 46].

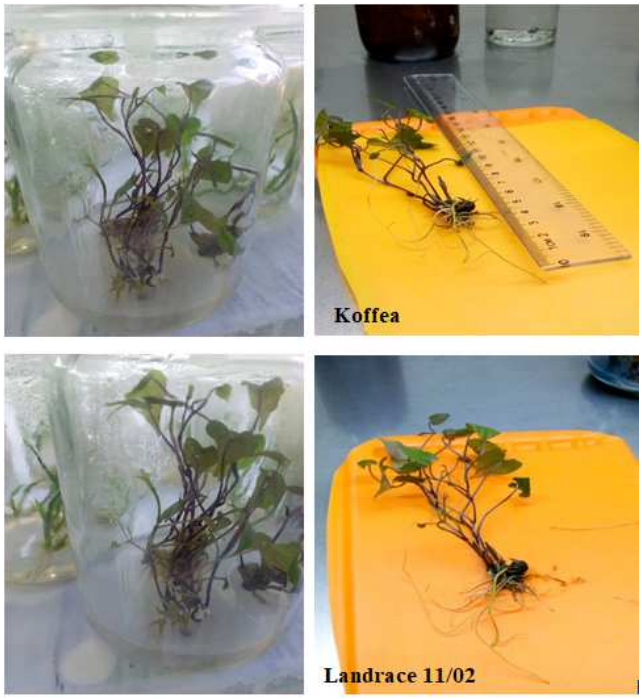


***Figure 2.*** *In vitro raised shootlets for rooting parameters after 45 days of culture on MS medium containing 3 mg/l IBA of both Koffea and 11/02 landraces of yam.*

***Table 2.*** *Effect of IBA for rooting of two yam cultivars.*

| Landraces | IBA conc. (mg/l) | Rooting (%) | Root Height (cm) (Mean±SD) | Root Number (Mean±SD) |
|---|---|---|---|---|
| | 0 | 38.33±0.02$^{de}$ | 5.54±0.14$^{e}$ | 5.65±0.18$^{f}$ |
| | 1 | 43.00±0.03$^{cd}$ | 8.64±0.53$^{bc}$ | 8.14±0.53$^{c}$ |
| | 2 | 46.33±0.01$^{c}$ | 9.35±0.57$^{b}$ | 7.60±0.30$^{de}$ |
| Koffea | 3 | 54.33±0.08$^{b}$ | 10.76±0.16$^{a}$ | 10.03±0.49$^{b}$ |
| | 4 | 43.00±0.03$^{cd}$ | 7.11±0.38$^{d}$ | 8.40±0.44$^{c}$ |
| | 5 | 32.67±0.06$^{e}$ | 7.76±0.57$^{cd}$ | 7.23±0.31$^{e}$ |
| | 6 | 37.00±0.02$^{de}$ | 7.04±0.21$^{d}$ | 7.09±0.11$^{e}$ |
| | 0 | 37.00±0.03$^{de}$ | 5.94±0.37$^{e}$ | 5.50±0.21$^{f}$ |
| | 1 | 49.00±0.05$^{bc}$ | 7.61±0.83$^{d}$ | 8.17±0.41$^{c}$ |
| | 2 | 55.00±0.05$^{b}$ | 8.59±0.83$^{bc}$ | 8.06±0.38$^{cd}$ |
| 11/02 | 3 | 68.00±0.03$^{a}$ | 10.42±0.32$^{a}$ | 8.09±0.17$^{cd}$ |
| | 4 | 45.67±0.01$^{c}$ | 7.79±0.33$^{cd}$ | 10.58±0.26$^{a}$ |
| | 5 | 34.33±0.05$^{e}$ | 7.28±0.34$^{d}$ | 7.44±0.21$^{e}$ |
| | 6 | 36.00±0.03$^{e}$ | 7.33±0.93$^{d}$ | 7.32±0.34$^{e}$ |
| CV (%) | | 7.98 | 7.63 | 5.88 |
| 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 0.05 probability level, CV=Coefficient of Variation.

### *3.3. 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 koffea and 11/02 yam landraces, respectively (Figure 3). Data was recorded in the greenhouse within four weeks after transferred to the sterilized soil medium (2:1:1 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 11/02 performed well in *ex vitro* establishment than cv. koffea. The current outcome of 90% survival rate in 11/02 is parallel with finding of Behera *et al*., [12]; Behera *et al*., [13] and Behera *et al*., [14] who reported 90% survival rate of different *Dioscorea species* in *ex vitro* establishment.

On other hand, cv. koffea exhibited about 86% of survival rate at *ex vitro* condition and this finding was similar with finding of Obsi *et al*., [42] and almost similar survival rate (80%) was obtained by Kadota and Niimi, [34] when micro-propagated plants of *D. japonica* were transferred to pots containing (1:1) vermiculite and soil (v/v) mixture under greenhouse.


***Figure 3.*** *Acclimatized plantlets of two yam koffea and 11/02 landraces after a month in greenhous.*

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