[65]

**RESEARCH PAPER**

# *In vitro* Plantlet Regeneration from Nodal Explants of Anchote [*Coccinia abyssinica* (Lam.) Cong.] Genotypes

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## Abstract

*In vitro plantlet regeneration experiment using nodal explants in KUWE (G1) and 223098 (G2) genotypes of anchote was conducted by regulating plant growth regulators and nutrient-strength of Murashige and Skoog media. Shoot development was evaluated on media supplemented with different concentrations and combinations of 6-Benzylamimopurine and Kinetin. Root formation was assessed on full and half nutrient-strength media, each with various concentrations of Indol Butyric Acid. Multiple shoots in G1 (4.85) and G2 (5.65) along with 100% induction frequency were obtained at 0.5 mg/L 6-Benzylamimopurine alone and 1mg/L 6-Benzylamimopurine + 0.25 mg/L Kinetin, respectively. Long shoots in both G1 (6.57 cm) and G2 (5.92 cm) was obtained on hormone free medium. Full and half nutrient-strength media without hormone were capable of producing 100% root formation in G1 and G2, respectively. Maximum root numbers in G1 (22.97) and G2 (17.6) were obtained on full nutrient-strength media with 0.25 mg/L and 0.5 mg/L Indol Butyric Acid, respectively. Long roots in G1 (8.02 cm) and G2 (7.07 cm) were recorded from hormone free full nutrient-strength media. Roots developed on hormone free media exhibited normal morphological appearance. After four weeks of acclimatization, plantlets of 76% in G1 and 79% in G2 were survived. In general, plantlet regeneration from nodal explants of both genotypes of anchote has been successfully achieved and the procedures could be applied for in vitro production of propagation materials of the respective genotypes.*

**Keywords**: Direct regeneration, Genotype, MS media, Nodal explants, Plant growth regulators

## Introduction

Anchote [*Coccinia abyssinica* (Lam.) Cong.] is one of the most potentially important indigenous root tuber crops of Ethiopia. It is an annual trailing vine belongs to the Cucurbitaceae family and found under cultivation and in the wild in the country (Demel *et al*., 2010; Tufa *et al*., 2017). One of the distinguishing natures of the crop is that almost all parts of it (tuber, tender shoots, immature fruits and seeds) are consumable. However, the cultivated type of anchote is best known and grown principally for its tuberous root (Tufa *et al.,* 2017). The tuber of anchote is the richest in calcium, protein, iron and Vitamin B2 contents of all other common widespread root tuber crops such as potato, sweet potato, taro, yam and cassava (Habtamu *et al.,* 2013; Tola, 2014; Aditya *et al.,* 2017). In addition, the low content of anti-nutritional

factors reflects the desirable quality of the tuber (Habtamu *et al.,* 2013; Habtamu and Kelbesa, 1997). The nutritive values of edible leaves and fruits of anchote are high even better than the commonly used tuber of the crop (Desta, 2011; Girma and Dereje, 2015; Ayalew *et al*., 2017). Apart from its nutritional importance, anchote is a medicinal plant and has been used as a folklore medicine to heal bone fractures, backaches, displaced joints and other diseases such as gonorrhea, skin eruptions, tuberculosis and cancer (Amare, 2003; Endashaw, 2007).

Cultivation of anchote is sporadic but widespread in western and southwestern parts of Ethiopia on altitude between 1300 and 2800 m.a.s.l with 762–1016 mm annual rainfall (Endashaw, 2007; Daba *et al.,* 2012). Anchote can be propagated both vegetatively (using tubers and vines) and by seed but propagation by seed is a common practice by local farmers

(Negash, 2019). The tuber yield of anchote at farmers level has been estimated 10-20 t/ha (Amsalu *et al.,* 2008). Whereas, under experimental condition, a maximum of 76-80 t/ha tuber yield was reported (Desta, 2011; Daba *et al.,* 2012). On the other hand, Bekele (2017) obtained not more than 26 t/ha of tuber yield in his trial using different accessions of anchote including those maximum yielders in Desta (2011) and Daba *et al.* (2012) experiments. Apart from other yield limiting environmental and agronomic factors, genetic segregation of yield contributing traits might have positive contribution in such a huge variation of anchote tuber yield. The high out-crossing nature of anchote due variation in developmental position and blooming time of male and female flowers has been demonstrated by Holstein (2015). Therefore, looking for *in vitro* propagation techniques is crucial to overcome such a challenge through providing quality propagation materials.

*In vitro* propagation using nodal explants is one of the simplest techniques of plant cell, tissue and organ culture to produce plenty of disease-free propagation materials which are genetically identical with the mother plant within short period of time. However, *in vitro* response of crop species and genotypes has been reported to vary greatly with their requirements for optimum culture environment (George and Debergh, 2008). Regardless of the type of explants, *in vitro* response variation between genotypes has been investigated in many crop species: cassava (Mapayi *et al*., 2013), eggplant (Sharmin *et al*., 2008), sweet potato (Geleta and Tileye, 2011), *Telfairia occidentalis* (Adesoye *et al.,* 2012). Little *in vitro* propagation studies in anchote carried out recently have also indicated genotype variation on the requirements for optimum culture media compositions (Folla *et al.*, 2013; Rahel, 2013; Kahia *et al.*, 2016). In considering such genotype based variations on the requirement of optimum *in vitro* culture media, this research work was initiated to investigate plantlet regeneration from nodal explants of KUWE (G1) and 223098 (G2) genotypes of anchote through regulating plant growth regulators and nutrient-strength of the medium.

# Materials and Methods

## Study area and plant materials

The experiment was conducted at the Plant Tissue Culture Laboratory of National Agricultural Biotechnology Research Centre (NABRC), Ethiopian Institute of Agricultural Research (EIAR), which is located about 44 km far to the west of Addis Ababa, the capital city of Ethiopia. Seeds of two anchote accessions, KUWE (G1) and 223098 (G2), each from different genotype cluster (Desta, 2011) were collected from Debre Zeit Agricultural Research Centre (DZARC). Mother plants were established in a greenhouse using pots filled with 2:1:1 ratio of soil, sand and compost, respectively.

## Media preparation

Murashige and Skoog (1962) (MS) basal nutrients with 3% sucrose was used as the basic components of the media. The pH of the medium was adjusted to 5.8 upon addition of plant growth regulators using hydrochloric acid (1N HCl) and sodium hydroxide (1N NaOH). Then, agar type I was added at a rate of 4.5 g/L while boiling to solidify the media. The prepared media were autoclaved for 20 minutes at a temperature of 121 $^{\circ}$C and 15 psi pressure and left for three days in the culture room before use to check for any contamination.

## Surface disinfection of explants

The second and third young nodes from five weeks old healthy greenhouse grown plants of each genotype were collected. Primary washing for five minutes using tap water and liquid detergent along with the addition of 1-2 drops of Tween-20 was done to remove dirt and reduce the level of contaminants attached on the surface of the nodes. Nodes were further surface disinfected under the laminar airflow hood with 70% alcohol for 30 seconds and 1% Clorox bleaches (5.25% NaOCl) for 5 minutes. The concentration and time exposure of NaOCl was determined through preliminary optimization experiment. Upon the completion of each surface disinfection process, nodes were rinsed four to five times with sterilized double distilled water to remove the effect of surface sterilizing agents. During all the surface disinfection processes, nodes were hand shaken

by putting them in a beaker with lid to facilitate contact between floating nodes and disinfected agents.

## Shoot induction and multiplication

Node explants of about 1 cm size were prepared by trimming extra large size including damaged parts at the cut ends of the nodes due to sterilizing agents. Nodal Explants were then cultured by placing them vertically in a magenta jar containing 50 ml of shoot induction and multiplication medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP) (0.5, 1, 1.5 and 2 mg/L) and Kinetin (Kn) (0.25, 0.5 and 1 mg/L). Hormone free MS medium was used as a control. There were a total of 40 treatments (five BAP levels x four Kn levels x two genotypes). Shoot induction frequency, number of shoots per explant, average shoot length and number of nodes per shoot were collected at about four weeks of culture initiation to evaluate shoot induction and multiplication response of the genotypes under the given concentrations and combinations of plant growth regulators (PGRs).

## Root induction and development

Normal and healthy shoots of 2 cm and above in length were selected and cultured on hormone free MS medium for five days to avoid the carryover shooting hormones. Shoots were then cultured in a magenta jar containing 50 ml of full nutrient-strength of MS medium (FMS) and half nutrient-strength of MS medium (HMS) supplemented with different concentration levels of indol butyric acid (IBA) (0.25, 0.5, 0.75 and 1 mg/L). Hormone free MS medium was also used as a control. There were a total of twenty treatments (two genotypes x two MS medium nutrient-strength x five IBA concentration levels). Data on root induction frequency, number of roots per shoot and length of root were collected after four weeks of culture on the rooting media.

## Culture conditions

All the culturing processes were undertaken under the aseptic condition of the laminar air flow hood. Unnecessary callus developed at the base of shoots was trimmed with the help of surgical blade before shoots subjected to root induction treatments. All cultures were kept in a growth room under 16 hours light (2700 lux light intensity) and 8 hours dark cycle at a temperature of $25^0C \pm 2^0C$.

## Acclimatization

Well rooted shoots with the absence of any defects were selected and rinsed several times in a container containing tap water to remove the attached agar. Plantlets were then planted in a greenhouse on a soil medium prepared from 2:1:1 ratio of soil, sand and compost, respectively. Acclimatization trays/pots contained plantlets were covered with a plastic sheet. The plastic sheet cover was gradually removed and at the end of third weeks plantlets were completely exposed to the greenhouse environment. Number of survived plantlets was recorded at the end of fourth weeks of acclimatization to evaluate survival rate of plantlets.

## Design and statistical analysis

All factorially combined treatments were arranged in Completely Randomized Design (CRD). Each data was collected from a total of twenty sample units, five observations per culture jar replicated four times. Data of all the quantitative parameters were subjected to statistical analysis with SAS computer statistical tool (version 9.1). List Significant Difference (LSD) test was conducted to compare treatment means, which revealed a significant difference of F-test in the ANOVA, at $p \leq 0.05$ probability level.

# Results and Discussion

## Shoot induction and multiplication response of the genotypes

All the main and interaction effects of genotype, BAP and kinetin significantly ($p \leq 0.05$) affected shoot induction parameters, except the main effect of genotype on shoot induction frequency, number of shoots and number of nodes. Mean values of shoot induction parameters as affected by the three

way interaction of genotype, BAP and kinetin are presented in Table 1.

Shoot formation was observed in all treatments including the controls of both genotypes. However, the level of shoot induction frequency varied with the type and concentration of cytokinins used. Both genotypes cultured on MS media supplemented with 0.5 mg/L BAP + 0.25 mg/L Kn, 1 mg/L BAP alone and 1 mg/L BAP + 0.25 mg/L Kn resulted in 100% induction frequency. Media supplemented with 0.5 mg/L BAP with and without 0.5 mg/L Kn in G1 and 0.5 mg/L Kn alone in G2 also resulted in 100% shoot formation (Table 1). Lower shoot induction frequency in G2 (45%) and G1 (65%) was recorded from hormone-free medium (Control treatment). A significant reduction in shoot induction frequency of both genotypes was observed as the level of BAP increased beyond 1 mg/L within the range regardless of the kinetin concentration levels (Table 1). Folla *et al.* (2013), Kahia *et al.* (2016) and Rahel (2013) reported 3 µM BAP, 0.5 µM BAP and 2 mg/L BAP, respectively as optimum BAP concentrations for maximum shoot induction frequency in nodal explants of other genotypes of anchote. However, all the authors did not get more than 80-85% shoot induction frequency which is significantly lower as compared to the findings of the current study. This variation might be developed due to differences in genotype. *In vitro* regeneration variations due to differences in crop genotype has been well demonstrated by Piqueras and Debergh (1999).

The highest number of shoots per explant (5.56) was recorded in G2 at 1mg/L BAP + 0.25 mg/L Kn while in G1, a maximum of 4.85 shoots per explant was recorded at 0.5 mg/L BAP alone (Table 1). Media without shooting hormone (control treatment) produced only a single shoot in both genotypes. A general decrease in shoot number was observed as the level of BAP increases beyond 0.5 mg/L in G1 and 1 mg/L in G2 regardless of kinetin concentrations. Similar trends on the number of shoots from nodal culture of other genotypes of anchote were reported by Kahia *et al.* (2016), Folla *et al.* (2013), and Rahel (2013). However,

the optimum concentrations and/or combinations of BAP and kinetin that the authors reported greatly vary with the current findings. The variation between genotypes in terms of multiple shoot production along with the requirement of optimum concentration and combination of cytokinins might be due to their totipotentiality and endogenous level of PGRs as stated by Piqueras and Debergh (1999). Such a genotype based variations has been also well demonstrated in sweet potato (Romuald and Anna, 2013). Long shoots in G1 (6.57 cm) and G2 (5.92 cm) along with highest number of nodes (4.75 in G1 and 4.5 in G2) were recorded from hormone-free medium. Comparatively, most of the media supplemented with kinetin produced longer shoots in both genotypes (Table 1). Whereas, shoots developed on BAP supplemented media generally exhibited bushy nature with very short internodes as compared to shoots obtained from the control and kinetin supplemented media (Fig 1A-C). The shoot length response variation between BAP and kinetin might be related with the variation in their stimulating effect on cell elongation as demonstrated by George and Debergh (2008). A similar phenomenon was also observed in other genotypes of anchote, particularly at an increased concentration level of BAP (Kahia *et al.*, 2016; Folla *et al.*, 2013). Moreover, the negative effect of BAP on plantlet height was demonstrated by Sanavy and Moieni (2003) in different potato cultivars. Whereas, the positive effects of kinetin on shoot elongation of *Stevia rebaudiana* has been reported by Sridhar and Aswath (2014).

A general decrease in shoot number was observed in both genotypes as the level of BAP increases, regardless of the kinetin concentration levels. This might be due to unnecessary callus development at the basal section of explants which upon time grown and suppressed multiple shoot formation (Fig 1D). The callusing nature of BAP, particularly at higher concentration levels, has been investigated in different *Coccinia* species: *Coccinia abbysinica* (Folla *et al.*, 2013; Rahel, 2013; Kahia *et al.,* 2016), *Coccinia cordifolia* (Roy *et al.*, 2012), *Coccinia indica* (Shekhawat *et al.,* 2014).

Table 1. Interaction effects of 6-benzylaminopurine (BAP) and Kinetin (Kn) on shoot induction and multiplication parameters of KUWE (G1) and 223098 (G2) genotypes of anchote

| Treatment | | | Shoot induction and multiplication parameters (mean ± SD) | | | |
|---|---|---|---|---|---|---|
| Genotype | BAP (mg/L) | Kn (mg/L) | ShIF | NoSh | ShL | NoN |
| G1 | 0 | 0 | 65 ± 10.00[f] | 1.00 ± 0.00[v] | 6.57 ± 0.35[a] | 4.75 ± 0.50[a] |
| | | 0.25 | 70 ± 11.55[ef] | 1.77 ± 0.17[qrs] | 6.20 ± 0.40[ab] | 3.55 ± 0.31[def] |
| | | 0.5 | 75 ± 10.00[def] | 1.42 ± 0.15[s-v] | 4.62 ± 0.22[c] | 3.30 ± 0.22[efg] |
| | | 1 | 95 ± 10.00[ab] | 1.62 ± 0.25[q-u] | 3.67 ± 0.34[de] | 2.50 ± 0.22[lmn] |
| | 0.5 | 0 | 100 ± 0.00[a] | 4.85 ± 0.30[b] | 3.50 ± 0.26[ef] | 3.77 ± 0.33[bcd] |
| | | 0.25 | 100 ± 0.00[a] | 4.25 ± 0.30[c] | 4.02 ± 0.33[d] | 2.67 ± 0.09[j-m] |
| | | 0.5 | 100 ± 0.00[a] | 3.70 ± 0.26[d] | 2.95 ± 0.13[hij] | 2.02 ± 0.21[pq] |
| | | 1 | 90 ± 11.55[abc] | 2.47 ± 0.21[k-n] | 3.02 ± 0.17[g-j] | 2.32 ± 0.30[m-p] |
| | 1 | 0 | 100 ± 0.00[a] | 2.85 ± 0.19[h-k] | 2.87 ± 0.26[h-k] | 2.70 ± 0.21[i-l] |
| | | 0.25 | 100 ± 0.00[a] | 2.00 ± 0.40[opq] | 2.97 ± 0.17[hij] | 2.25 ± 0.50[n-q] |
| | | 0.5 | 85 ± 10.00[bcd] | 3.35 ± 0.41[def] | 2.32 ± 0.36[m-p] | 2.75 ± 0.21[i-l] |
| | | 1 | 80 ± 0.00[cde] | 1.62 ± 0.33[q-u] | 2.80 ± 0.29[h-l] | 2.55 ± 0.19[k-n] |
| | 1.5 | 0 | 85 ± 10.00[bcd] | 3.30 ± 0.24[d-g] | 2.02 ± 0.17[p-s] | 3.97 ± 0.09[bc] |
| | | 0.25 | 80 ± 16.33[cde] | 2.50 ± 0.57[k-n] | 2.45 ± 0.10[l-o] | 2.90 ± 0.14[h-k] |
| | | 0.5 | 90 ± 11.55[abc] | 2.27 ± 0.32[m-p] | 2.95 ± 0.10[hij] | 2.27 ± 0.31[n-q] |
| | | 1 | 85 ± 10.00[bcd] | 3.05 ± 0.13[f-i] | 2.67 ± 0.15[j-m] | 2.57 ± 0.09[k-n] |
| | 2 | 0 | 90 ± 11.55[abc] | 2.37 ± 0.33[l-o] | 1.82 ± 0.21[q-t] | 3.05 ± 0.13[ghi] |
| | | 0.25 | 75 ± 10.00[def] | 2.25 ± 0.29[nop] | 2.05 ± 0.19[o-r] | 3.15 ± 0.19[gh] |
| | | 0.5 | 80 ± 16.33[cde] | 2.87 ± 0.27[g-k] | 1.77 ± 0.26[rst] | 2.97 ± 0.17[g-j] |
| | | 1 | 85 ± 10.00[bcd] | 1.32 ± 0.27[tuv] | 2.12 ± 0.30[n-r] | 2.10 ± 0.14[opq] |
| G2 | 0 | 0 | 45 ± 10.00[g] | 1.00 ± 0.00[v] | 5.92 ± 0.67[b] | 4.50 ± 0.57[a] |
| | | 0.25 | 95 ± 10.00[ab] | 1.30 ± 0.24[uv] | 4.72 ± 0.46[c] | 3.02 ± 0.09[g-j] |
| | | 0.5 | 100 ± 0.00[a] | 2.60 ± 0.42[j-n] | 3.42 ± 0.38[efg] | 2.72 ± 0.26[i-l] |
| | | 1 | 90 ± 11.55[abc] | 2.47 ± 0.05[k-n] | 3.07 ± 0.27[g-j] | 2.47 ± 0.38[lmn] |
| | 0.5 | 0 | 80 ± 0.00[cde] | 3.60 ± 0.34[de] | 2.15 ± 0.13[n-r] | 3.62 ± 0.15[cde] |
| | | 0.25 | 100 ± 0.00[a] | 1.90 ± 0.20[pqr] | 3.10 ± 0.29[f-i] | 2.25 ± 0.21[n-q] |
| | | 0.5 | 95 ± 10.00[ab] | 3.00 ± 0.41[f-j] | 2.50 ± 0.39[k-n] | 2.02 ± 0.05[pq] |
| | | 1 | 80 ± 0.00[cde] | 1.75 ± 0.29[q-t] | 2.75 ± 0.21[i-l] | 1.92 ± 0.09[q] |
| | 1 | 0 | 100 ± 0.00[a] | 4.60 ± 0.36[bc] | 1.97 ± 0.25[p-s] | 3.97 ± 0.27[bc] |
| | | 0.25 | 100 ± 0.00a | 5.65 ± 0.41[a] | 2.92 ± 0.09[hij] | 4.12 ± 0.29[b] |
| | | 0.5 | 90 ± 11.55[abc] | 2.40 ± 0.49[l-o] | 3.07 ± 0.17[g-j] | 2.77 ± 0.28[i-l] |
| | | 1 | 85 ± 10.00[bcd] | 1.67 ± 0.23[q-u] | 3.02 ± 0.12[g-j] | 2.45 ± 0.13[l-o] |
| | 1.5 | 0 | 90 ± 11.55[abc] | 2.75 ± 0.30[i-l] | 2.22 ± 0.38[n-q] | 3.32 ± 0.20[efg] |
| | | 0.25 | 90 ± 11.55[abc] | 2.45 ± 0.41[k-n] | 2.10 ± 0.34[n-r] | 2.05 ± 0.21[pq] |
| | | 0.5 | 95 ± 10.00[ab] | 2.30 ± 0.26[m-p] | 2.25 ± 0.29[nop] | 2.57 ± 0.15[k-n] |
| | | 1 | 75 ± 10.00[def] | 1.65 ± 0.34[q-u] | 3.17 ± 0.22[fgh] | 2.45 ± 0.37[l-o] |
| | 2 | 0 | 75 ± 10.00[def] | 3.20 ± 0.23[e-h] | 1.52 ± 0.28[t] | 2.67 ± 0.33[j-m] |
| | | 0.25 | 90 ± 11.55[abc] | 2.95 ± 0.25[f-j] | 2.07 ± 0.22[o-r] | 3.25 ± 0.21[fgh] |
| | | 0.5 | 70 ± 11.55[ef] | 2.70 ± 0.47[i-m] | 1.62 ± 0.39[st] | 2.50 ± 0.29[lmn] |
| | | 1 | 65 ± 10.00[f] | 1.50 ± 0.24[r-u] | 1.82 ± 0.23[q-t] | 2.05 ± 0.25[pq] |
| | CV (%) | | 10.82 | 12.10 | 9.71 | 9.12 |

Means assigned with the same letter within a column are not significantly (p < 0.05) different to each other; ShIF = Shoot Induction Frequency, NoSh = Number of Shoots, ShL = Shoot Length, NoN = Number of Nodes
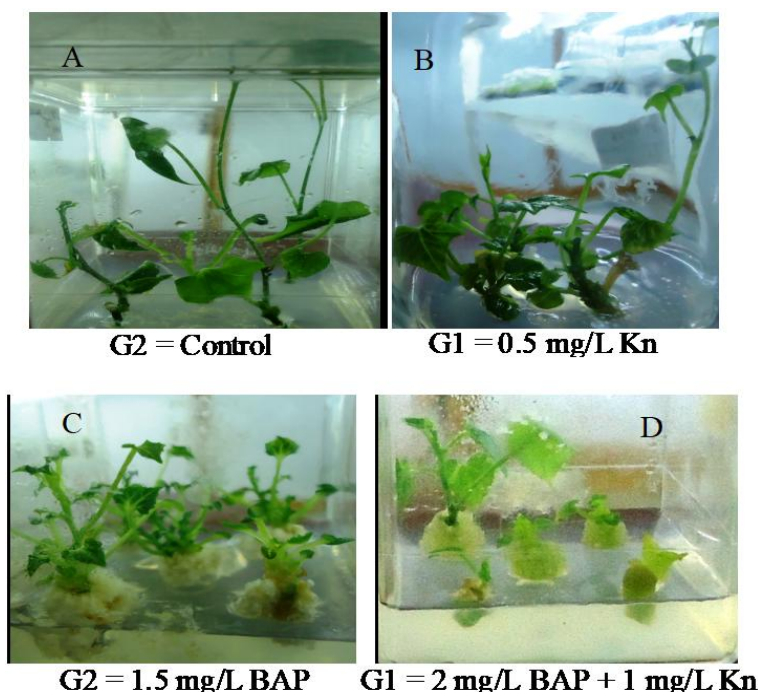
Figure 1. Morphological appearance of shoots induced from nodal explants in KUWE (G1) and 223092 (G2) genotypes of anchote observed at fourth week of culture initiation: Long shoots with no basal callus formation (A and B), Bushy nature of shoots obtained at an increased level of BAP concentration (C) and Suppressed shoot formation due to unnecessary callus formation (D).

## Effect of MS media nutrient-strength and IBA on root induction and development

Interaction of genotype, media nutrient-strength and IBA significantly (p ≤ 0.05) affected root induction and development parameters. Half nutrient-strength MS media (HMS) with 0.5 mg/L IBA resulted in 100% root formation in G1. Whereas in G2, 100% root formation was recorded from full nutrient-strength MS media (FMS) supplemented with 0.75 mg/L IBA and HMS + 0.25 mg/L IBA. Similarly, FMS and HMS without rooting hormone were capable of producing 100% root induction frequency in G1 and G2, respectively (Table 2). Ishag *et al.* (2009) also obtained 100% root induction frequency in tomato shoots without external application of rooting hormones as well as with the application of IBA at 0.5 mg/L and lower concentrations. The finding of the current study is, however, far apart with the previous findings in anchote. Kahia *et al.* (2016) reported 40%

root formation with HMS without rooting hormone. Rahel (2013) obtained 18% root induction frequency with the control treatment of FMS. Whereas, Folla *et al.* (2013) did not obtain any root on the control treatment using HMS. Regardless of the variation between genotypes, these authors reported a maximum of 76-91% root formation in anchote micropropagation even with the application of rooting hormones. The reason for 100% root formation without rooting hormone might be related with the endogenous auxin level of the genotypes and such genotypes eliminate the cost for external application of rooting hormones.

The number and length of roots recorded in both genotypes significantly varied with the media nutrient-strength and IBA concentration (Table 2). The highest number of roots per shoot (22.97) was recorded in G1 on FMS + 0.25 mg/L IBA while the minimum number of roots per shoot (3.15) was obtained from the

HMS control treatment of the same genotype. Comparatively long roots in both genotypes (8.02 cm in G1 and 7.07 cm in G2) were obtained from hormone-free full nutrient-strength medium. While, the shortest roots in G1 (2.25 cm) and G2 (2.7) were obtained from full nutrient-strength media with 1 mg/L IBA and half nutrient-strength medium with 0.75 mg/L IBA, respectively.

Regardless of the variation between genotypes and media nutrient-strength, a general decrease in number of roots per shoot and average shoot length were observed as the IBA concentration increased from 0.25 to 1 mg/L (Table 2). Apart from reduction in number and length of roots, roots formed at an increased concentration of IBA were also observed morphologically abnormal as compared to roots formed with no defects at lower concentrations of IBA (Fig 2A-C). This might be due to unnecessary callus formation at the base of the shoots. Such root formation challenges due to unnecessary callus formation at an elevated concentration of IBA was also observed by Folla *et al.* (2013) in anchote and Shekhawat *et al.* (2014) in *Coccinia indica*.

Table 2. Interaction effects of MS media nutrient-strength and idol butyric acid (IBA) on the root formation of KUWE (G1) and 223098 (G2) genotype of anchote

| Treatment | | | Mean value ± SD | | |
|---|---|---|---|---|---|
| Genotype | MS-strength | IBA (mg/L) | RF (%) | NoR | RL (cm) |
| G1 | FMS | 0 | $100 \pm 0.00^a$ | $12.15 \pm 0.25^f$ | $8.02 \pm 0.33^a$ |
| | | 0.25 | $85 \pm 10.00^{bcd}$ | $22.97 \pm 0.27^a$ | $7.25 \pm 0.24^b$ |
| | | 0.5 | $90 \pm 11.55^{abc}$ | $10.52 \pm 0.34^h$ | $5.97 \pm 0.36^{de}$ |
| | | 0.75 | $80 \pm 0.00^{cde}$ | $11.25 \pm 0.33^g$ | $2.82 \pm 0.21^k$ |
| | | 1 | $60 \pm 0.00^g$ | $5.40 \pm 0.26^k$ | $2.25 \pm 0.26^l$ |
| | HMS | 0 | $90 \pm 11.55^{abc}$ | $3.15 \pm 0.31^l$ | $6.52 \pm 0.51^c$ |
| | | 0.25 | $90 \pm 11.55^{abc}$ | $11.32 \pm 0.57^g$ | $5.60 \pm 0.29^{ef}$ |
| | | 0.5 | $100 \pm 0.00^a$ | $13.17 \pm 0.36^e$ | $4.42 \pm 0.40^h$ |
| | | 0.75 | $75 \pm 10.00^{def}$ | $10.52 \pm 0.46^h$ | $4.15 \pm 0.26^{hij}$ |
| | | 1 | $80 \pm 0.00^{cde}$ | $6.77 \pm 0.38^j$ | $3.92 \pm 0.09^{ij}$ |
| G2 | FMS | 0 | $80 \pm 0.00^{cde}$ | $10.52 \pm 0.36^h$ | $7.07 \pm 0.45^b$ |
| | | 0.25 | $90 \pm 11.55^{abc}$ | $16.27 \pm 0.25^c$ | $5.37 \pm 0.40^{fg}$ |
| | | 0.5 | $95 \pm 10.00^{ab}$ | $17.6 \pm 0.58^b$ | $4.52 \pm 0.25^h$ |
| | | 0.75 | $100 \pm 0.00^a$ | $13.75 \pm 0.19^d$ | $4.07 \pm 0.26^{hij}$ |
| | | 1 | $85 \pm 10.00^{bcd}$ | $6.37 \pm 0.43^j$ | $6.17 \pm 0.33^{cd}$ |
| | HMS | 0 | $100 \pm 0.00^a$ | $7.85 \pm 0.54^i$ | $5.10 \pm 0.47^g$ |
| | | 0.25 | $100 \pm 0.00^a$ | $13.22 \pm 0.22^e$ | $4.22 \pm 0.26^{hi}$ |
| | | 0.5 | $95 \pm 10.00^{ab}$ | $11.90 \pm 0.31^f$ | $4.37 \pm 0.38^{hi}$ |
| | | 0.75 | $70 \pm 11.55^{efg}$ | $16.05 \pm 0.34^c$ | $2.70 \pm 0.25^{kl}$ |
| | | 1 | $65 \pm 10.00^{fg}$ | $14.02 \pm 0.29^d$ | $3.75 \pm 0.39^j$ |
| | CV (%) | | 9.20 | 3.16 | 6.82 |

Means assigned with the same letter within a column are not significantly ($p < 0.05$) different to each other; RF = Rooting Frequency, NoR = Number of Root, RL = Root Length
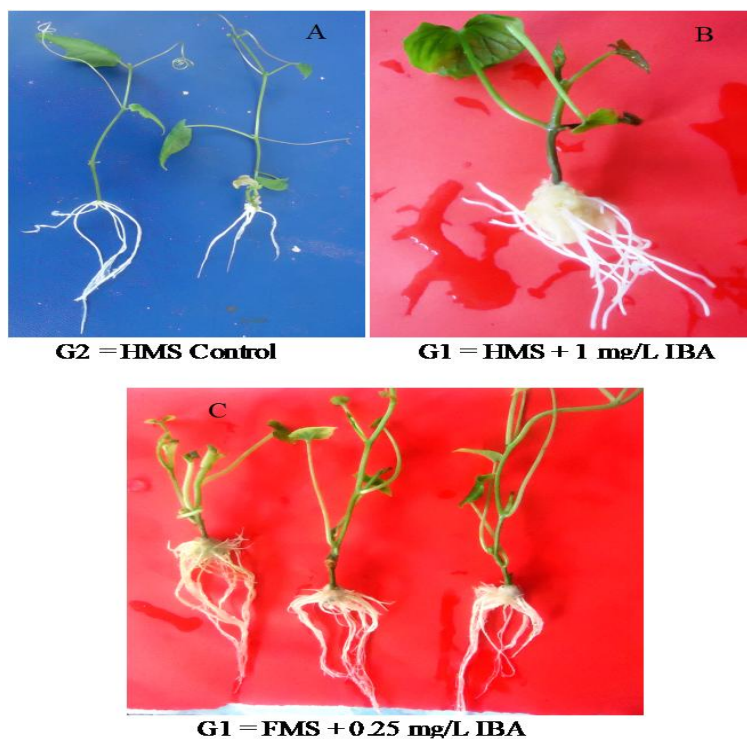
Figure 2. Different types of root morphology in KUWE (G1) and 223092 (G2) observed after four weeks of culture on rooting media: Normal morphological appearance of roots obtained from the control (A) and lower concentration of IBA (C) and root formation interference due to unnecessary callus development at the base of shoots (B)

## Acclimatization of plantlets

Upon four weeks of acclimatization on a potting medium prepared from sand, soil and compost in a 2:1:1 ratio, plantlets survival rate of 76% in G1 and 79% in G2 were recorded. Survival rates of plantlets obtained in this study is slightly lower than obtained by Kahia *et al.* (2016) and Folla *et al.* (2013), who reported survival rates of 83% and 82%, respectively. This might be happened due the variation in the type and ratio of potting mixtures.

## Conclusion

*In vitro* regeneration of plantlets in KUWE (G1) and 223098 (G2) genotypes of anchote using nodal explants has been investigated in detail in this study along with determination of optimum media composition for each genotype. Though several treatments resulted in 100%

shoot formation, MS media supplemented with 1 mg/L BAP + 0.25 mg/L Kn in G2 and 0.5 mg/L BAP alone in G1 were best in terms of multiple shoot formation along with the maximum rate of shoot formation. Apart from some IBA supplemented media, full and half nutrient-strength MS media without rooting hormone produced 100% root formation in G1 and G2, respectively. Even if significant variation existed between treatments in number and length of roots, roots of both genotypes obtained in hormone-free MS media exhibited normal morphological appearance. Therefore, application of rooting hormone on the respective genotypes is wastage. Plantlets of 76% in G1 and 79% in G2 were survived after a month of acclimatization on 2:1:1 ratio of soil, sand and compost, respectively. These findings could be applied for mass production of quality propagation materials through *in vitro* nodal culture of anchote genotypes

investigated in this study. However, further experiment might be required to improve the rate of shoot multiplication and the survival rate of plantlets during acclimatization.

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