# Effect Of Genotype On In Vitro Propagation Of Elite Sugarcane (Saccharum Officinarum L.) Varieties Of Ethiopian Sugar Estates

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**ABSTRACT:** In Ethiopia, sugar industry is increasing at an alarming rate and is expected to play a significant role in poverty reduction. Thus, tissue culture has irreplaceable potential as it enables rapid and large scale production of disease free planting material and creates novel genetic variation for improvement of the varieties. The experiment was carried out on two elite sugarcane varieties using leaf explants to investigate the effect of genotype on callus initiation and subsequent in vitro plant regeneration. The two varieties showed a statistically different response to various hormones and hormonal combinations with regard to most of the parameters measured. For callus initiation, vars. B52-298 and NCO-334 performed best on 3 mg/l and 2 mg/l of 2, 4-D respectively. 2 mg/l BA and 1 mg/l BA are better concentration of hormones for better shoot performance for B52-298 and NCO-334 variety respectively and hence no need of hormonal combination. Roots perform better on 1/2MS medium supplemented with 4 mg/l NAA for NCO-334 and 1mg/l IBA for B52-298 variety. The finding of the study is very important as it serves as a baseline for undertaking comprehensive sugarcane tissue culture research and other activities.

**Keywords:** acclimatization, auxin, callus, cytokinin, explants, genotype, root, shoot

## Abbreviations

%CRS= Percentage of Callus Regenerating Shoot
%SRR= Percentage of Callus Regenerating Shoot
%ERC= Percentage of Explants Regenerating Callus
2, 4-D= 2, 4-Dichlorophenoxy Acetic Acid
BA= Benzyl Adenine
IBA=Indole Butyric Acid
MS= Murashige and Skoog
NAA= Naphtalene Acetic Acid

## INTRODUCTION

Sugarcane (Saccharum officinarum L.) is an important commercial crop in many countries. Considering its importance in the agricultural industry and its contribution to the country's economy, the Ethiopian government has planned an ambitious plan to expand the sugarcane plantation of the estates to over 300,000 hectare and set to establish 11 new sugar factories in the coming five years. Ethiopia's sugarcane productivity was among the highest in the world, which is recently declining rapidly. The most important reasons for lower yield are the non availability of disease free elite seeding stock and incapability to make use of advanced technologies in sugarcane propagation [5]. The bulky cane cuttings used for planting harbor many pests and diseases over vegetative cycles that decreases cane yield and quality drastically [10]. Hence, micropropagation through tissue culture holds great potential to alleviate the problems. Flynn [6] and Soodi [18] have reported that the mericlones derived seed cane was superior in sprouting, cane yield, sugar concentration and sugar yield and quality. Plant regeneration from tissue culture of sugarcane has been successfully applied to breeding programs for rapid screening of clones for disease resistance, salt tolerance, drought tolerance, herbicide resistance and early maturity and high sugar [10]. Callus induction is a very important phenomenon in tissue culture. It is the most important explant for genetic modification [15]. Since in tissue culture different genotypes of the same species respond differently to media [16], it is recommended that an efficient protocol is needed for every new variety or clone of sugarcane to get rapid callus induction, shoot initiation, shoot multiplication and root induction and elongation [4]. Thus, it is of paramount importance to optimize specific in vitro regeneration protocols for the major commercial sugarcane varieties so that the benefit of somaclonal variation will be applied for the ever increasing sugar industry of the country. Therefore, the study was undertaken with the objective of investigating the effect of genotype on callus initiation and in vitro plant regeneration of the two elite sugarcane varieties.

## MATERIALS AND METHODS

**Genotypes used**: Two sugarcane varieties were used from two sugar estates of Ethiopia (Metahara and Wonji). The varieties were B52-298 and NCO-334. These varieties were chosen based on their superior yield performances across the two estates and they occupy, on average, close to 45.5% of the total production area of the two estates. The two varieties have wide range of adaptability, excellent ratooning ability, no germination and flowering problem, high tillering capacity.

**Culture media preparation**: The basal medium employed for the culture of sugarcane was Murashige and Skoog [14]. Sugar was added at the concentration of 30g/l for callus and shoot initiation and 60 g/l for rooting. The pH was adjusted in all cases to 5.8 by using 1 N KOH and 1 N HCl. Phytagel at 0.2 % ( w/v) for shoot initiation and agar at 0.8 % (w/v) for root initiation was used to make semi-solid media. 40 ml media volume were poured into washed and dried culture bottles (jars) and then capped and labeled properly. The jars containing the media were then autoclaved at $120^0$C for 20 minutes at 15-psi pressure and transferred to the inoculation room till their use.

**Explant preparation**: Healthy looking young (6 months old) sugarcane plants were selected. The young leaves, from the top portion of the plants were removed and the spindles (5cm tall and 0.5cm in diameter) were excised from the top. The excised spindles were washed thoroughly with tap water and then with soap solution to remove all the traces of dust particles. The spindles were washed again in sterile water for 10 minutes. The spindles were then immersed in 25% (w/v) 'Barakina' (5% Chlorine) and Tween-20 for 15 minutes. The detergent solution was decanted and the explants were rinsed three times with autoclaved distilled water to remove all the traces of the sterilent. The spindles were again immersed in 25% (w/v) 'Barakina' for 20 minutes inside the laminar airflow cabinet. The detergent solution was decanted and the explants were rinsed three times with autoclaved distilled water to remove all the traces of the sterilent.

**Callus initiation**: Before carrying out any culture process, the laminar hood was cleaned by scrubbing with 70% ethanol solution and was irradiated with UV radiations for 25 minutes. Following sterilization, the spindles were unfurled to remove the part which is damaged by the sterilent till the size of the spindle is approximately equal to pencil size. Leaf explants with the size of 5mm by 5mm were inoculated into each jar. All cultures were incubated in a dark room for four weeks. Callus initiation treatments: control, 2, 4-D (2 mg/l, 3 mg/l, and 4 mg/l).

**Shoot induction and regeneration:** Shoot induction and regeneration was established on MS media supplemented with different concentration of cytokinins and auxins singly or in combination: control, BAP (0.5, 1.0, 1.5, and 2.0 mg/l), Kin (0.5, 1.0, 1.5, 2.0 mg/l), 2 mg/l BA + 0.5 mg/l IBA, and 1 mg/l BA + 0.5 mg/l NAA. All the cultures were incubated in a growth room with a 16h photoperiod (2000-3000 Lux) and the temperature (at 25 ± $3^0$C) with 80% relative humidity in the culture room.

**Rooting:** Shoots having three to four leaves and measuring about 5-6 cm in length was excised from culture tube and transferred to half-strength (1/2 MS) medium supplemented with different concentrations of auxins: - control, IBA (1.0, 1.5, and 2.0 mg/l), and NAA (3.0, 4.0 and 5.0 mg/l).

**Acclimatization and transfer of plantlets to soil**: The rooted plantlets were taken out of the culture bottles using forceps. They were then thoroughly washed in tap water to remove any remaining medium to avoid any future infection of plantlet. The plantlets were carefully planted in the plastic trays filled with autoclaved garden soil, farmyard manure and sand (2:1:1). They were then thoroughly watered and kept in polyhouse under humidity of 80% for about 3 weeks.

**Data collected:** data were recorded on callus induction percentage, callus weight, shoot induction percentage, number of shoot, shoot length, root induction percentage, number of root, root length and survival percentage.

**Data Analysis**: The treatments were arranged in two factor completely randomized design and the data were analyzed using MSTAT-C Software for a two-factor completely randomized design with six replications for callus initiation and three replications for shoot and root initiation. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at P≤0.05.

## RESULTS

**Callus induction**: Callus induction was observed within two weeks after inoculation of the explants on MS medium containing three different concentrations of 2, 4-D (2.0, 3.0 and 4.0 mg/l). Callus initiation was highly influenced by the interaction of the varieties and 2, 4-D concentrations. The highest value (92.5%) was observed at 3 mg/l of 2, 4-D for var. B52-298 while the lowest value (65%) was recorded for var. NCO-334 at the same concentration of 2, 4-D. Variety B52-298 produced less amount of callus at 2 mg/l of 2, 4-D; while best callus percentage (90.83%) was recorded on MS media supplemented with 2 mg/l of 2, 4-D for NCO-334 variety, in which 3 mg/l of 2, 4-D produced the least callus percentage. This indicated that callus initiation response in these two varieties is different with respect to the amount of 2, 4-D to be used.

**Callus weight**: Varietal response to auxin/ 2, 4-D level with respect to callus weight followed similar trend to both varieties responded to callus induction. Callus weight was highly significantly different (P≤0.05) between the two varieties. Callus weight was highly significantly affected by the amount of 2, 4-D and the type of variety. B52-298 produced maximum callus weight (600mg) on MS media supplemented with 3 mg/l 2, 4-D and less callus weight (200mg) was recorded on 2 mg/l 2, 4-D. For NCO-334 variety, maximum weight of callus (430mg) was recorded on MS media supplemented with 2 mg/l 2, 4-D and the least callus weight (130mg) was obtained for the same variety on 4 mg/l 2, 4-D (Table 1).

**Table 1. Interaction effects of varieties and different concentrations of 2, 4-D on callus induction and callus weight from leaf explants of two sugarcane varieties**

| Treatments | Mean %ERC | | Mean Callus Weight(g) | |
|---|---|---|---|---|
| | Variety | | Variety | |
| 2, 4-D | B52-298 | NCO-334 | B52-298 | NCO-334 |
| 2 mg/l | 77.50 ± 1.23[b] | 90.83 ± 0.68[a] | 0.20 ± 0.89[d] | 0.43 ± 0.772[b] |
| 3 mg/l | 92.50 ± 0.26[a] | 77.50 ± 2.10[b] | 0.60 ± 1.05[a] | 0.22 ± 1.109[cd] |
| 4 mg/l | 78.33 ± 1.28[b] | 65.00 ± 0.76[c] | 0.26 ± 0.98[c] | 0.14 ± 0.236[e] |
| CV (%) | 5.41 | | 8.31 | |
| P | P≤0.001 | | P≤0.001 | |
| LSD | 5.125 | | 0.037 | |

Means followed by the same letter are not significantly different at 5% significance level

**Shoot regeneration:** Shoot regeneration started with the appearance of green dots on callus within a week on regeneration medium and generally produced normal stem and leaves. No response was observed on control or hormone free MS medium.

**Shoot induction percentage**: A highly significant (P≤0.05) interaction between varieties and hormones level was observed with regard to shoot induction. Callus cultured on shoot induction media regenerated shoots with highly significant differences among treatments. The highest percentage of callus regenerated shoots was recorded in var. B52-298 on media supplemented with 2 mg/l BA + 0.5mg/l IBA (89.67%) and the second highest on 2 mg/l BA (85%); whereas maximum percentage of callus regenerated shoots at 1 mg/l BA + 0.5 mg/l NAA (83%) and 1 mg/l BA (82.5%) for NCO-334 variety (Table 2). Highly significant differences (P≤0.05) were observed for treatments on shoot induction. Callus cultured on shoot induction media regenerated shoots with highly significant difference among treatments. The percentage of callus regenerating shoot for interaction between varieties and different hormones combinations ranged from 64.67% in 0.5 mg/l kin for NCO-334 variety to 89.67% in 2 mg/l BA + 0.5 mg/l IBA for B52-298 variety (Table 3). The highest percentage of callus regenerated shoots was recorded in var. B52-298 on media supplemented with 2 mg/l BA + 0.5 mg/l IBA (89.67%) and 2 mg/l BA (85%); whereas maximum percentage of callus regenerated shoots at 1 mg/l BA + 0.5 mg/l NAA (83%) and 1.0 mg/l BA (82.5%) for NCO-334 variety (Table 3).

**Number of shoots**: With average number of shoots per callus, genotypic specificity was apparent as the interaction of varieties and hormones combinations was highly significant (P≤0.05) (Table 2). For variety B52-298, the highest shoot number was obtained on MS + 2 mg/l BA + 0.5 mg/l IBA (25.17) and 2 mg/l BA (23.33) and for variety NCO-334 the maximum number of shoot was recorded on 1mg/l BA + 0.5mg/l NAA (20.5) and 1.0mg/l BA (19.33).

**Shoot length**: The interaction of varieties and hormones combinations was highly significant (P≤0.05) on average shoot length. B52-298 variety produced maximum (4cm) shoot length on media supplemented with 2.0 mg/l BAP and 2 mg/l BA + 0.5 mg/l IBA while NCO-334 variety, 1 mg/l BA + 0.5 mg/l NAA recorded maximum shoot length and moderate on media containing 1.0 mg/l BA and 2.0 mg/l Kin (Table2). It is important to note that hormonal concentrations and combinations producing maximum number of shoots (2 mg/l BA + 0.5 mg/l IBA and 2 mg/l BA for var. B52-298 and 1 mg/l BA + 0.5 mg/l NAA and 1 mg/l BA for var.NCO-334) produce moderate or even sometimes optimum shoot length.

**Root induction (Rhizogenesis)**: Roots regeneration started 10 to 15 days after micro shoots were transferred to root induction media for both varieties.

**Root induction percentage**: Highly significant (P≤ 0.05) interaction between the varieties and different auxins was observed for root induction percentage. Optimum percentage of shoots regenerated roots in NCO-334 variety on media supplemented with 4 mg/l NAA (88%) and 5 mg/l NAA (85.33%); whereas maximum percentage of shoots regenerated roots at 1 mg/l IBA and 1.5 mg/l IBA (85%) for B52-298 variety (Table 3).

**Number of roots**: The interaction between varieties and different hormones was highly significant (P≤0.05) for number of roots. The highest number of roots was recorded for NCO-334 variety on media supplemented with 5 mg/l NAA (15.67) and 4 mg/l NAA produced moderate number of roots; whereas maximum number of roots was counted on media containing 1 mg/l IBA (14.33) and 1.5 mg/l IBA produced 11 roots for var. B52-298 (Table 3).

**Root length**: Root length was significantly (P≤0.05) affected by interaction of varieties and hormones and was highly significantly (P≤0.05) affected by hormones (Table 3). Variety B52-298 produced maximum root length (7cm) on MS media containing 5 mg/l NAA and another maximum root length (6.33cm) was measured on 1.5 mg/l IBA (Table 3). However, as we can see on Table 3, hormone levels that produced maximum number of roots in the above results produced moderate root length.

**Table 2. Interaction effects of varieties and different concentrations of hormones on shoot regeneration**

| | Mean %CRS | | Mean # Shoot | | Shoot length(cm) | |
|---|---|---|---|---|---|---|
| | Variety | | Variety | | Variety | |
| Hormones | B52-298 | NCO-334 | B52-298 | NCO-334 | B52-298 | NCO-334 |
| 2mg/l BA + 0.5 mg/l IBA | 89.67 ± 0.96$^{a}$ | 73.33 ± 1.73$^{c-f}$ | 25.17 ± 1.13$^{a}$ | 14.50 ± 0.54$^{cd}$ | 4.00 ± 0.24$^{a}$ | 3.10 ± 0.86$^{cde}$ |
| 1 mg/l BA +0.5mg/l AA | 76.67 ± 0.71$^{cd}$ | 83.00 ± 1.33$^{b}$ | 19.17 ± 0.71$^{b}$ | 20.50 ± 0.85 $^{b}$ | 3.00 ± 0.55$^{cde}$ | 4.20 ± 0.10$^{a}$ |
| 0.5 mg/l BA | 70.00 ± 0.74$^{fg}$ | 66.67 ± 2.36$^{gh}$ | 14.67 ± 0.26$^{c}$ | 13.17 ± 2.05$^{cde}$ | 3.12 ± 2.16$^{cde}$ | 2.83 ± 0.58$^{de}$ |
| 1.0 mg/l BA | 69.67 ± 0.67$^{fg}$ | 82.50 ± 0.83$^{b}$ | 15.67 ± 1.53$^{c}$ | 19.33 ± 1.31$^{b}$ | 2.67 ± 0.74$^{e}$ | 3.67 ± 1.25$^{ab}$ |
| 1.5 mg/l BA | 78.00 ± 1.23$^{c}$ | 72.67 ± 1.05$^{def}$ | 18.17 ± 3.06$^{b}$ | 14.17 ± 2.28$^{cde}$ | 2.93 ± 0.90$^{de}$ | 2.83 ± 1.84$^{de}$ |
| 2.0 mg/l BA | 85.00 ± 2.40$^{b}$ | 70.00 ± 0.95$^{fg}$ | 23.33 ± 1.22$^{a}$ | 13.00 ± 0.63$^{cde}$ | 4.00 ± 1.16$^{a}$ | 2.67 ± 1.58$^{e}$ |
| 0.5 mg/l Kin | 67.33 ± 1.21$^{gh}$ | 64.67 ± 2.10$^{h}$ | 12.00± 0.81$^{def}$ | 10.00 ± 2.50$^{f}$ | 3.17± 1.31$^{b-e}$ | 3.00 ± 0.78$^{cde}$ |
| 1.0 mg/l Kin | 75.17 ± 2.71$^{cde}$ | 69.00 ± 1.35$^{fgh}$ | 11.83 ± 1.76$^{ef}$ | 11.83 ± 1.46$^{ef}$ | 3.50 ± 2.71$^{abc}$ | 3.33 ± 1.31$^{bcd}$ |
| 1.5 mg/l Kin | 71.67 ± 1.33$^{efg}$ | 71.67 ± 1.17$^{efg}$ | 15.00 ± 3.17$^{c}$ | 14.67 ± 0.25$^{c}$ | 3.48 ± 1.17$^{abc}$ | 3.50 ± 1.38$^{abc}$ |
| 2.0 mg/l Kin | 68.33 ±1.64 $^{gh}$ | 66.67 ± 0.38$^{gh}$ | 13.50 ±0.11$^{cde}$ | 11.83 ± 0.14$^{ef}$ | 3.33 ± 1.33$^{bcd}$ | 3.67 ± 2.11$^{ab}$ |
| CV (%) 8.59 | | 3.58 | | | 8.95 | |
| P P≤0.001 | | P≤0.001 | | | P≤0.001 | |
| LSD 0.4638 | | 4.352 | | | 2.300 | |

**Acclimatization**: The acclimatization response recorded for these two varieties in the current study was somewhat lower as compared to most literatures. For B52-298 variety, 60% of the plants transferred to the plastic trays survived; while 55% of the plants transferred survived for NCO-334-variety.

## DISCUSSIONS

Genotypic specificity was evident with regard to the performance of callus induction as it was better on 3 mg/l of 2, 4-D for var. B52-298 and on 2 mg/l of 2, 4-D for var. NCO-334. Variation of callus induction response with variety was reported by Gandonou et al. [7]; Rashid et al. [15]; Behera &Sahoo [4]. Both varieties perform poor on 4 mg/l of 2, 4-D. Control showed no response for callusing in which all explants cultured on control (0mg/l) dried out 7 days after explantation. Ali et al. [1]; Ather et al. [3]; and Tarique et al. [19] indicated that among the auxins, 2, 4- D at 3.0 mg/l was more potent for callus induction and its subsequent growth. Behera &Sahoo [4] and Ali et al. [2] observed callus initiation as well as proliferation at 2.5 and 3mg/l 2,4-D. Gopitha et al. [9] obtained best callus induction at 3.0 mg/l, 2,4-D with 10% coconut milk. Contrary to the current study, Khan et al. [12] observed maximum callus weight on 4 mg/l 2, 4-D for the varieties they tested. Rashid et al. [15] obtained maximum callus size on MS media supplemented with 2, 2.5, and 3 mg/l 2, 4-D. More number of shoots was observed in var. B52-298 on media supplemented with 2 mg/l BA + 0.5 mg/l IBA and 2 mg/l BA and on 1 mg/l BA + 0.5 mg/l NAA and 1.0 mg/l BA for NCO-334 variety. Thus, 2 mg/l BA and 1 mg/l BA are better concentration of hormones for shoot performance for B52-298 and NCO-334 variety respectively and hence no need of hormonal combination. The same concentration of hormones produced optimum shoot length for both varieties. This is in line with the work of Behera & Sahoo [4] in which best shoot multiplication performance was showed on MS medium supplemented with BAP (2.0 mg/l) + IBA (0.5mg/l) followed by BAP (2.0mg/l) + IBA (1.0mg/l). According to Tarique et al. [19], 1.0 mg/l BAP + 0.5 mg/l NAA were the best result for induction and multiplication of shoot. BAP in the concentration of 1.0 mg/l was found best in terms of highest number of shoot regeneration [1, 9, 15]. Genotype pecific response to number of shoot regeneration was reported by Gandonou et al. [7], and Behara & Sahoo [4]. Behara & Sahoo [4] reported that BAP at 2.0 mg/l + IBA at 0.5mg/l and BAP at 2 mg/l + IBA at 1.0mg/l gave 12.4 ± 1.90 and 10.5 ± 1.31 average number of usable shoots respectively. According to Tarique et al. [19], MS media supplemented with 1.0 mg/l BA + 0.5 mg/l NAA and 1.0 mg/l BA + 0.5 mg/l IBA were best regarding regeneration of shoot from the callus tissue produced13.8 and 11.43 shoots

respectively. Similar to previous studies, BAP was found to be an important cytokinin in the development of shoots in line with a number of previous findings [1, 9, 15].

**Table 3. Interaction effects of varieties and different concentrations of auxins on root initiation, root number and root length of the two varieties**

| Treatments | Mean % SRR | | Mean # Root | | Root Length(cm) | |
|---|---|---|---|---|---|---|
| | Variety | | Variety | | Variety | |
| Auxins | B52-298 | NCO-334 | B52-298 | NCO-334 | B52-298 | NCO-334 |
| 1.0 mg/l IBA | 85.00 ± 0.26$^{a}$ | 71.67 ± 0.99$^{bc}$ | 14.33 ± 0.85$^{a}$ | 8.33 ± 1.25$^{d}$ | 3.53 ± 0.94$^{d}$ | 7.67 ± 0.14$^{a}$ |
| 1.5 mg/l IBA | 85.00 ± 0.12$^{a}$ | 64.00 ± 1.08$^{d}$ | 11.00 ± 0.77$^{bc}$ | 8.83 ± 1.54$^{d}$ | 3.33 ± 0.61$^{d}$ | 6.50 ± 0.32$^{b}$ |
| 2.0 mg/l IBA | 64.67 ± 0.71$^{d}$ | 64.67 ± 0.43$^{d}$ | 9.17 ± 1.37$^{d}$ | 9.93 ± 1.58$^{cd}$ | 3.50 ± 0.79$^{d}$ | 3.83 ± 0.37$^{d}$ |
| 3.0 mg/l NAA | 65.33 ± 0.23$^{cd}$ | 64.67 ± 1.18$^{d}$ | 8.67 ± 1.21$^{d}$ | 9.67 ± 1.36$^{cd}$ | 5.10 ± 1.88$^{c}$ | 5.00 ± 1.61$^{c}$ |
| 4.0 mg/l NAA | 66.00 ± 0.69$^{cd}$ | 88.00 ± 1.38$^{a}$ | 9.50 ± 0.63$^{cd}$ | 12.00 ± 1.13$^{b}$ | 6.60 ± 1.73$^{b}$ | 3.63 ± 0.41$^{d}$ |
| 5.0 mg/l NAA | 73.33 ± 1.22$^{b}$ | 85.33 ± 0.64$^{a}$ | 9.00 ± 0.29$^{d}$ | 15.67 ± 0.55$^{a}$ | 7.17 ± 1.52$^{ab}$ | 3.50 ± 0.87$^{d}$ |
| CV (%) | 4.92 | | 8.73 | | 9.68 | |
| P | P≤0.001 | | P≤0.001 | | P≤0.05 | |
| LSD | 6.063 | | 1.545 | | 0.8064 | |

The current study result of shoot length is in line with the works of Behara & Sahoo [4] and Tarique et al. [19]. With regard to mean shoot length, Behara & Sahoo [4] recorded 6.2 ± 0.37 cm and 4.0 ± 0.61cm for the two top performing hormone combinations, BAP (2.0 mg/l) + IBA (0.5mg/l) and BAP (2.0mg/l) + IBA (1.0mg/l) respectively. According to Tarique et al. [19], 4.9 and 4.7cm shoot length was recorded on MS media supplemented with 1.0 mg/l BA + 0.5 mg/l NAA and 1.0 mg/l BA + 0.5 mg/l IBA respectively. Roots performance was better on 1/2MS media supplemented with 4mg/l NAA and 5mg/l NAA for NCO-334 variety and on 1/2MS media supplemented with 1 mg/l IBA and 1.5 mg/l IBA for B52-298 variety. Thus, respective lower concentrations should be used from economic point of view. Control (media without hormone) showed no response for rooting in the same way as indicated by Rashid et al. [15]. According to Khan et al. [13], Gopitha et al. [9] and Behera & Sahoo [4], 3mg/l NAA supplemented media proved best for production of roots. Ali et al. [2] and Tarique et al. [19] observed best result of root formation on MS medium supplemented with 4-5.0 mg/l of NAA. Khan et al. [11] observed best rooting on media containing MS+ 1 mg/1 IBA + 6% sucrose for sugarcane. Ali et al. [1] recommended 1.0 mg/l IBA and 2.0 mg/l IBA for 100% rooting response and Khan et al. [12]observed profuse roots on MS supplemented with 1mg/l IBA and 1.5 mg/l IBA + 6% sugar. Khan et al. [12] observed 41 and 34 roots on MS media supplemented with 1mg/l IBA and 1.5 mg/l IBA + 6% sugar respectively. Behera & Sahoo [4] recorded the highest number roots per micro shoots (13.4 ±1.5) at 2.5mg/l NAA. Rashid et al. [15] obtained maximum root number (3.6) on ½ strength MS media for 1.0 mg/l IBA. Sabaz et al. [17] used 1 mg/l IBA as the best root initiating growth hormone with highest number of 41 roots per plant. Gopitha et al. [9] observed the highest number of average roots per micro shoots (15) in 1/2MS medium supplemented with 3mg/l NAA. Tarique et al. [19] recorded the highest number of roots (13.47) at 5.0 mg/l of NAA. Khan et al. [12] recorded 2.5cm and 1.8 cm root length on MS supplemented with 1mg/l IBA and 1.5 mg/l IBA + 6% sugar respectively. Behera & Sahoo [4] obtained average root length of 4.0 ± 0.94 cm for the variety Nayana on MS media supplemented with 2.5mg/l NAA. Rashid et al. [15] observed 3.5 cm root length when grown at 1/2MS medium supplemented with 1.0 mg/l IBA. Gopitha et al. [9] recorded average length of root 4.9 cm in 1/2MS medium supplemented with 3 mg/l NAA. As to the acclimatization response, relatively lower survivability percentages were recorded as compared to previous acclimatization response observed on sugarcane. Gopitha et al. [9]; Tarique et al. [19]; Behera & Sahoo [4] and Rashid et al. [15], obtained acclimatization response which ranged from 65 to 85%.The reason for lower acclimatization response may be associated with the environment in which the varieties were acclimatized; which means that the poly house was already constructed for banana acclimatization and no such house appropriate for acclimatizing sugarcane varieties was there. Besides, there may be varietal difference for acclimatization response as compared to other varieties tested in the previous studies. Above all, time constraint was the major factor in that it was not possible to carry out various acclimatization treatments. From the above discussions, we can conclude that the two tested varieties responded specifically to hormonal treatments for most of the parameters measured. Thus, indicating the need to develop specific protocols for each variety of sugarcane, even though it is economically costly. Generally, the information generated by current study is highly valuable that it serves as a stepping stone for future comprehensive sugarcane tissue culture activities that less matured Ethiopian plant tissue culture needs.

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