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In Vitro Rooting and Acclimatization of Micropropagated Elite Sugarcane (*Saccharum officinarum* L.)Genotypes - N52 and N53

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Abstract

Availability of sufficient quantity and quality of sugarcane planting materials from conventional seed source is one of the major challenges in the Ethiopian sugar estates. To circumvent this challenge, tissue culture technology is found to be the best alternative for which *in vitro* propagation protocol is a key pre-request. Thus, the present study was aimed to optimize protocol for *in vitro* rooting and acclimatization of two elite sugarcane genotypes i.e., N52 and N53. Experiments were laid out in a completely randomized design with factorial treatment arrangements. Half strength MS liquid media supplemented with combination of Sucrose (0, 40, 50, 60 and 70 g/l) and NAA (0,3,5 and 7 mg/l) along with two sugarcane genotypes (N52, N53) were used for rooting while substrate containing sand, soil and farmyard manure in six different ratios (1:1:0, 1:1:1, 1:2:1, 2:1:1, 1:1:2 and 1:2:0) were used for acclimatization. With regard to *in vitro* rooting, ½ strength liquid MS medium + 50 g/l sucrose + 3 mg/l NAA induced the highest rooting (100%) with 23.5 ± 1.29 average root number per shoot and 4.95 cm ± 0.06 cm root length in genotype N52 while 5 mg/l NAA + 50 g/l sucrose induced the highest (100%) rooting response with an average of 21.76 ± 0.57 root number per shoot with 4.54 cm ± 0.06 cm root length in sugarcane genotype N53. In acclimatization, best survival rate (94% in N52 and 100% in N53) was achieved on substrate mixtures containing sand + soil in 1:1: ratios. Thus, it can be deduced that this protocol can be used successfully for *in vitro* rooting and acclimatization of these genotypes.

Keywords: *In vitro* rooting; Acclimatization; Liquid medium; NAA; Sucrose; N52 and N53.

Introduction

Sugarcane (*Saccharum officinarum* L.) is an important industrial cash crop of Ethiopia. It was introduced to the country in the 16th century [1]. However, commercial sugarcane production was commenced in 1952 by Dutch company, Handles-Vereening Amsterdam (HVA) at Wonji shoa sugar factory [2]. Currently, it is cultivated in three sugar factories over an area of 37, 000 ha with total production of 300,000 ton of sugar and 11.1 million liters of ethanol annually [3], which accounts 100% of Ethiopian sugar production [4]. However, most of the sugarcane plantations of sugar industries are confronting with problems of low cane yields due to poor quality seed material, prevalence of diseases and pests, slow commercialization of improved sugarcane varieties. Therefore, to alleviate the problems, use of efficient propagation system is a must for mass multiplication of disease free seed material of improved sugarcane varieties in a short period.

Plant tissue culture is currently a powerful tool that plays a major role in rapid multiplication of disease free planting material of newly improved varieties through *in vitro* technique on continuous year rounded basis [5]. Shoot tip culture is relatively simple *in vitro* method for rapid propagation of selected sugarcane materials and the clean or pathogen free plant materials [6]. In line with this, protocols have been developed for rapid multiplication of newly released and commercially important genotypes of sugarcane through shoot tip cultures in Ethiopia [4,7,8]. The ultimate success of commercial micropropagation depends largely on successful rooting and acclimatization of *in vitro* derived plantlets. Rooting process is genetically determined and influenced by culture environment factors such as mineral concentration, type and concentration of growth regulators and sucrose concentration in the culture media. Thus genotype specific protocol is needed as the plant growth regulators and sucrose requirements for morphogenetic responses of *in vitro* rooting vary from genotype to genotype in sugarcane [5,9].

Similarly, in the acclimatization stage, it is necessary to ensure optimal *ex vitro* conditions to obtain high survival rates. The selection of a suitable substrate is one of a decisive factor which substantially affects the survival rate, growth and development of *ex vitro* acclimatized plantlets and mainly depends on plant species. Although, in acclimatization of micropropagated plantlets on suitable media. Therefore, the study was carried out with objectives to optimize *in vitro* rooting and acclimatization protocol for two elite sugarcane genotypes.

Materials and Methods

The study was conducted at the National Agricultural Biotechnology Laboratory of the Ethiopian Institute of Agricultural Research, in Holetta. Two elite sugarcane genotypes (N52 and N53) were used for the study. The genotypes were obtained from the Ethiopian Sugar Corporation, Research and Training Division and they were selected based on their cane yield performance and sugar quality. The planting material (setts) with two buds were treated with hot water at 50°C for 2 hours followed by immersing in fungicide (Bayleton® DF 50%) solution at rate of 1 g/l for 5 minutes. The treated setts were planted in plastic pots containing mixture of autoclaved forest soil, farm yard manure and river sand in the ratio of 1:1:1 and allowed to grow in a screen house for five months. Actively growing shoot tops were used as source of shoot tip explant. The method of explant preparation and surface sterilization was adopted from [7,10] with some modifications. Actively growing shoot tops were taken from

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5-months-old screen house grown healthy mother plants and used as explants. The entire leaves were removed, and the segments were taken to the laboratory for surface sterilization and explant preparation. Trimmed shoot tops were washed thoroughly under running tap water for 30 minutes, and resized to 10 cm length by cutting off at the two ends. Then the explants were further washed for 30 minutes with tap water containing a drop of liquid detergent solution plus two drops of tween-20 with continuous shaking and rinsed three times with double distilled water. Later, the explant was taken to a laminar air flow cabinet and immersed in 0.1% (w/v) Bavistin® DF 50% (Carbendizem) fungicide solution, ascorbic acid (0.2% w/v) and citric acid (0.4% w/v) for 30 minutes followed by three times rinsing each for five minutes with sterile double distilled water. The shoot tips were washed again with 70% ethanol for one minute and rinsed with sterile double distilled water three times each for five minute to remove residual ethanol from the shoot tip surface. Finally, 10 cm sized explants were surface sterilized with 50% (v/v) aqueous solution of Sodium hypochlorite (5.25% w/v active chlorine) containing a few drops of a wetting agent (tween-20) with gentle shaking for 25 minutes. After pouring out sodium hypochlorite solution, the explants were rinsed with sterile double distilled water three times each for five minutes to remove all the trace of the sterilant. Subsequently, shoot tip explants having 1.5 cm long size were cultured in test tube containing 20 ml of sterilized and agar (4.5 g/l) solidified MS medium [11] fortified with BAP, Kinetin and NAA (0.5 mg/l each) plant growth regulators [12] and 20 g/l sucrose as carbon source. The pH of the medium was adjusted to 5.8 followed by autoclaving at 121°C at 105 Kpa pressure for 20 minutes. Cultures were incubated in growth room at temperature of 25°C ± 2°C under 16 hours photoperiod with photo flux density of 30 µmol m^2/ s provided by cool white fluorescent light and 70% to 80% relative humidity.

In vitro rooting

For rooting studies, the regenerated microshoots were further multiplied on liquid MS medium containing 2 mg/l BAP + 0.5 mg/l Kinetin (N52) and 1.5 mg/l BAP and 0.5 mg/l Kinetin (N53) [13]. Then shoots were maintained on plant growth regulators (PGRs) free MS medium with 2 g/l activated charcoal for two weeks before transferring to a liquid rooting media in order to avoid the carry over effect of hormones from the multiplication media on rooting. Individual microshoot comprising uniform shoot length (4 cm) derived from the multiplication experiment was used for the study (Figure 1a and 1b). In this experiment, the rooting response of *in vitro* regenerated shoots was determined on half strength liquid medium supplemented with different concentrations of NAA (0, 3, 5 and 7 mg/l) and sucrose (0, 40, 50, 60 and 70 g/l) without agar. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 1.05 kg/$cm^3$for 20 minutes. For each treatment 3 test tubes, each with two shoots supported by filter paper bridge (whatman filter paper) were lined up randomly in CRD with four replications. All shoots were incubated on rooting medium for four weeks in growth room adjusted at temperature of 25°C ± 2°C under 16 hours photoperiod with photo flux density of 30 µmolm^2/s provided by cool white fluorescent light and 70-80% relative humidity. After four weeks of culture growth, data were recorded on number of roots per shoot, length of roots (cm) and number of rooted shoots.

Acclimatization

In vitro rooted plantlets were taken out of the culture test tubes and thoroughly washed with water to remove all the traces of the rooting medium. After trimming out excess leaves and roots, the plantlets were transferred to trays containing autoclaved mixture of river sand, forest soil and well decomposed farmyard manure in different proportion or ratio i.e., 1:1:0, 1:1:1, 1:2:1, 2:1:1, 1:1:2 and 1:2:0. Before planting, the trays filled with the soil mixtures were properly irrigated with water. For acclimatization, the plantlets were maintained in the greenhouse for 4 weeks at about 25°C to 30°C under high humidity (>85%) by covering the trays with transparent polyethylene sheets and red cheese cloth and then sprayed with water intermittently. The cover was removed from the tray after 10 days. Starting from the 1st week of acclimatization, numbers of surviving and dead plantlets were recorded on weekly basis until the 4th week of acclimatization. Data on survival percentage was collected after 30 days. The acclimatized plantlets were further transferred to medium polyethylene bags (15 cm × 20 cm) containing forest soil and river sand in 1:1 ratio and allowed to grow in the green house for further hardening for 30 days. The experiment was carried out in five replications with 50 explants for each treatment in a completely randomized design (CRD).

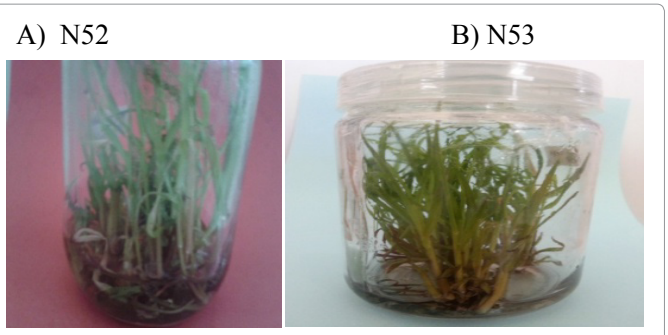

Figure 1: Starting materials (shoots) for *in vitro* rooting.

Data analysis

The analysis of variance for different variables of the two experiments was performed by SAS version 9.2 [14] and for significantly different treatments, mean separation was done with REGWQ (Ryan-Einot-Gabriel-Welsch) at or below the probability level of 0.05.

Results and Discussion

Effect of NAA and sucrose on *in vitro* rooting of regenerated shoots

Analysis of variance showed that the interaction effects of the three factors: Genotype*NAA*Sucrose is very highly significant on root length and number of roots per shoot while rooting percentage is affected by the interaction of Genotype* NAA (Table 1). Similarly, the analysis of variance (ANOVA) showed that the main effects of NAA and sucrose;

Source of variation	DF	Rooting percentage	Root length (cm)	Number of roots per shoot
		MS	MS	MS
Genotype	1	27.56^{ns}	31.83***	52.10***
NAA	3	630.59***	16.40 ***	799.74***
Sucrose	4	5246.20***	70.01***	1390.98***
Genotype*NAA	3	194.44**	3.87***	11.95***
Genotype*Sucrose	4	88.72^{ns}	2.29***	8.35***
NAA*Sucrose	12	88.69*	2.81***	64.03***
Genotype*NAA*Sucrose	12	52.95^{ns}	0.96***	34.09***
CV %		8.88	5.35	5.93

*** = very highly significant at P ≤ 0.0001, ** = highly significant at P ≤ 0.01, * = significant at P ≤ 0.05, NS = Non-significant at P ≥ 0.05, DF = Degree of freedom, NAA = α-naphthalene acetic acid, MS = mean square, CV = Coefficient of variation

Table 1: Effect of NAA and sucrose on *in vitro* rooting.

interaction effect of genotype and NAA, and the interaction effect of NAA and sucrose were statistically highly significant ($p<0.0001$) on rooting frequency of sugarcane micro-shoots. In both genotypes, no rooting response was observed on half strength liquid MS medium devoid of NAA and sucrose (control) (Table 2).

Again, there was no rooting of micro-shoot recorded on half strength liquid MS medium supplemented with different level of NAA (3, 5 and 7 mg/l) without the presence of sucrose in both genotypes (Table 2). These results indicated that significance of sucrose as a source of energy in rooting medium for sugarcane micro-shoot root induction. On the contrary, root induction occurred in both genotypes when the micro-shoots were cultured in half strength liquid MS medium supplemented with various concentrations of sucrose without NAA (Table 2). This result showed that the two genotypes are rich in endogenous auxins that at least enable them to induce root. Both genotypes showed significant increase in rooting frequency because of the increase in the concentration of sucrose from 0.0 mg/l to 40 mg/l in every level of NAA. There was significant level of reduction of rooting frequency from 100% to 87.84% as the concentration of NAA and sucrose increased from 3 to 7 mg/l and 50 to 70 g/l, respectively (Table 2). This might be due to inhibition of rooting at higher concentration of NAA. Higher concentrations of NAA reduce root induction as it promotes the biosynthesis of ethylene which has inhibitory effect in sugarcane rooting [15,16] had also reported that higher concentration of NAA (7 mg/l) and sucrose (70 g/l) inhibit root induction frequency.

ANOVA also showed highly significant ($p<0.0001$) effect of all main and interaction effect of genotype, NAA and sucrose on root length and number of roots per shoot of the two sugarcane genotypes studied. No root was initiated on 1/2 MS liquid medium that lack NAA and sucrose in both genotypes, N52 and N53 (Table 2). It was observed that there was differential response of the two genotypes used. N52 was responsive to lower concentration of NAA than N53. Genotype N52 gave a maximum of 4.95 cm and 23.5average root length and average root number per shoots, respectively on 1/2 MS liquid medium fortified with 3 mg/l NAA and 50 g/l sucrose (Table 2 and Figure 2a) while only 1.68 cm average root length with 16.43 average roots number per shoot were recorded in N53 in the same medium composition (Table 2). On the other hand, in genotype N53, the highest of 4.54 average root length and 21.76 average roots number per shoot were produced on 1/2 MS

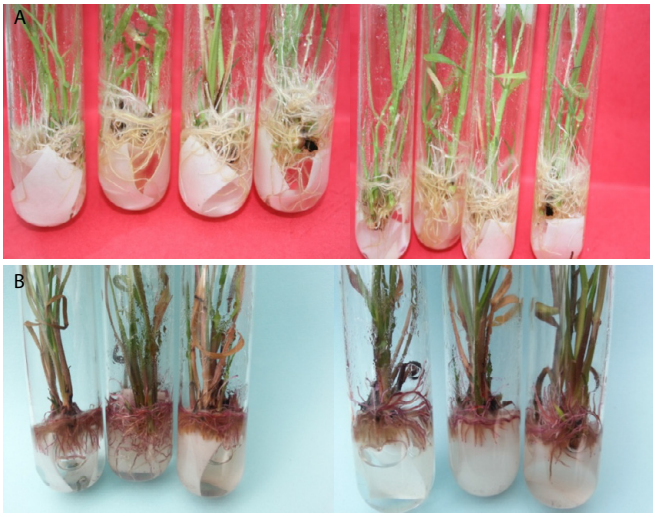

Figure 2: *In vitro* rooting of sugarcane microshoots.
A) Genotype N52 at 3 mg/l NAA + 50 g/l Sucrose
B) Genotype N53 at 5 mg/l NAA + 50 g/l sucrose

Genotypes							
Treatments			**N52**			**N53**	
NAAmg/l	**Sucrose**	**Rooting percentage**	**Root length**	**Number of root per shoot**	**Rooting percentage**	**Root length**	**Number of root per shoot**
	g/l		(cm)			(cm)	
0	0	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00
	40	83.31d ± 8.90	4.24cd ± 0.17	6.50mn ± 0.58	83.31d ± 8.90	3.45h ± 0.31	4.00o ± 0.23
	50	85.41cd ± 13.90	4.36b-d ± 0.13	6.25mn ± 0.50	85.41cd ± 13.90	4.36b-d ± 0.24	5.45no ± 0.17
	60	89.56a-d ± 8.64	4.91a ± 0.07	5.75n ± 0.96	89.56a-d ± 8.64	4.49bc ± 0.08	8.28l ± 0.10
	70	83.31d ± 8.90	4.64ab ± 0.16	5.75n ± 0.50	83.31d ± 8.90	3.38h ± 0.21	7.45lm ± 0.24
3	0	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00
	40	100a ± 0.00	3.81fg ± 0.39	16.25gh ± 0.96	100a ± 0.00	1.52m ± 0.10	11.35k ± 1.22
	50	100a ± 0.00	4.95a ± 0.06	23.50 a ± 1.29	100a ± 0.00	1.68m ± 0.03	16.43f-h ± 0.39
	60	93.74a-d ± 8.64	4.14de ± 0.21	17.25e-g ± 0.96	93.74a-d ± 8.64	2.74jk ± 0.20	18.05d-f ± 0.97
	70	89.56a-d ± 8.64	3.55gh ± 0.19	15.25hi ± 0.96	89.56a-d ± 8.64	1.82m ± 0.14	14.13ij ± 0.75
5	0	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00	0.00e ± 0.00	0.00 ± 0.00	0.00p ± 0.00
	40	97.91ab ± 5.90	3.91ef ± 0.14	16.25gh ± 0.96	97.91ab ± 5.90	3.77fg ± 0.05	19.28cd ± 0.69
	50	100a ± 0.00	4.58bc ± 0.13	18.00d-f ± 0.82	100a ± 0.00	4.54 bc ± 0.06	21.76b ± 0.57
	60	93.74a-d ± 8.64	2.90j ± 0.08	22.75ab ± 0.50	93.74a-d ± 8.64	2.28l ± 0.05	13.95ij ± 0.58
	70	91.65a-d ± 8.93	2.78jk ± 0.05	18.25d-e ± 0.50	91.65a-d ± 8.93	1.77m ± 0.04	13.48j ± 0.34
7	0	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00	0.00e ± 0.00	0.00o ± 0.00	0.00p ± 0.00
	40	95.83a-c ± 7.73	3.26hi ± 0.09	18.25de± 0.96	95.83a-c ± 7.73	1.04n ± 0.14	12.73kj ± 0.73
	50	95.83a-c ± 7.73	3.05ij ± 0.13	18.50c-e ± 1.00	95.83a-c ± 7.73	2.34l ± 0.07	15.43hi ± 0.57
	60	95.83a-c ± 7.73	2.75kj ± 0.13	19.00c-d ± 1.41	95.83a-c ± 7.73	1.73m ± 0.14	20.02c ± 0.96
	70	87.48b-d ± 7.73	2.55kl ± 0.06	16.10gh ± 0.60	87.48b-d ± 7.73	1.62m ± 0.13	19.05cd ± 0.30
CV %		8.88	5.77	6.32	8.88	5.77	6.32

*Values in the same column and with different letters(s) are significantly different from each other according to REGWQ at $P<0.05$

Table 2: Effect of genotype, NAA and sucrose on rooting percentage, number of roots per shoot and root length.

liquid medium supplemented with 5 mg/l NAA and 50 g/l sucrose (Table 2 and Figure 2b) with this medium composition, N52 resulted in 4.58 average root length and 18.00 average roots number per shoot (Table 2).

The result also showed that an increase in the concentration of NAA from 0.0 mg/l to 3 mg/l at fixed quantity (50 g/l) of sucrose increased the average root length and roots number of N52 significantly to 4.95 cm and 23.5, respectively. In the same trend increasing the concentration of NAA from 0 mg/l to 5 mg/l maintaining the concentration of sucrose at 50 g/l increased the average root length and number of roots to 4.54 cm and 21.76, respectively in genotype N53. Conversely, further increasing the concentration of NAA and sucrose to 7 mg/l reduced the shoot length and number of root noticeably to 3.05 cm and 16.1 in N52 and to 2.34 cm and 19.05 in N53, respectively. This was due to the fact that high auxin concentration inhibits sugarcane root elongation through production of ethylene in the culture jar [15].

Similarly, increase in sucrose concentration from 0 g/l to 50 g/l along with a definite concentration of NAA (5 mg/l), significantly increased the average root length and root number to 4.58 cm and 18.00 in genotype N52, and to 4.54 cm and 21.76 in genotype N53, respectively. However, further increase in sucrose concentration to 70 g/l resulted in reduction in average root length and number of roots in both sugarcane genotypes (Table 2). Earlier reports also confirmed that higher concentrations of sucrose in the medium have a negative impact on overall rooting due to accumulation of rooting inhibitors, the reduction of rooting promoters in the medium, and the transformation of added sugars in to insoluble and storage form [16].

The current result obtained in genotype N53 was in agreement with earlier results reported by Raman et al. [17]. They obtained the longest root of 6.7 cm and the highest average roots number of 6.8 in sugarcane genotypes CoS 96268 and CoS 95255, respectively on 1/2 MS liquid medium containing 5 mg/l NAA in combination with 50 g/l sucrose; whereas NAA at 7 mg/l reduced the root length. The result obtained in N53 was also in harmony with the previous findings of Yadav et al. [18]. They reported best root growth with 5.8 and 5.7 average number of roots per shoot in sugarcane genotypes CoSe 01235 and CoS 99259, respectively Baksha et al. [19] also obtained a maximum average root length of 4.5 cm and average root number of 17 per shoot in sugarcane genotype lsd 31 on ½ MS medium with 5 mg/l of NAA.

The results recorded in the present investigation in genotype N52 was consistent with findings of Gopitha et al. [20] who obtained the highest average root length (4.9 cm) and average root number per shoot (15.1) on ½ MS liquid medium fortified with 3 mg/l NAA with 50 g/l sucrose in genotype Co671 [21] revealed a maximum of 2.50 cm average root length and 6.8 average numbers of roots at 3.0 mg/l NAA for genotype BL-4 [22] found a maximum of 13.4 average numbers of roots per shoot with 4.0 cm average root length on ½ MS medium containing 3 mg/l NAA [23] reported the highest root induction with best root growth at ½ MS medium containing 1 mg/l NAA and 60 g/l sucrose. The current result also disagrees with the report of Khan et al. [24]. They obtained a maximum roots (35) and average root length (3.05 cm) from genotype HSF-240, at 0.5 mg/l IBA with 60 g/l of sucrose. The same author also reported a maximum of 34 roots with 1.8 cm average root length from genotype CPF-237 at 1.5 mg/l IBA with 60 g/l of sucrose. However, there were other research reports of Khan et al. [24] that showed a different result, where low IBA concentration (0.5 mg/l) combined with 60 g/l sucrose gave the best results in average root number 35 per plant and root length of 3.05 cm (Figure 3).

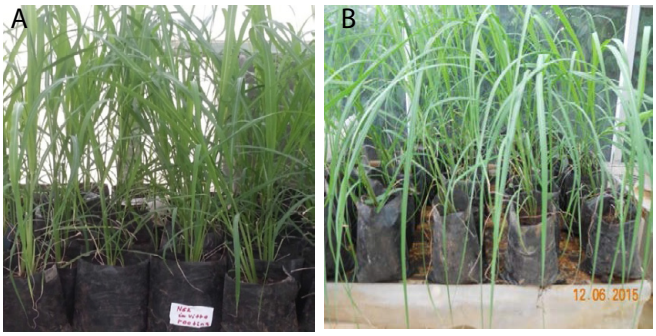

Figure 3: *In vitro* rooted & *ex vitro* acclimatized plantlets.
A) Survived genotype N52 B) Survived genotype N53

Effect of different substrate mixture on *ex vitro* acclimatization of *in vitro* regenerated sugarcane plantlets.

Statistical analysis of variance showed that the main effect of substrates mixture and interaction effect of genotype and substrates mixture were highly significant (P<0.0001) on survival rate of *ex vitro* acclimatized shoots (Table 3). There was a significance difference observed between the two genotypes at substrate mixture composed of sand + soil + FYM in 1:1:1 ratio (Table 4). Genotype, N52 exhibited 90% survival while genotype N53 showed only 70%. Similar trend was observed at substrate mixture of sand + soil + FYM at 1:2:1 ratio, where, N52 showed 90% survival rate whereas N53 exhibited significantly low (78%) survival rate. The highest survival rates 100% and 94%, were observed in substrates mixture of sand + soil + FYM at 1:2:0 ratio, in genotype N53 and N52, respectively; however, there was no significance difference between them. Similarly, no significance difference was observed between genotype N53 (94%) and N52 (86%) on substrate mixture made up of sand + soil + FYM at 2:1:1 ratio (Table 4). The lowest (76%) survival was obtained in substrate mixture with 1:1:2 ratio for genotype N52 while, the lowest (70%) survival rate

Source of variation	DF	Survival rate (%)
		MS
Genotype	1	60.00^{NS}
Substrate mixture	5	632.00***
Genotype*Substrate mixture	5	320.00***
CV %		5.91

*** = very highly significant at P ≤ 0.0001, NS = Non-significant at P ≥ 0.05, DF = Degree of freedom, MS = mean square, CV = Coefficient of variation

Table 3: Effect of different substrate mixture on survival rate of *ex vitro* acclimatized sugarcane plantlets.

Substrates mixture (Sand + Soil + FYM)	Survival Rate (%)	
	Genotype	
	N52	N53
T1 = 1:1:0	$92^{ab} \pm 4.47$	$96^{ab} \pm 5.48$
T2 = 1:1:1	$90^{ab} \pm 0.00$	$70^{d} \pm 0.00$
T3 = 1:2:1	$90^{ab} \pm 0.00$	$78^{cd} \pm 13.04$
T4 = 2:1:1	$86^{bc} \pm 5.48$	$94^{ab} \pm 5.48$
T5 = 1:1:2	$76^{cd} \pm 5.48$	$78^{cd} \pm 4.47$
T6 = 1:2:0	$94^{ab} \pm 5.48$	$100^{a} \pm 0.00$
CV%	5.91	5.91

FYM= farmyard manure, value in the same column with different letters are significantly different from each other according to REGWQ at P<0.05.

Table 4: The effect of different substrates mixtures on *ex vitro* acclimatization of *in vitro* generated plantlet of sugarcane.

was observed in substrate mixture with 1:1:1 ratio for genotype N53. Substrate mixtures comprised of sand + soil + FYM at 1:1:0 and 1:2:0 ratios had no significance difference in survival rate of plantlets for both genotypes. They had 94 and 92% in genotype N52 and 100 and 96% in genotype N53, respectively.

The current result also revealed that there was no significance difference in survival rate of N53 among substrates mixtures containing different proportion of sand + soil + farmyard manure (FYM) in 1:1:0 (T1), 2:1:1(T4) and 1:2:0 (T6) ratios (Table 4). Likewise, in genotype N52, the survival rate difference was not significant among substrates mixtures composed of sand + soil + FYM in 1:1:0 (T1), 1:1:1(T2), 1:2:1(T3) and 1:2:0 (T6) ratios (Table 4). However, plantlets grown on substrate mixture devoid of FYM (T1 and T6) exhibited vigorous growth and deep green leaves while those plantlets grown on substrate mixture containing FYM (T2 and T3 in genotype N52 and T4 in genotype N53) had weak growth and yellowish leaves. This could be due to the fact that farmyard manure increases the pH of the substrate mixture to 7.4 to 7.5 [23], while the optimum substrate mixture pH value for container grown plants should range from 5.5 to 6.5 [25]. Similar result was reported by Yasmeen et al. [26].

The result obtained in the present study in genotype N53 was in agreement with previous findings of Warakagoda et al. [27] who reported 100% survival rate of sugarcane plantlets acclimatized on substrates mixtures containing sand + coir dust in 1:2 ratio [28] reported more than 90% survival when plantlets were transplanted in vermiculite. Seventeen percent plantlet survival was also reported under *ex vitro* condition by Baksha et al. [29]. Similarly Baksha et al. [19] found survival rate of 75% for sugarcane plantlets acclimatized on pots containing a mixture of soil + sand in 2:1 ratio. Best acclimatization response was obtained in a mixture of sand + soil + peat at 1:1:1 after three week of transplanting to greenhouse [30,31] also found 96% survival rate for sugarcane plantlets acclimatized on substrate mixture composed of FYM and soil in 2:8 ratio. Eighty five percent survival was obtained from sugarcane plantlets acclimatized on potting mixture composed of soil + sand + compost in 1:1:1 ratio [15,32] also declared 72% survival rate of micropropagated plantlet.

Conclusion

Half MS liquid medium fortified with 3 mg/l NAA and 50 g/l sucrose was the optimal combination for *in vitro* rooting of *in vitro* generated shoots of sugarcane genotype N52 While ½ strength MS medium supplemented with 5 mg/l NAA and 50 g/l sucrose was the best combination for *in vitro* rooting of shoots of sugarcane genotype N53. Substrates mixture comprising sand and soil substrate in 1:1 ratio was found to be an ideal substrate mixture for best *ex vitro* acclimatization with higher plantlets survival rate for both sugarcane genotype, N52 and N53.

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