

## *In Vivo* Proliferation of *In Vitro* Propagated Sugarcane (*Saccharum officinarum* L.) genotypes at Tana- Beles Sugar Development Project, North-Western Ethiopia

Belay Tolera\*

Ethiopian Sugar Corporation, Research and Training Division, Variety Development Research Directorate, Biotechnology Research Team, Wonji Research Center, P.O. Box 15, Wonji, Ethiopia

### Abstract

In spite of the costly procurement and secondary acclimatization activities, the Ethiopia Sugar estates use huge quantity of micropropagated sugarcane plantlets to complement the conventional propagation method. The current study was aimed at finding rapid and cost effective propagation method for sugarcane planting materials multiplication to complement *in vitro* propagation method. In the study, acclimatized sugarcane plantlets were treated with Diammonium phosphate fertilizer (DAP), plant growth regulators and leaf trimming treatments. Plantlets lacking fertilizer, plant growth regulators and without trimming were used as free check. Data were collected on the number of tillers per shoot, average shoot length and number of leaves per shoot after 30 days. Analysis of variance revealed that the interaction effects of genotypes, trimming, DAP and plant growth regulators application was very highly significant ( $p < 0.0001$ ). Treatment combinations containing DAP at  $0.16 \text{ gm L}^{-1} \text{ m}^2$  with plant growth regulators GA3, BAP and kinetin each at  $0.04 \text{ mg L}^{-1} \text{ m}^2$  and trimming one-third of the leaves gave optimum *in vivo* shoot proliferation responses. On this treatment combination, B52-298 gave  $6.45 \pm 0.51$  tillers per shoot with  $4.39 \pm 1.44 \text{ cm}$  average shoot length and  $5.12 \pm 0.23$  leaves per shoot while NCo-334 produced  $5.77 \pm 0.79$  tillers per shoot with  $7.21 \pm 0.11 \text{ cm}$  average shoot length and  $5.51 \pm 0.05$  leaves per shoot. Similarly, N14 gave  $5.36 \pm 0.55$  tillers per shoot with  $5.71 \pm 0.15 \text{ cm}$  average shoot length and  $5.41 \pm 0.30$  leaves per shoot on the same treatment combination. Thus, the current result can be used as rapid and cost effective sugarcane planting material multiplication system to complement the costly micropropagation technology and hence minimize the cost of sugar production.

**Keywords:** Conventional propagation; *In vitro* propagation; *In vivo* proliferation; Plant growth regulators; Sugarcane genotypes

### Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most versatile cash crops grown extensively all over the world [1,2]. It is a monocotyledonous [3,4], tall growing perennial tropical grass (C4 plant) that tillers at the base to produce unbranched stems [5,6]. It is one of the most efficient converters of solar energy into sugar and other renewable forms of energy and hence produced primarily for its ability to store high concentrations of sugar in the internodes of the stem [6]. The crop is originated in the New Guinea region, spread along the human migration route [3] and today, it is grown in over 120 countries with estimated annual global sugar production of 1.7 billion metric tonnes [7]. Sugarcane is the sole base material for the Ethiopian Sugar Industry that plays a great role in the socio-economy of the country, given its agricultural and industrial investments, foreign exchange earnings, its high employment, and its linkages with major suppliers, support industries and customers. Currently, sugarcane is cultivated on more than 60,000 ha and the four sugar mills produce about 300,000 tonnes of sugar which only covers about 60% of the annual demand for domestic consumption while the deficit is imported from abroad. In spite of this fact, Ethiopia is endowed with favorable climate, enormous land and water resources for large scale irrigated sugarcane agriculture [8]. Besides, sugarcane has now emerged as a multiproduct crop used for food (sugar), energy and raw material for a number of by-products. Accordingly, the Ethiopian Government is implementing a large scale expansion and new green field sugar development programs with the objective of boosting the country's annual sugar production both to satisfy the domestic sugar demand and exploit the international sugar market.

However, availability of adequate amount of quality disease free planting material within short time is the major limitation via

conventional propagation to attain the intended plan. On the other hand, decline in cane and sugar yield, increased cost of production, large plantation area under very few sugarcane varieties used over many years, lack of methods for fast commercialization of improved and adapted varieties, obsolescence of productive commercial varieties due to disease, lack of alternative techniques for rejuvenation and disease cleansing of the old contaminated sugarcane varieties are the other challenges to attain the planned objectives using the conventional route of propagation [8]. The conventional seed cane multiplication method where stem cuttings with two to three buds are used as planting material has various limitations. The multiplication rate of sugarcane planting material is very slow (1:6 to 1:8) [9]. In addition, it requires large quantity of seed and land demanding [2].

Furthermore, the cutting implements used for seed cane preparation play a significant role in disease transmission. Besides the costly transport of the bulky cane cuttings, it harbors many pests and diseases with accumulation of diseases over vegetative cycles leading to further yield and quality decline over the years [9,10]. Currently, Micropropagation technology is a realistic and reliable tool to solve the limitations of conventional propagation method [10-16].

**\*Corresponding author:** Belay Tolera, Ethiopian Sugar Corporation, Research and Training Division, Variety Development Research Directorate, Biotechnology Research Team, Wonji Research Center, P.O. Box 15, Wonji, Ethiopia, Tel: +251-910181644; E-mail: [belaytolera@yahoo.com](mailto:belaytolera@yahoo.com)

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Micropropagation Technology is a technique through which group of genetically identical plants all derived from a selected individual multiply vegetatively and rapidly by aseptic culture of meristematic regions under defined nutritional and controlled environmental conditions *in vitro*. Nowadays, unlike the conventional propagation method, it is the only practical means of achieving rapid and large scale production of disease free quality planting materials in sugarcane [17-19] and alternative approach for fast multiplication of a variety in its original form. It is very effective in entire disease cleansing, rejuvenation and subsequent mass propagation of well adapted and promising varieties facing gradual deterioration in yield, quality and vigor due to accumulation of pathogens during prolonged vegetative cultivation and hence sustains the productive potential of sugarcane crops for a longer period [20,21].

Furthermore, micropropagated sugarcane plants were reported to give superior in cane and sugar yield as compared to their donors under similar agronomic management systems [15,16,22,23]. Considering all the drawbacks of conventional method and potential of tissue culture techniques, researchers have developed low cost protocols for *in vitro* mass propagation of sugarcane. However, the Ethiopian Sugar Corporation is procuring micropropagated sugarcane plantlets from other organizations where the cost of procurement and acclimatization activities is the major limiting factor. The cost of procuring micropropagated sugarcane plantlets is about US\$0.143 while the cost of acclimatization is about US\$ 0.024 per plantlet. In addition, erratic supply owing to limited technical and planning skills of the supplier organizations and occasional bulk delivery of the plantlets with limited preparatory activities of the recipient organization resulted in low survival rate and hence radically reduce the quantity of available plants for planting though the demand for quality planting material is high for the expansion and new sugar development projects of the sugar estates. Therefore, this experiment was carried out with the objective to evaluate the effects of trimming, plant growth regulators and fertilizer application on *in vivo* proliferation of tissue culture raised acclimatized sugarcane plantlets of three sugarcane genotypes with a view to complement micropropagation technology to cut down the cost of *in vitro* propagation and avail large quantity quality planting materials of sugarcane plantlets within short period of time.

## Materials and Method

The study was conducted at Tana Beles Sugar Development Project of the Ethiopian sugar estate located in North-Western Ethiopia at 11°30' latitude and 36°41' longitude with an elevation of 1110 m.a.s. It receives mean annual rainfall of 1447 mm with a mean maximum and minimum temperature of 32.5 and 16.4°C, respectively. *In vitro* propagated three sugarcane genotypes namely B52-298, N14 and NCO-334 delivered from Mekele Technology Institute Tissue Culture Laboratory were subjected to acclimatization for 45 days in the Lath house (60% shade rate) and the acclimatized plantlets were used as experimental materials for the study. The sugarcane genotypes are well adapted, having high cane and sugar yield and are among a few very productive ones widely grown in all the Ethiopian sugar estates and most projects. The experimental materials were of the same batch having uniform size and checked for the absence of tillers before using for the experiment. For planting media, mixture of sand and forest soil at the ratio of 1:1 were used to fill polyethylene bags (white, 8 cm dia. by 12 cm height) arranged under open nursery conditions. The experiment was laid out in a completely randomized design (CRD) with four factorial treatment combinations arrangements: Sugarcane genotypes, fertilizer, plant growth regulators and trimming. In this experiment, the effect of

trimming, fertilizer and plant growth regulators on number of tillers per shoot, average shoot length and number of leaves per shoot of the sugarcane genotypes was tested. Accordingly, two levels of trimming (trimming 1/3 of the leaves and without trimming), two levels of DAP fertilizer (application of 0.16 g L<sup>-1</sup>m<sup>-2</sup> and without DAP fertilizer) and two levels of plant growth regulators combination (GA3, BAP and kinetin each at 0.04 mg L<sup>-1</sup> m<sup>-2</sup> and without plant growth regulators) with three sugarcane genotypes (NCo-334, B52-298 and N14) resulting in 2'2'2'3=24 treatment combination arrangements each replicated thrice is used. Each treatment combination contains 150 plantlets. Data on number of tillers per shoot, average shoot length and number of leaves per shoot was collected from 30 randomly selected sugarcane plantlets per treatment combination after 30 days of first treatment application. Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) software (*version 9.1.3*) and treatments' means were separated using the procedure of REGWQ (Ryan-Einot-Gabriel-Welsch Multiple range Test).

## Results and Discussion

Analysis of variance (ANOVA) showed that the interaction effects of genotype, trimming, plant growth regulators (PGRs) and diammonium phosphate (DAP) fertilizer was found to be very highly significant ( $p < 0.0001$ ) on all the responses tested, i.e., number of tillers per shoot, average shoot length and number of leaves per shoot (Table 1). The three sugarcane genotypes also showed marked variation for all the responses tested (Table 2). For the three sugarcane genotypes, the lowest number of tillers per shoot was found on treatment lacking trimming, plant growth regulators (PGR) and DAP fertilizer, i.e., on the control treatment (Table 3: T1). On the contrary, for all the three sugarcane genotypes, the highest (optimum) number of tillers per shoot was recorded on trimmed (1/3 of the leaves) plantlets having plant growth regulators (GA3, BAP and Kinetin each at 0.04 mg L<sup>-1</sup> m<sup>-2</sup>) and DAP fertilizer at 0.16 gm L<sup>-1</sup> m<sup>-2</sup>) (Table 3: T8 and Figure 1: 1a-1c). This result revealed that the combined application of plant growth regulators (GA3, BAP and Kinetin) and DAP fertilizer along with trimming of

Source of variations	DF	Mean Squares		
		Number of Tillers per shoot	Average Shoot length	Number of Leaves per shoot
Genotype	2	6.0871***	32.2969***	3.6666***
Trimming	1	3.9293***	6.6248***	0.7688***
Genotype*Trimming	2	0.0079ns	0.0529***	0.0139***
PGR	1	61.3463***	26.6450***	5.0881***
Genotype*PGR	2	3.1750***	1.0849***	0.1372***
Trimming*PGR	1	0.0003 <sup>ns</sup>	0.0481***	0.0685***
Genotype*Trimming*PGR	2	1.2759***	0.3451***	0.0040***
DAP	1	35.7294***	89.6461***	11.8585***
Genotype*DAP	2	1.2080***	2.1355***	0.5254***
Trimming*DAP	1	1.0464***	0.0968***	0.0685***
Genotype*Trimming*DAP	2	1.2326***	0.0466***	0.0974***
PGR*DAP	1	4.5200***	1.9602***	0.0018***
Genotype*PGR*DAP	2	1.5853***	0.1580***	0.3092***
Trimming*PGR*DAP	1	0.2358**	0.3613***	0.2450***
Genotype*Trimming*PGR*DAP	2	0.4085***	0.3739***	0.0280***

DF=Degree of Freedom; \*\*\* =very highly significant ( $p < 0.0001$ ) at  $\alpha = 0.05$  significant level; \*\* =significant ( $P < 0.002$ ) at  $\alpha = 0.05$  significant level; ns=no significant variation.

**Table 1:** ANOVA for the effects of genotype, trimming, plant growth regulators and DAP fertilizer on number of tillers per shoot, shoot length and number of leaves per shoot of sugarcane genotypes.

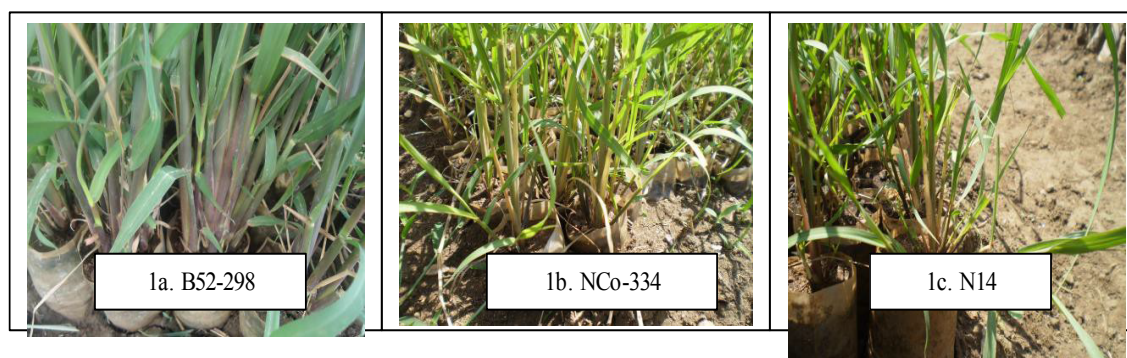
S.N	Genotypes	REGWQ Grouping of Genotypes		
		Average number of tillers per shoot	Average shoot length (cm)	Average number of leaves per shoot
1	B52-298	4.78 <sup>a</sup>	3.84 <sup>b</sup>	4.28 <sup>c</sup>
2	NCo-334	4.39 <sup>b</sup>	4.98 <sup>a</sup>	4.34 <sup>b</sup>
3	N14	4.78 <sup>c</sup>	2.66 <sup>c</sup>	4.98 <sup>a</sup>

**Table 2.** Effects of Trimming, DAP fertilizer and PGRs on sugarcane genotypes' average number of tillers, average shoot length and number of leaves per shoot.

Treatment codes	Treatments			Response variables								
	Trimming	PGR (gm L <sup>-1</sup> m <sup>-2</sup> )	Fertilizer (DAP) (gm L <sup>-1</sup> m <sup>-2</sup> )	Number of tillers per shoot (Mean ± SE)			Average Shoot Length (cm) (Mean ± SE)			Number of Leaves per shoot (Mean ± SE)		
				B52-298	Nco-334	N14	B52-298	Nco-334	N14	B52-298	Nco-334	N14
T1	0	0	0	2.42 ± 0.26 <sup>t</sup>	1.73 ± .32 <sup>v</sup>	1.92 ± 0.40 <sup>u</sup>	1.11 ± 0.47 <sup>v</sup>	2.31 ± 0.59 <sup>t</sup>	1.46 ± 0.74 <sup>n</sup>	2.01 ± 0.42 <sup>t</sup>	3.20 ± 0.29 <sup>n</sup>	4.31 ± 0.18 <sup>j</sup>
T2	0	0	0.16	3.91 ± 0.72 <sup>n</sup>	2.52 ± 0.87 <sup>t</sup>	5.15 ± 0.66 <sup>g</sup>	2.97 ± 1.04 <sup>k</sup>	5.36 ± 0.42 <sup>e</sup>	4.23 ± 0.27 <sup>h</sup>	4.41 ± 0.38 <sup>l</sup>	4.32 ± 0.08 <sup>l</sup>	5.13 ± 1.00 <sup>e</sup>
T3	0	0.04	0	4.24 ± 1.02 <sup>t</sup>	4.71 ± 0.78 <sup>l</sup>	4.51 ± 0.18 <sup>k</sup>	1.29 ± 1.01 <sup>u</sup>	4.24 ± 0.19 <sup>h</sup>	2.28 ± 0.51 <sup>t</sup>	3.24 ± 0.40 <sup>p</sup>	3.38 ± 0.41 <sup>p</sup>	4.44 ± 0.18 <sup>l</sup>
T4	0	0.04	0.16	5.25 ± 0.11 <sup>f</sup>	5.23 ± 0.46 <sup>g</sup>	4.09 ± 0.32 <sup>m</sup>	2.53 ± 1.40 <sup>o</sup>	6.63 ± 0.32 <sup>b</sup>	2.43 ± 0.26 <sup>m</sup>	4.84 ± 0.21 <sup>f</sup>	4.36 ± 0.93 <sup>i</sup>	5.32 ± 0.51 <sup>c</sup>
T5	1	0	0	2.89 ± 0.70 <sup>d</sup>	2.46 ± 0.31 <sup>s</sup>	3.71 ± 0.91 <sup>o</sup>	1.33 ± 0.91 <sup>t</sup>	3.38 ± 0.26 <sup>j</sup>	1.73 ± 0.15 <sup>f</sup>	3.83 ± 0.42 <sup>m</sup>	3.78 ± 0.25 <sup>m</sup>	4.65 ± 0.40 <sup>g</sup>
T6	1	0	0.16	4.69 ± 0.01 <sup>l</sup>	3.38 ± 0.73 <sup>p</sup>	5.14 ± 1.32 <sup>h</sup>	3.46 ± 1.15 <sup>l</sup>	6.12 ± 0.70 <sup>c</sup>	4.85 ± 0.34 <sup>f</sup>	4.27 ± 0.41 <sup>k</sup>	4.64 ± 0.51 <sup>h</sup>	4.72 ± 0.66 <sup>g</sup>
T7	1	0.04	0	5.94 ± 0.12 <sup>b</sup>	5.31 ± 0.12 <sup>e</sup>	4.72 ± 0.21 <sup>i</sup>	2.16 ± 0.80 <sup>q</sup>	4.59 ± 1.02 <sup>g</sup>	1.55 ± 1.73 <sup>s</sup>	2.41 ± 0.51 <sup>s</sup>	3.91 ± 1.13 <sup>j</sup>	4.22 ± 0.12 <sup>l</sup>
T	1	0.04	0.16	6.45 ± 0.51 <sup>a</sup>	5.77 ± 0.79 <sup>c</sup>	5.36 ± 0.55 <sup>d</sup>	3.79 ± 1.44 <sup>h</sup>	7.21 ± 0.11 <sup>a</sup>	5.71 ± 0.15 <sup>d</sup>	5.12 ± 0.23 <sup>d</sup>	5.51 ± 0.05 <sup>a</sup>	5.41 ± 0.30 <sup>b</sup>
CV (%)				7.09			11.2			8.4		

**Remark:** The number "0" in all treatments indicates absence of the particular treatment while "1" in trimming treatment indicates trimming 1/3 of the leaves.

**Table 3:** *In vivo* proliferation responses of sugarcane genotypes to Trimming, Plant Growth Regulators and DAP fertilizer



**Figure 1:** *In vivo* proliferation of sugarcane genotypes on optimum treatment combinations.

the leaves enhanced *in vivo* Proliferation of sugarcane plantlets. Among the three sugarcane genotypes, B52-298 produced the highest number of tillers per shoot (6.45 ± 0.51) (Table 3; Figure 1a) followed by NCo-334 and N14 that gave 5.77 ± 0.79 (Table 3; Figure 1b) and 5.36 ± 0.55 (Table 3; Figure 1c) tillers, respectively. Similarly, the lowest average shoot length and number of leaves per shoot was obtained on treatment without trimming, PGRs and DAP fertilizer (Table 3: T1). However, the presence of the three treatments (trimming, PGRs and DAP fertilizer) together gave the maximum average shoot length (cm) and number of leaves per shoot for all the three sugarcane genotypes (Table 3: T8 and Figure 1). Accordingly, B52-298, NCo-334 and N14 produced 4.39 ± 1.44, 7.21 ± 0.11, 5.71 ± 0.15 and 5.12 ± 0.23, 5.51 ± 0.05, 5.41 ± 0.30 average shoot lengths and number of leaves, respectively. Among the three sugarcane genotypes, NCo-334 gave the maximum average shoot length (7.21 ± 0.11) and number of leaves per shoot (5.51 ± 0.05) followed by N14 (5.71 ± 0.15; 5.41 ± 0.30) and B52-298 (4.39 ± 1.44;

5.12 ± 0.23) (Table 3). The increase in the levels of DAP fertilizer from 0 to 0.16 gm L<sup>-1</sup> m<sup>-2</sup> increased all the response variables (number of tillers per shoot, shoot length and number of leaves per shoot) in all the sugarcane genotypes tested. In B52-298, the number of tillers, average shoot length and number of leaves increased from 2.42 ± 0.26 to 3.91 ± 0.72: 1.11 ± 0.47 to 2.97 ± 1.04 and 4.41 ± 0.38 to 2.01 ± 0.42, respectively. Similarly, the same trend was observed for NCo-334 and N14 (Table 3). In the absence of trimming (0 level) treatment, combined application of DAP fertilizer and PGRs (Table 3: T4) increased the number of tillers per shoot, average shoot length and number of leaves per shoot than the sole application of PGRs (Table 3: T3) in all the genotypes. It is evident that the results of the combined effects of the three factors (Table 3: T8) is by far better than the combined effects of either of the two factors (Table 3: T4, T6 and T7). This *in vivo* proliferation method was used successfully for propagation of sugarcane plantlets at Tana Beles sugar development project of the Ethiopian sugar estate. Thus, it can be



used for cost effective multiplication of tissue culture raised sugarcane plantlets in tendom with tissue culture mass propagation laboratory at pilot plant stage and there was no difference in plants generated through this system as compared to tissue culture raised plantlets under field condition except the method had limitations that the multiplication rate is not as high as in the tissue culture laboratory but can multiply plants manifold. Using this result, a saving of 52 to 62.4 million US\$ can be made from plantlets procurement per annum and hence reduce the cost of sugar production in the country.

## Conclusion

The conventional method of sugarcane planting material propagation has diverse limitations while procurement and acclimatization of large quantity micropropagation sugarcane planting materials to complement the conventional method is costly. Thus, to complement the costly micropropagation technology, *in vivo* proliferation system (IVPS) of three sugarcane genotypes 'B52-298, NCo-334 and N14' has been developed. The result proved that *in vivo* proliferation of sugarcane is highly dependent on the interaction effects of genotype, Diammonium phosphate (DAP) fertilizer, plant growth regulators and trimming of the leaves. Treatment combination containing DAP fertilizer at 0.16 gm L<sup>-1</sup> m<sup>-2</sup>, PGRs: GA3, BAP and Kinetin each at 0.04 mgL<sup>-1</sup>m<sup>-2</sup> and trimming 1/3 of the leaves was found to give optimum *in vivo* proliferation responses for the three sugarcane genotypes in all the responses tested. On this treatment combination, B52-298 produced 6.45 ± 0.51 tillers per shoot with 4.39 ± 1.44 cm average shoot length and 5.12 ± 0.23 leaves per shoot while NCo-334 gave 5.77 ± 0.79 tillers per shoot with 7.21 ± 0.11 cm average shoot length and 5.51 ± 0.05 leaves per shoot. Similarly, N14 gave 5.36 ± 0.55 tillers per shoot with 5.71 ± 0.15 cm average shoot length and 5.41 ± 0.30 leaves per shoot on the same treatment combination. Thus, the current findings will help minimize the current challenges of sugarcane production by rapidly availing adequate amount of quality planting material of sugarcane while reducing the cost of plantlets procurement and hence the cost of sugar production. To increase the efficiency of propagation and reduce the cost of production, the current result needs to be further improved in the future using different concentrations and types of plant growth regulators, fertilizer types and rates as well as varying cultural practices that enhance the proliferation system.

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