# Effect of Rate and Time of Nitrogen Application on Growth and Quality of Seed Cane Produced from Tissue Cultured Plantlets at Tana Beles Sugar Development Project, Ethiopia

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**Abstract**
Sugarcane is cultivated throughout the tropical and sub-tropical regions of the world in a wide variety of soils and climates. A field experiment was conducted to determine the optimum rate and time of N fertilizer application on seed cane produced from tissue cultured plantlets at Tana Beles sugar development project. The treatments were laid out in randomized complete block design with three replications. The experiment was designed with four levels of nitrogen fertilizer (0, 65,130,195 kg N ha$^{-1}$) applied into three equal split doses (0.5 months after transplanting, 2.5 months after transplanting and 5.0 months after transplanting), two equal split doses (2.5 months after transplanting and 5 months after transplanting) and a single application of the whole dose once (5 months after transplanting). Among the parameters of seed cane crop, plant height, stalk length, stalk weight, stalk diameter, sett yield, leaf sheath moisture, leaf N and K content and sprout percent were significantly affected by applied N fertilizer. Whereas, stalk population, number of internodes per stalk and leaf P content were not significantly affected by different rates and times of N application. Highest plant height, stalk length, stalk weight, sett yield and leaf K content was attained with 195 kg N ha$^{-1}$applied in three equal split doses. On the other hand maximum leaf sheath moisture, leaf N content and sprout count was attained from plants treated with a lately single dose application of 195 kg N ha$^{-1}$ at 5 months after transplanting. Significantly higher Stalk girth/diameter, was recorded when 195 kg N ha$^{-1}$ applied in two equal split doses. However, the results of sett yield and sprout percent which received 195 kg N ha$^{-1}$ applied in different time were not significantly different from the plants which received 130 kg N ha$^{-1}$ applied in different time. Therefore, it is advisable to use 130 kg N ha$^{-1}$ applied in two equal split doses at 2.5 months after transplanting and 5 months after transplanting.
**Keywords**: Tissue cultured plantlets, Nitrogen fertilization, Sett yield, and Sprouting percent.

## Introduction

Sugarcane can be grown in a wide variety of soil types ranging from sandy loam to heavy clay (Nazir, 1994). It is one of the most important widely grown cash crops in Ethiopia (Yoseph, 2006). The crop is commercially cultivated during 1954 by Dutch Company, Handles-Vereening Amsterdam (HVA) (Yoseph, 2006). Currently sugarcane is cultivated in Ethiopia on about 70, 000 hectares of land with 4 million quintal of sugar production in 2015 (Girma Abejehu, 2016, Personal communication).

In conventional propagation method of sugarcane where stem cuttings with two to three nodes are used as a planting material, however, there are various limitations. Propagation from stem cuttings facilitates spread of pathogens with accumulation of disease over vegetative cycles leading to reduction in yield and quality (Fitch *et al.,* 2001). Unlike the conventional propagation method, micro-propagation using shoot tip or apical meristem culture is widely used to produce virus–free plants (George *et al.,* 2008) with rapid multiplication of new variety (Khan *et al.,* 2008) and for rejuvenation and mass production of true to type and uniform planting materials from sugarcane plants.

Among the elements applied to sugarcane nitrogen is the primary nutrient limiting in sugarcane production throughout the world (Wiedenfeld and Enciso, 2008). Seed cane production, which is an integral component of sugar production, often receives less priority than the crop plant in many sugarcane plantations (Koehler, 1984). On the other hand, it is emphasized that improving seed cane production is the basis for satisfactory crop stand establishment. Nevertheless, most of the research works on seed cane has focused on the mechanics of cutting and fungicidal treatment of setts and little effort had been made to improve cultivation of seed cane (Koehler, 1984). Nitrogen fertilization enhances the growth of sugarcane and enables the plants to take up other nutrients (Barnes, 1974). Hebert (1956) asserted the level of nitrogen to the optimum requirements of the seed cane plants correspondingly increases the quality of setts. At Tana Beles sugar development project, no study has been made concerning the rate and time of nitrogen fertilizer application to seed cane raised through tissue culture. Therefore the objective of this study was to determine the rate and time of nitrogen application for seed cane production raised through tissue culture plantlets.

## Materials and Methods

### Description of the study site

Tana Beles sugar development project is located at $11^0$07'N and $36^0$20'E latitude with an altitude of 1119 m.a.s.l, in Amhara regional state, Awi Zone, Jawi Woreda, at a distance of 650 km far from Addis Ababa. The study area is bounded by Belaya mountain Aboygara mountain, Bakussa escapement and Fendika ridge from the South, North, West and East directions respectively. The study area receives average annual rainfall of 1490 mm, mean minimum and maximum temperatures of 16.4 $^0$C and 32.5$^0$C, respectively(Zeleke and Netsanet, 2015).

### Treatments and experimental design

Treatment designation, rate and time of fertilizer application are described below.

$T_1$-control (untreated), $T_2$- 65 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_3$- 65 kg N ha$^{-1}$applied in two equal split doses at 2.5 and 5 months of cane age; $T_4$-a full dose of 65 kg N ha$^{-1}$ applied at 5 months of cane age; $T_5$-130 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_6$-130 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_7$-a full dose of 130 kg N ha$^{-1}$ applied at 5 months of cane age; $T_8$-195 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_9$-195 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_{10}$-a full dose of 195 kg N ha$^{-1}$ applied at 5 months of cane age.

The treatments were arranged in randomized complete block design (RCBD) and replicated three times. Triple supper phosphate (TSP) was applied during transplanting to all plots uniformly as starter with the rate of 250 kg ha$^{-1}$. At all times of fertilizer application, the treatments were applied manually along the either sides of each plant row, covered with a thin layer of soil followed by a light irrigation. After 8 months of seed cane age three budded seed setts were prepared from N treated seed cane plants grown in phase one and mixing of sett samples was properly done. Planting was done in end to end way. The design, field lay out and arrangement of treatments was similar with phase one. All cultural practices were done according to the practices of the plantation project.

### Data collection

**Plant height measurement –** Eight plants were selected at random with four plants from each two middle rows and their height was measured at eight months of plant age. The height of the plants was measured from the zero height to the third leaf (the first visible dewlap).

**Percent sheath moisture and N, P, K content in the dry matter of leaf blade –** For N, P, K and percent sheath moisture determination, leaf blade and sheath samples were collected at 8:00 am following the procedure of Martin-prevel *et al.,* (1987). Sixteen samples of leaf were taken from each plot at six months of transplanting. The middle 200 mm section of each leaf was taken from leaf number 3-6, the midribs removed and the remaining was used for analysis (Calcino *et al.*, 2000). The ground samples were digested following the procedure for wet destruction and nitrogen was determined by micro kjeldahl distillation apparatus (FAO, 1980).

**Stalk population count -** Plants/stalk counting was made from the two middle rows of each plot at eight months of plant age.

**Stalk weight -** Stalk weight was determined by taking a bundle of eight stalks from middle rows of each plot, and then the average weight of each stalk was made.

**Stalk length -** The length of eight stalks per plot was measured after discarding the bottom three and upper two internodes. Data was arranged and average stalk length was recorded.

**Stalk girth -** The girth/diameter of these eight stalk samples from two middle rows of each plot was measured using caliper. Each stalk which is approximately divided in to three equal segments was taken.

**Number of internodes per stalk -** The number of internodes per stalk was also counted from the stalks prepared for stalk length measurement.

**Set Yield per hectare -** Set Yield was calculated using the following formula:

Y= (a-1)x X 6896.55*/bc (Tadesse, 1993).

Where Y- sett Yield (number of three-budded setts) per hectare

a -Average number of internodes per stalk

b - Number of buds per sett

c - Furrow length per plot (16 m for two rows in this case)

x - Number of stalks utilizable for seed per two rows of a plot

* - Total length of furrows per hectare (m) at a distance of 1.45 m between them

**Sprout count -** The number of shoots emerged were counted from the two middle rows of each plot at 45 days of plant age after planting of seed setts cut from fertilized seed cane.

### Data analysis

Data collected from experiment were subjected to analysis of variance using proc GLM technique with SAS soft ware (SAS, 2002). Means of the treatments was separated using DMRT (Duncan's Multiple Range Test).

 

Correlations of parameters were studied standard procedure suggested in SAS software.

**Results and Discussion**

**Plant height**

Plant height as affected by different levels and times of N application is presented in Table 1. Plant height recorded at 8th month of plant age was significantly ($P \leq 0.01$) affected due to the rate and time of N application. During 8 months of plant age the highest increment in height was observed on the plants which received 195 kg N ha$^{-1}$ applied in three equal split doses (0.5, 2.5 and 5.0 MATP) as T8 followed by 195 kg N ha$^{-1}$applied in two equal split doses (2.5 and 5.0 MATP) as T9 and full dose of 195 kg N ha$^{-1}$ applied at 5.0 MATP as T10. The increased in the plant height due to nitrogen fertilizer may caused by increase in number of nodes or inter nodes elongation or both. Similar result was reported by Mohammed (1997). The increase in plant height with respect to increased N application rate indicates maximum vegetative growth of the plants under higher N availability. Also Taye and Yifru (2010) reported that the smallest plant height was achieved from untreated plant. This result was also supported by the finding of Tadesse (1993) as the application of N at early growth stage of the seed cane plants enhanced better vegetative growth. On the contrary, plants which did not receive N fertilizer (T1) were the shortest of all others. This result was in agreement with the report of Humbert (1968) who reported that plants deficient in N exhibited retarded growth. Similarly, Sime (2013) reported a close relationship between growth and applied nitrogen in which high amount of applied N resulted the highest plant height and vise versa on the experiment done at Fincha.

Table 1: Plant height of seed cane at 8 months of age as affected by rate and times of N application.

| Trt. No. | Plant height (m) |
|---|---|
| T1 | 1.82$^{b}$ |
| T2 | 1.9$^{b}$ |
| T3 | 1.87$^{b}$ |
| T4 | 1.83$^{b}$ |
| T5 | 2.26$^{a}$ |
| T6 | 2.24$^{a}$ |
| T7 | 2.23$^{a}$ |
| T8 | 2.35$^{a}$ |
| T9 | 2.34$^{a}$ |
| T10 | 2.31$^{a}$ |
| Mean | 2.12 |
| CV (%) | 7.54 |
| MSE | 0.152 |

Means followed by the same lower case letter along columns are statistically non significant according to DMRT, CV= coefficient of variation, MSE= mean square error.

$T_1$-control (untreated), $T_2$- 65 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_3$- 65 kg N ha$^{-1}$applied in two equal split doses at 2.5 and 5 months of cane age; $T_4$-a full dose of 65 kg N ha$^{-1}$ applied at 5 months of cane age; $T_5$-130 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_6$-130 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_7$-a full dose of 130 kg N ha$^{-1}$ applied at 5 months of cane age; $T_8$-195 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_9$-195 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_{10}$-a full dose of 195 kg N ha$^{-1}$ applied at 5 months of cane age.

**Sheath moisture content and leaf tissue composition**

Percent N, P, and K of the leaf tissue and sheath moisture content during the sampling time in seed cane crop as affected by different levels and times of N application is presented in Table 4. There was significant difference ($P \leq 0.01$) in leaf sheath moisture content of seed cane due to time and rate of fertilizer application. The leaf sheath moisture content significantly increased with increasing nitrogen (Table 2).

The highest leaf sheath moisture content (83.64 %) was obtained in seed cane plants which received full dose of 195 kg N ha$^{-1}$ applied at 5.0 MATP ($T_{10}$) followed by seed cane plants that received 195 kg N ha$^{-1}$ in three equal split doses (0.5, 2.5 and 5.0 MATP) ($T_8$) which was 82.99% and plants received full dose of 130 kg N ha$^{-1}$ applied at 5.0 MATP as $T_7$ (82.97 %). Whereas, the lowest content of leaf sheath moisture was recorded on seed cane plant which did not receive N fertilizer as $T_1$ (control). Here, the result indicated that the amount of nitrogen fertilizer exerts greatest influence on moisture level of leaf sheath.

Similarly, Borden's (1944) showed that increased succulence of cane plants with increased N application, and this according to Borden meant a dilution of juice quality. The optimum value of sugar cane leaf sheath moisture content is more than 85% and 80% during formative and grand growth phases respectively (Verma, 2004). According to the result, leaf sheath moisture content of the plant was above the optimum values of grand growth phases except plants treated with $T_1$ (control) which was 78.9 %.

Leaf tissue analysis indicated statistically significant (P≤0.01) difference in leaf N and K contents due to time and rate of N application. The highest content of leaf N was recorded for seed cane plants that received full dose of 195 kg N ha$^{-1}$ applied at 5.0 MATP as $T_{10}$ followed by seed cane plants that received 195 kg N ha$^{-1}$ in three equal split doses (0.5, 2.5 and 5.0 MATP) as $T_8$ and 195 kg N ha$^{-1}$ applied in two equal split doses (2.5 and 5.0 MATP) as $T_9$ which were highest amount of applied N (Table 4). Increment of Leaf-N due to nitrogen application to sugarcane was also reported in previous findings (Ibrahim, 1979). The lowest amount of leaf N and K was obtained for the control ($T_1$) plants. This result is supported by the work of Tadesse (1993) who reported the finding of lowest leaf K and N on plants which received no N fertilizer at Wonji-Shoa. On the other hand the highest content of leaf K was recorded for seed cane plant which received 195 kg N ha$^{-1}$ in three equal split doses (0.5, 2.5 and 5.0 MATP) as $T_8$ followed by 195 kg N ha$^{-1}$ applied in two equal split doses (2.5 and 5.0 MATP) as $T_9$ and a full dose of 195 kg N ha$^{-1}$ applied at 5.0 MATP as $T_{10}$.

As the result indicated, application of the nutrient (N) increased the concentration of that nutrient in the plant. The N fertilizer not only consistently increased the N percentages but also increased the K percentages of the leaves. The result clearly indicated that if optimum N is applied to plants in the presence of suitable K level, plant productivity increases which could be due to increase in N use efficiency of the plants (Helal *et al.*, 1975).

Table 2. Percent N, P, and K of the leaf tissue and sheath moisture content during the sampling time in seed cane crop as affected by different rate and times of N application.

| Trt. No. | Leaf sheath Moisture (%) | N, P and K in Leaf tissue (%) | | |
|---|---|---|---|---|
| | | N | P | K |
| T1 | 78.9$^{b}$ | 1.53$^{d}$ | 0.3$^{a}$ | 1.605$^{e}$ |
| T2 | 80.61$^{b}$ | 1.74$^{bc}$ | 0.3$^{a}$ | 1.6767$^{de}$ |
| T3 | 82.31$^{a}$ | 1.7$^{cd}$ | 0.3$^{a}$ | 1.77$^{cd}$ |
| T4 | 82.83$^{a}$ | 1.76$^{bc}$ | 0.3$^{a}$ | 1.7667$^{cd}$ |
| T5 | 82.32$^{a}$ | 1.79$^{bc}$ | 0.317$^{a}$ | 1.86$^{bc}$ |
| T6 | 82.82$^{a}$ | 1.81$^{bc}$ | 0.31$^{a}$ | 1.91$^{b}$ |
| T7 | 82.97$^{a}$ | 1.85$^{bc}$ | 0.3$^{a}$ | 1.8233$^{bc}$ |
| T8 | 82.99$^{a}$ | 2.056$^{a}$ | 0.3$^{a}$ | 2.0367$^{a}$ |
| T9 | 81.88$^{a}$ | 1.93$^{ab}$ | 0.31$^{a}$ | 1.94$^{ab}$ |
| T10 | 83.64$^{a}$ | 2.06$^{a}$ | 0.3$^{a}$ | 1.9367$^{ab}$ |
| Mean | 80.85 | 1.82 | 0.305 | 1.83 |
| CV (%) | 2.73 | 5.86 | 5.12 | 3.88 |
| MSE | 2.197 | 0.111 | 0.016 | 0.071 |

Means followed by the same lower case letter along columns are statistically non significant according to DMRT. CV = Coefficient of variation; MSE = mean square error.

$T_1$-control (untreated), $T_2$- 65 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_3$- 65 kg N ha$^{-1}$applied in two equal split doses at 2.5 and 5 months of cane age; $T_4$-a full dose of 65 kg N ha$^{-1}$ applied at 5 months of cane age; $T_5$-130 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_6$-130 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_7$-a full dose of 130 kg N ha$^{-1}$ applied at 5 months of cane age; $T_8$-195 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_9$-195 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_{10}$-a full dose of 195 kg N ha$^{-1}$ applied at 5 months of cane age.

**Stalk characteristics, stalk count and sett yield at harvest**

Stalk characteristics, stalk count per hectare and sett yield per hectare at harvest of the seed cane crop as affected by different rate and times of N application is presented in the Table 3. The different levels of nitrogen applied to seed cane did not significantly (P ≤ 0.05) affect the number of internodes per stalk and stalk population. This result is in line with the work of Bikila *et al.* (2014) and Tadesse (1993) for number of internodes per stalk and stalk population, respectively. Bikila *et al.* (2014) reported that Pre-cutting nitrogen application rate and time did not affect the internodes number. No significant response of millable stalk population count to nitrogen fertilizer application was also reported at Wonji-Shoa for plant cane by the work of Ambachew and Abiy (2008).

On the other hand the response of stalk length and sett yield of seed cane showed significant (p≤ 0.01) difference for different rates of nitrogen fertilizer applied at the time of harvest. This result was similar with the work of Sime (2013) for sett yield in which significant differences between treatments (P<0.01) for sett yield was observed on experiment conducted at Finchaa due to different rate and time of N application. The same result was obtained from the work of Bikila *et al.* (2014) and Tadesse (1993) for stalk length. As they reported from the experiments conducted at Fincha and Wonji-Shoa, the different levels of pre-cutting nitrogen application on seed cane of sugarcane did not significantly (P ≤ 0.05) affect the stalk height of seed cane at the time of harvest. Based on the result, the highest value was recorded on cane plants that received 195 kg N ha$^{-1}$ in three equal split doses (0.5, 2.5 and 5.0 MATP) ($T_8$) for both stalk length and sett yield. The lowest value for both parameters was recorded on plants that received $T_1$ (untreated).

Stalk weight and stalk girth/diameter exhibited significant ($p \leq 0.05$) difference due to treatment (Table 5). Based on this result, significantly the highest stalk weight and stalk girth/diameter was recorded on seed cane plants when 195 kg N $ha^{-1}$ applied in three equal split doses at 0.5, 2.5 and 5.0 MATP ($T_8$) and 195 kg N $ha^{-1}$ applied in two equal split doses at 2.5 and 5.0 MATP ($T_9$), respectively. Increase in cane tonnage due to increase in nitrogen fertilizer application was reported by other workers (Samuels and alers-alers1963).

Table 3. Stalk characteristics, stalk count and sett yield per hectare at harvest of the seed cane crop as affected by different levels and times of N application

| Trt. No. | Stalk length (m) | No.ofInter nodes | Stalk weight (kg) | Stalk girth (cm) | Stalk population (count) | Sett yield (count) |
|---|---|---|---|---|---|---|
| T1 | $1.32^{b}$ | 12.3a | $0.49^{c}$ | $2.06^{c}$ | $135641^{a}$ | $615947^{d}$ |
| T2 | $1.39^{b}$ | 14a | $0.7933^{bc}$ | $2.21^{bc}$ | $139376^{a}$ | $693581^{cd}$ |
| T3 | $1.38^{b}$ | 14.7a | $0.89667^{ab}$ | $2.17^{bc}$ | $138946^{a}$ | $715756b^{cd}$ |
| T4 | $1.34^{b}$ | 14.7a | $0.85667^{ab}$ | $2.2^{bc}$ | $136790^{a}$ | $690708^{cd}$ |
| T5 | $1.77^{a}$ | 17a | $1.08^{ab}$ | $2.36^{ab}$ | $162654^{a}$ | $970833^{ab}$ |
| T6 | $1.75^{a}$ | 17.3a | $1.09667^{ab}$ | $2.31^{ab}$ | $158487^{a}$ | $950909^{abc}$ |
| T7 | $1.73^{a}$ | 17.3a | $1.07667^{ab}$ | $2.3^{ab}$ | $156188^{a}$ | $936062^{abc}$ |
| T8 | $1.85^{a}$ | 16.7a | $1.2667^{ab}$ | $2.32^{ab}$ | $195702^{a}$ | $1132997^{a}$ |
| T9 | $1.84^{a}$ | 16.3a | $1.2267^{a}$ | $2.45^{a}$ | $174724^{a}$ | $1001101^{a}$ |
| T10 | $1.81^{a}$ | 17.7a | $1.10667^{ab}$ | $2.32^{ab}$ | $170700^{a}$ | $973371^{ab}$ |
| Mean | 1.62 | 15.8 | 0.98 | 2.27 | 156920 | 868126 |
| CV(%) | 7.17 | 18.34 | 15.37 | 5.14 | 18.76 | 16.65 |
| MSE | 0.053 | 2.73 | 0.20 | 0.12 | 39401 | 144601 |

Means followed by the same lower case letter along columns are statistically non significant according to DMRT. CV = Coefficient of variation, MSE = Mean square erreor.

T1-control (untreated), T2- 65 kg N ha-1 applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; T3- 65 kg N ha-1applied in two equal split doses at 2.5 and 5 months of cane age; T4-a full dose of 65 kg N ha-1 applied at 5 months of cane age; T5-130 kg N ha-1 applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; T6-130 kg N ha-1 applied in two equal split doses at 2.5 and 5 months of cane age; T7-a full dose of 130 kg N ha-1 applied at 5 months of cane age; T8-195 kg N ha-1 applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; T9-195 kg N ha-1 applied in two equal split doses at 2.5 and 5 months of cane age; T10-a full dose of 195 kg N ha-1 applied at 5 months of cane age.

**Sprout count**

Use of N fertilized planting material for commercial cane production showed significant difference ($p \leq 0.01$) in sprouting ability of the cane in Luvisol (light soil) of Tana Beles. Similar result was obtained by Sime (2013) at Finchaa sugar estate. The highest percentage of sprouting was recorded on plants treated with a full dose of lately (5 MATP) applied 195 kg N $ha^{-1}$ designated as $T_{10}$ and its sprouting percentage was 89.31 % (Table 6). On the other hand the lowest percentage of sprouting (60.067%) was recorded for plants treated with no N fertilizer (control) which was designated as $T_1$. Plant treated with 195 kg N $ha^{-1}$ applied at 5 MATP ($T_{10}$) showed 29.24% increase over the control (untreated).

At all the three levels of N tested, the application of late (5 MATP), single and full dose of N fertilizer resulted the best sprouting percentage than two and three equally split dose of N fertilizer application. This result was supported by Gurura.J.H. (2001), as well fertilized setts germinate rapidly with vigorous seedlings and a high proportion of roots and shoots. Sime, (2013) also reported that, application of N at early growing stage, regardless of the rate, it could be utilized for vegetative growth and may have not been enough to produce reserve nutrients for the sprouting buds as compared to late application. On the other hand, late application of N fertilizer, the seed cane for planting might have made available the applied N before it was used up for other metabolic activities. In late applications, N is found stored temporarily in the nodes just below the buds Cornelison and cooper (1994). Therefore, the sprouting buds might have easily utilized this N for emergence and successive growth. That is why the lately single dose of N application has given the best result of sprouting compared to others.

 

Table 6. Sprouting (%) of buds on planting material obtained from seed cane crops grown with different levels and times of N application on light soil of Tana Beles

| Trt. No. | Percent sprout count at 45 DAP |
|---|---|
| T1 | 60.067[d] |
| T2 | 62.543[c] |
| T3 | 67.8[d] |
| T4 | 69.9[cd] |
| T5 | 79.14[bcd] |
| T6 | 80.8[abc] |
| T7 | 86.443[ab] |
| T8 | 82.21[a] |
| T9 | 82.69[a] |
| T10 | 89.31[a] |
| Mean | 76.09 |
| CV (%) | 8.49 |
| MSE | 6.465 |

Means followed by the same lower case letter along columns are statistically non significant according to DMRT; DAP = Days After Planting; CV = Coefficient of variation; MSE = Mean square error.
$T_1$-control (untreated), $T_2$- 65 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_3$- 65 kg N ha$^{-1}$applied in two equal split doses at 2.5 and 5 months of cane age; $T_4$-a full dose of 65 kg N ha$^{-1}$ applied at 5 months of cane age; $T_5$-130 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_6$-130 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_7$-a full dose of 130 kg N ha$^{-1}$ applied at 5 months of cane age; $T_8$-195 kg N ha$^{-1}$ applied in three equal split doses at 15 days after transplanting, at 2.5 and 5 months of cane age; $T_9$-195 kg N ha$^{-1}$ applied in two equal split doses at 2.5 and 5 months of cane age; $T_{10}$-a full dose of 195 kg N ha$^{-1}$ applied at 5 months of cane age.

**Conclusion and Recommendation**

Application of 195 kg N ha$^{-1}$ in three equal split doses at 15 DATP, 2.5 MATP and 5 MATP showed the best plant height, stalk weight, stalk length, sett yield and leaf K content. Late (5.0 MATP) application of 195 kg N ha$^{-1}$ in a single dose showed increased leaf sheath moisture, leaf nitrogen content and sprout count of the planting material produced. Stalk girth was higher when application of two equal split doses of 195 kg N ha$^{-1}$ was made. However, the results of sett yield and sprout percent which received 195 kg N ha$^{-1}$ in three equal split doses (0.5, 2.5 and 5.0 MATP) and full dose (5.0 MATP) respectively, were not significantly different from the plants which received 130 kg N ha$^{-1}$ in two equal split doses at 2.5 MATP and 5 MATP.

Since this type of experiment was conducted for the first time at this place, the result of the experiment provides a good foundation for the project to nurture tissue culture plantlets for seed cane production. Fertilization of seed cane plants by using high dose of N has a potential to increase yield and quality of seed setts, which play a significant role on commercial sugar cane production. Therefore, it is advisable to use 130 kg N ha$^{-1}$ in two equal split doses at 2.5 MATP and 5 MATP in order to attain high yield and good quality seed cane produced from tissue culture plantlets. Further research is required on the rate and time of N fertilizer application including rate of P fertilizer for seed cane production raised through tissue culture plantlets in different sugar cane verities, locations and soil types of Tana Beles sugar development project.

**References**

Ambachew, Dametie; and Abiy Fantaye. (2008). Determination of optimum Nitrogen rate for sugarcane at Wonji-Shoa sugarcane plantation. *1st Ethiopian Sugar Industry Annual Conference.* Adama. *Sept. 23-24, 2008.*

Barnes, A.C. (1974). *The sugarcane* . London. Leonard Hill Books.

Bikila, M., Dechassa, N., and Alemayehu, Y (2014) Effects of Pre Cutting Nitrogen Application Rate and Time on Seed Cane Quality of Sugarcane(Saccharum officinarum L.) Crop at Finchaa Sugar Estate. *Adv. Crop Sci. Tech.*, 2:3-7.

Borden, R. J (1944). A search for guidance in the nitrogen fertilization of the sugarcane crop. II. The first ratoon crop. *Hawaiian Planters' Record.*, 48: 271 -275.

Calcino, D., G. Kingston and M. Haysom (2000). Nutrition of the plant in: Manual of Cane Growing. Ed. M. Hogarth and P. Allsopp. Bureau of Sugar Experiment Station, Indooroopilly, Brisbane, Australia.

Cornelison , A. H. and Cooper, H. F. (1994). Time of application of nitrogen test. *Hawaiian plant. Rec.*, 45: 115-118.

Food and agriculture organization (FAO). (1980). Soil and plant testing as a basis of fertilizer recommendation.

*FAO Soil bull*. 38/1.

Fitch, M. M. M., Leherer Komor, E. and Moore, P. H. (2001). Elimination of sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immune assy. *Plant pathology*, 50:676-680.

George, E. F., Machakova, I. and Zazimalova, E. (2008). *Plant propagation by tissue culture*. United Kingdom. Springer press.

Gururaj. H. (2001). *Sugar cane in agriculture and industry*. Banglore, Prism Books Pvt Ltd.

Hebert, L. P. (1956). Effect of seed pieces and rate of planting on yield of sugarcane. *Congress of International Society of Sugar and Cane Technologist*, 1: 301-310.

Helal, M., Koch, K. and Mengel, K. (1975). Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. *Agron. J*., 351:310-313.

Humbert, R. P. (1968). *The growing of sugarcane*. Amsterdam. Elsevier press.

Ibrahim, H. S. (1979). Effect of N fertilizers on sugarcane in Sudan, J. *Experimental Agriculture.*, 14:273–276.

Khan S.A., Rashid, A., Chaudhary M. F., Chaudhary, Z. and Afroz, (2008) A "Rapid Micropropagation of three elite Sugarcane (Saccharum officinarumL.)Varieties by shoot tip culture" *African Journal of Biotechnology*, 21:74-80.

Koehler, P.H. (1984). Seed cane production at the lihue plantaion company. *Hawiian sugar technology*. 14: 12-13.

Martin-Prevel, P., Gagnard, J. and Guitier, P. (1987). *Plant analysis as guide to the nutrients of temperate and tropical crops.* New York, Lavoissier Pub.

Mohammed. (1997**).** Effect of nitrogen and phosphorus fertilization on growth and yield of some graminacea forage. *Journal of Agric. Sci.,* 5(2): 25-33.

Nazir, M.S. 1994. Sugarcane. In: Crop production. E. Bashir and Bantel R. (Eds.). National Book Foundation, Islamabad, Pakistan, pp 421-422.

Samuels, G., and Alers-Alers. (1963). Effect of time of N application on sucrose content of sugarcane ratoons. *Journal of Agriculture of the University of Puerto Rico,* 48:304-307.

SAS (Statistical Analysis System). (2002). Institute of Applied Statistics SAS programming Language, version 9.2. Cary, North Carolina.

Sime, Mengistu. (2013). Effect of Different Nitrogen Rates and Time of Application in Improving Yield and Quality of Seed Cane of Sugarcane (Saccharum spp. L.) VarietyB41/227. *International Journal of Scientific and Research Publications,* 3(1): 3-6.

Tadese Negi. (1993*). Effect of rate and time of nitrogen application on growth parameters and quality of setts of seed cane plants of sugar cane at Wonji-Shoa*. M.Sc. thesis. Alemaya University. Alemaya.

Taye and Yifru Abera. (2010). Response of maize (Zea maize L.) to tied ridges and planting methods aat Goro, southern Ethiopia. *American-Euroasian J. Agronomy* 3(1): 21-24.

Verma. (2004). *Sugarcane Production Technology in India*. International. India: Book Distributing Co.

Wiedenfeld, B., and Enciso, J. (2008). Sugarcane responses to irrigation and N in semiarid South Texas. *Agron. J.*, 100: 665-668.

Yoseph Assefa. (2006). *Sugarcane stalk borer in Ethiopia: Ecology and Phytogeography*. Ph.D. thesis, University of Kwazulu- Natal, South Africa.

Zeleke, Teshome; and Netsanet, Ayele. (2015). Soil fertility assessment for fertilizer recommendation for sugarcane plantations at Tana Beles sugar development project in Ethiopia. *African Journal of Agricultural Science and Technology* (AJAST). 3 (11): 245-246.