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Research Article

EFFECT OF RATE AND TIME OF NITROGEN APPLICATION ON GROWTH 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 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⁻¹applied in three equal split doses. On the other hand maximum leaf sheath moisture and leaf N content 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 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 to obtain maximum seed cane yield.

Keywords: Tissue cultured plantlets, Nitrogen fertilization and Sett yield

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

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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 optimum rate and time of nitrogen application for seed cane production raised through tissue culture plantlets for the attainment of maximum seed cane yield.

Materials and Methods

Description of the study site

Tana Beles sugar development project is located at 11°07'N and 36°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° C. respectively(Zeleke and Netsanet, 2015).

Treatments and experimental design

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

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⁻¹ applied 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; 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. 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.

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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 threebudded 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

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

0.01) affected due to the was significantly (P 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 ¹applied 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 Tave 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
Τ6	2.24 ^a
Τ7	2.23 ^a
Τ8	2.35 ^a
Т9	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⁻¹applied 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; 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; 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 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.

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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 T₉ 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 T₈ followed by 195 kg N ha⁻¹ applied in two equal split doses (2.5 and 5.0 MATP) as T₉ 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).

Trt. No.	Leaf sheath Moisture (%)	N, P and K in Leaf tissue (%)		
		N	Р	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}
Т3	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}
Т8	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

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.

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.

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⁻¹ applied 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; 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 Precutting 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

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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 0.05) difference due to treatment (Table 3). 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 (T₈) and 195 kg N ha⁻¹ applied in two equal split doses at 2.5 and 5.0 MATP (T₉), respectively. Increase in cane tonnage due to increase in nitrogen fertilizer application was reported by other workers (Samuels and alers-alers1963).

Trt.	Stalk length	No.ofInter	Stalk weight	Stalk g	girth	Stalk	Sett	yield
No.	(m)	nodes	(kg)	(cm)		population (count)	(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}	

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

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Τ8	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 error.

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.

Conclusion and Recommendations

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 and leaf nitrogen content of the seed cane plant. 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 which received 195 kg N ha⁻¹ in three equal split doses (0.5, 2.5 and 5.0 MATP) was 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.

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