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Evaluation of grain protein content in *Eragrostis tef* for different N fertilizer application under irrigated condition

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The decline in soil fertility and shortage of rainfall has been the main reason for the low productivity of *Eragrostis tef* (Zucc.), particularly in the northern part of Ethiopia. A field experiment was conducted to examine the impact of planting method and nitrogen fertilizer rates on the yield and protein content of irrigated Tef. The experiment consisted of four planting methods (pelleting, broadcasting, row planting, and transplanting) and six N fertilizer rate (0, 23, 46, 69,92 and 115 kg ha⁻¹) combined in Factorial Randomized Complete Block Design (RCBD) with four replications. Seven response variables, heading date, maturity date, plant height, number of productive tillers, above ground biomass yield, grain yield, and grain protein content of Tef have been collected and analyzed. Analysis of variance showed that all parameters, with the exception of grain protein content, were significantly affected (P<0.05) by the interacting effects of planting methods and nitrogen levels. The highest values of all parameters were obtained from transplanting and N rate of 92 kg ha⁻¹, although Tef plants receiving this treatment were extremely tall and were late in heading and maturity periods, which may have a negative implication Tef under rainfed system. In irrigated Tef, the nitrogen rate exponentially increased grain protein content until 92 kg ha⁻¹N, which optimizes the nutritional quality of the crop, unlike the rainfed agricultural system.

Key words: pelleting, transplanting, nutritional quality, irrigated tef

INTRODUCTION

Tef is an indigenous and a major cereal staple food crop in Ethiopia which is annually cultivated on more than three million hectares of land (Bekele et al., 2019). Tef in Ethiopia shared more than any other cereal crops primarily contributed as a source of protein, minerals and amino acids particularly methionine and cysteine (Council, 1996; Habtegebrial & Singh, 2006). Tef performs well under diverse soil types, but has an extremely low grain yield, with the national average yield of only 1.6 ton ha⁻¹ (Bezabeh, 2015). The low productivity of Tef is due to the use of low and improper use of synthetic fertilizers, inadequate and erratic rainfall, weeds, low soil fertility and water logging on poorly drained soils (Efrem, 2001; Gelaw & Qureshi, 2020; Solomon et al., 2019). Vertisols are among the dominant soil types in Ethiopia since they occupy about 12.6 million hectares in the country from which 7.6 million ha are in the highlands where Tef crop is extensively cultivated (Debele & Deressa, 2016). The poor drainage of Vertisols due to the high clay content of the soil severely affects the infiltration or percolation of water to lower soil horizons particularly during the rainy season (Debele & Deressa, 2016). In addition, the Ethiopian Vertisols tend to exhibit low total N content mainly due to leaching and denitrification (Tekalign Mamo & Kamara, 1988). Hence, due to removal by erosion and leaching, the availability of N to plants especially to the cereal crops is very low (Gashu et al., 2020). The application of N is, therefore, one of the major inputs used by farmers to boost productivity under these soil types (Daba, 2017). Another study in the northern Ethiopia showed that in addition to poor soil fertility, low moisture stress was the major limiting factor to crop development particularly to Tef (Haileselassie et al., 2011). Nitrogen is involved in plant protein synthesis, which is a vital process-determining crop yield (Suter & Békés, 2021). Even though the excessive nitrogen fertilizer application can lead to lodging and grain losses of Tef, the increasing rate of N highly and positively correlates with yield and soil mineral nutrients which increases the grain quality (Tesfahun, 2018b). Effects of nitrogen to the grain protein content of crops are most wide spread in soils particularly northern part Ethiopia and their deficiency is common in cereal-grown areas where Tef is growing under monoculture cropping system(Cakmak, 2008; Çakmak et al., 2004; Ethio, 2021; Kassahun, 2015). In the last few decades, research conducted results revealed that N amount, methods and time of application determines not only the fate of applied N, but also optimizes the crop output under rainfed Tef production (Dereje et al., 2018; Gedamu et al., 2023; Habtegebrial et al., 2007; Negassa & Abera, 2013). The findings from these studies showed that the increasing rates of N application influences the yield parameters while the shortage of N supply decreases yield (Fikre et al., 2018; Tadele, 2019).

In rainfed agriculture, the most common way of planting Tef is by broadcasting on the surface of the plot (Assefa et al., 2001). However, in this practice where 25-50 kg ha⁻¹ seed rate is used for the tiny seed of Tef, the grain yield is severely reduces due to high competition among plants and due to lodging or permanent displacement of the plant from the upright position (Assefa et al., 2001). Planting methods also affect the quality of products through its effects on the lodging incidence (Subbarao et al., 2015). A recent study showed that row planting and transplanting can facilitate the mechanical and hand weeding as well as reduce the lodging incidence (Mulatua, 2019). Earlier study indicated that the uptake and efficiency of nutrients are affected by soil type, planting methods, rate and time of nutrient applications (Tekalign

Mamo & Kamara, 1988). Ethiopia is one of the sub-Saharan African countries facing recurrent droughts leading to low crop productivity, where most regions of Ethiopia are suffering from insufficient and unreliable rainfall (McLay et al., 2015). Thus, food insecurity has remained amajor problem that is a great concern to the country (Yihun et al., 2013). The decline in the total amount and high rainfall variability are among the main causes for low crop productivity in different parts of Ethiopia, particularly the Tigray region (Tilahun, 2006). Yield losses of Tef due to low moisture are estimated to reach up to 40% during severe stress (Ayele, 1993). However, a yield reduction of up to 77% was reported when drought occurred during the anthesis stage (Takele, 2001).

Since rainfed agriculture does not provide sufficient moisture during the entire cropping season especially in the drought prone areas, the use of supplementary water in the form of irrigation is being promoted to cereal crops including Tef (Tsegay et al., 2015). Recently, studies have been made to investigate the effect of supplementary irrigation and other agronomic practices on the productivity of Tef (Araya & Stroosnijder, 2011; Birhanu et al., 2020; Tsegay et al., 2015; Yihun, 2015). Nevertheless, producing Tef under full irrigation is a new practice and needs specific agronomic packages other than rainfed agriculture. Several agronomic research studies developed Tef under irrigation, but there was a gap in determining appropriate planting methods and nitrogen fertilizer rates under fully irrigated areas. Thus, the objectives of this study were to evaluate the effect of planting methods and N fertilizer applications on yield and yield components and grain protein content of Tef plants under irrigation in northern part of Ethiopia.

MATERIALS AND METHODS

Description of the experimental site

The field experiment was conducted during December to March 2018 and 2019 cropping season at Mokoni Experimental Station, which is located in the Raya Azobo district, Southern Tigray in the northern Ethiopia Fig.1. Geographically located at the altitude of 1630 m.a.s.l, 12°40′0″N latitude and 39°44′0″ E). The area was selected for its suitability to Tef under full Irrigation system, the soil type of the site is Vertisols, and the area was characterized by bimodal rainfall pattern and received an annual rainfall of 550 mm with average maximum and minimum temperatures were 29.9 and 15.9°C, respectively (Sertse et al., 2021).

An improved Tef variety called *Quncho* was used for the entire study. Four planting methods namely pelleting, transplanting, row planting and broadcasting were used. Pelleting refers to the coating of Tef seeds with inert material to 15-time of the size of Tef seeds on a weight basis.

Six Nitrogen rates namely N1=0, N2=23, N3=46, N4=69, N5=92, N6=115 kg ha⁻¹ of nitrogen were used as nitrogen fertilizer levels. Planting methods and N rates were combined and named as treatments arranged as follows.

T1=(Transplanting,N1),T2=(Transplanting,N2), T3=(Transplanting,N3), T4=(Transplanting,N4), T5=(Transplanting,N5), T6=(Transplanting,N6), T7=(Pelleting,N1), T8=(Pelleting,N2), T9=(Pelleting,N3),T10=(Pelleting,N4), T11=(Pelleting,N5), T12=(Pelleting,N6), T13=(Row planting,N1), T14=(Row Planting, N2), T15=(Row Planting,N3), T16=(Row Planting,N4), T17=(Row Planting,N5), T18=(Row Planting,N6), T19=(Broadcasting,N1), T20=(Broadcasting,N2), T21=(Broadcasting,N3),T22=(Broadcasting,N4), T23=(Broadcasting,N5),and T24=(Broadcasting,N6) respectively. Treatments were arranged in RCBD with four replications. The plot size was 4m x 2m while the spacing between blocks and rows were 1.5 m and 1.2 m, respectively. According to the recommended rate, Tef sown in rows and broadcasting 10 kg ha⁻¹ of Tef seeds was used (Arefaine et al., 2020), a spacing at 20*5 cm was used for transplanting (Abraham et al., 2014b), and seed rate of 30 kg ha⁻¹ was used for pelleting (because it is three times larger coated than the normal seed rate recommended in rows). According to the recommended method of application for nitrogen except for zero level, N was applied in split at half at sowing and the remaining at tiller initiation (Chanie, 2017). All mineral fertilizers were applied according to the study area's soil test-based fertilizer recommendation (Berhe, 2020). All plots were hand-weeded and managed equally except treatment variability.

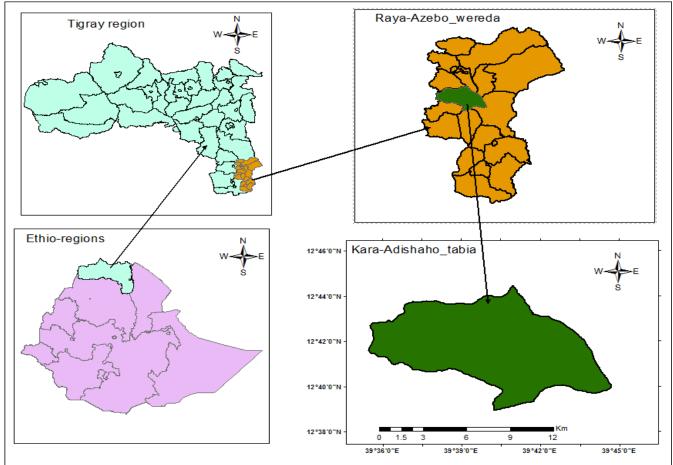


Figure 1. Location of the Study Area

Data collected as Irrigation Water Parameters

Crop water requirement (ETc) was calculated from ETo and estimated crop evaporation rates expressed as crop coefficients (KC), based on FAO procedures (Doorenbos & Pruitt, 1977). The length of the growing period for Tef was 120 days, and the estimated average rooting depth from field measurement was 40 cm adopted from Araya (2011). The Kc factor and crop water depletion factor (p) for water stress (stomata closure) were adopted from the FAO Irrigation and drainage paper 56 (Araya et al., 2011). For the study area the reference evapotranspiration (ETO) was adopted from FAO Penman-Monteith method (Allen et al., 1998).

ET crop = (KC * ET0).....Equation(1)

Where; ETcrop=crop evapo transpiration, Kc=crop coefficient and ETo=reference evapo transpiration.

Crop Phenology

Crop parameters measured were: (1) Heading date was calculated by counting the number of days from sowing to the time when 50% of the plants started to emerge the tip of panicles. (2) Maturity date was collected by the number of days from sowing to the time when the plants reached physiological maturity. (3) Plant height: the length of the stalk from the base to the tip of plants,twenty randomly selected per plot measured in cm. (4) Number of productive tillers: the total number of tillers per plant at physiological maturity stage from twenty plants selected randomly per plot. (5) Grain yield: the weight grain yield from the middle rows with the size of 3.6 m x 2 m. (6) above ground biomass yield: the shoot biomass of the entire plant from the middle rows as indicated above.

Plant tissue analysis and grain protein content measurement

Plant samples randomly collected at physiological maturity from each experimental plot for nitrogen were partitioned into grain and straw and cleaned from foreign materials such as dust with distilled water. Both straw yield and grain yield sampled plant parts were oven dried at 70°C for 24 h, ground and passed through 0.5 mm sieve for analysis of N content adopted from (Harfe, 2017). The concentration of nitrogen in both straw and grain was determined by micro-Kjeldahl digestion procedure as described by (Bremner & Mulvaney, 1983).

Grain protein content(GPC %): This was calculated according to (Rutherfurd, 2010).

GPC(%) = (% NG * 5.7)....Equation(2)

NG=nitrogen content in grain in kg ha-1

Data Analysis

After checking the ANOVA assumptions, all the response variables were subjected to two-way analysis of variance, and mean comparisons were performed by the least significance difference test at the 5% level of significance according to the procedure by(Gomez & Gomez, 1984), using R-statistical software (R Core Team, 2013),and results were plotted and displayed in tables and figures.

RESULTS AND DISCUSSION

Crop phenology

Heading date was significantly influenced by planting method, N rate and their interaction, Table 1 and Table 2 respectively, and showed an increasing tendency to increase N rates. The delay in the days to heading in Tef plants that received high N rate was not surprising since N promotes vegetative growth rather than the reproductive phase of plants. Days to heading were also affected by planting methods in which transplanted Tef plants were significantly (P<0.05) taken longer time than broadcasted once. The higher days to heading in transplanted plants compared to broadcasted ones might be due to the high root to shoot ratio in the transplanted plants that promote excessive vegetative growth. Due to this, transplanting method with the highest N rates (115 and 92 kg ha⁻¹) took significantly more delayed to heading as compared to

broadcasting and zero kg ha-1 N took earlier time to heading Table 3. The delay in days to heading due to N application was also reported for Tef (Dereje et al., 2018). This study disagrees with the finding of (Tesfaye et al., 2019) where combined application of higher rate NP significantly shortened heading days for Tef than lower rate. This may be due to the effect of phosphorus in the combination which is probably related to shorten days to heading and maturity, but higher N level (Hinsinger et al., 2011). The maturity date was significantly (P<0.05) affected by planting methods, N rates, and their interaction. Regarding method of planting, the longest maturity days were recorded from transplanting followed by row planting as compared to the control, i.e., broadcasting. The maturity date was not significantly changed at lower nitrogen rates (0-69 kg ha⁻¹N), but at higher nitrogen rates (69-115 kg ha⁻¹) was significantly affected compared with the absence of fertilizer. The interaction of nitrogen rates and planting methods also affected maturity days in Tef Table 3. The delay in days to maturity from high N rate and transplanting versus low N rate and broadcasting was due to the creation of conducive conditions for vegetative growth of the former. The delay in days to maturity due to N application was also reported from pepper (Qawasmi et al., 1999). However, earlier studies on Tef showed the opposite, where transplanting and high rates of blended fertilizer have accelerated the maturity time (Tesfahun, 2018a).

Plant height was significantly (P<0.05) influenced by the interacting effects of planting methods and nitrogen levels Table 1 and Table 3. Increasing N from zero to 115 kg ha⁻¹ led to a 71% increase in plant height, as shown in Table 3. The interaction effects of planting methods and N rate significantly impacted plant height (P<0.05) Table 3. The highest plant height at transplanting and at higher rate of N (92 and 115 kg ha⁻¹) may be due to transplanting increases the root structures, and prolongs to nutrient uptake and nutrient use efficiencies, and the higher nitrogen level may enhanced more for the vegetative part as compared to broadcasting and zero level of N in which limited root and shoot growth which eventually shortens the height of Tef plants. This study agrees with the findings of transplanting and row planting at a higher rate of N fertilizer studies on Tef under rainfed and irrigation system prolongs plant height and panicle length of Tef as compared to broadcasting at a lower level of N (Dereje et al., 2018; Gashu et al., 2020). Planting methods, N rates, and their interactions had a significant impact on the number of productive tillers (P<0.05). The transplanting planting method resulted in the highest number of productive tillers (44.5) and the highest N rate of 115 kg ha⁻¹ whereas the broadcasting planting method resulted in the lowest number of productive tillers (1.3) and 0 kg ha⁻¹ of N Table 4. This agrees with the finding that transplanting method increases more productive tillers than broadcasting methods which produce lower unproductive tillers due to limited moisture content at physiological maturity stage (Salifu, 2015). However, this finding disagrees with earlier studies where no effect of N on the number of productive tillers (Fayera et al., 2014). This may be due the experiment was at rainfed, and higher N fertilizer application N prolongs vegetative growth and at the maturity stage, the limited residual moisture may determine tillers unproductive.

AGBY refers to the total biomass of the plant, which includes the grain and the straw yield (yield Qt ha⁻¹). Tef straw is the most preferred and palatable crop residue for livestock feed (Mekuriaw & Harris-Coble, 2021). Although there were inconsistencies in the shoot biomass the highest shoot dry biomass was obtained from the transplanted planting receiving 92 kg ha⁻¹N while the least shoot biomass yield was recorded from broadcasted with no N fertilizer application Table 4. The significant increase in the shoot biomass of transplanted plants receiving high N rate was due to the positive effect of the treatment to the uptake of other micronutrients and water use efficiencies. The high shoot biomass from this treatment is also related to excessively tall plant height (Fayera et al., 2014). Wider spacings from transplanted Tef plants promote optimum growth as they are affected by the competition of neighboring plants for light, nutrients, and water. Our finding agrees with earlier studies in which factors

positively affected parameters; number of productive tillers, plant height, and higher panicle bearing culms eventually increases Tef shoot biomass yield (Abraham et al., 2014a). Similarly, the study on rice indicated that transplanting increased shoot biomass over row planting (Maqsood, 1998).

Table 1. presents the mean squares of Heading Date (HD), Maturity Date (MD), Plant Height (PH), Number of Productive Tillers (NPT), Above Ground Biomass Yield (AGBY) a quintal per hectare, Grain Yield (GY) a quintal per hectare and grain protein content (GPC) in percent as influenced by planting methods rates, and their interactions.

Sources of variation	df.	HD	MD	PH	NPT	AGBY	GY	GPC
Planting method	3	496***	1162***	3506***	5782***	4464***	510***	80***
N rate	5	139***	179***	657***	212***	3504***	399***	626***
Planting method : N rate	15	9***	64**	31***	42***	207***	27***	1.39 ^{ns}
Residuals	72	7	23	8	8	18	2	4.7

ns=non significant, **and *** indicated significant differences at probability levels of 0.05% and 0.01 respectively, df=degree of freedom

Similar to other cereal crops, grain yield is the most important trait in Tef husbandry since Tef is cultivated mainly for its grain. This finding showed that GY was significantly (P<0.05) affected by interaction effect of planting methods and N fertilizer rate Table 4. The highest GY (34 Qt ha⁻¹) was obtained from transplanted plants with 92 kg ha⁻¹ N while the lowest grain yield of 8 Qt ha⁻¹ was recorded from broadcasting and no nitrogen fertilizer application. For transplanted plants, the grain yield was consistently and significantly increased in response to increasing the rate of N from nil to 92 kg ha⁻¹. As indicated above for other traits, the significant increase in GY from transplanted plants may be due to lower competition for resources and reduced the incidence of insect pests and diseases. This result agrees with the finding (Wato et al., 2020) reported transplanting positively affected GY and lodging of rainfed Tef production in Ethiopia.

Grain protein (GPC) content of irrigated tef

Though the interaction effect of Planting methods and N rate was not significant (P>0.05), independent analysis showed planting methods and N rates significant (P<0.05) influenced to GPC of Tef Table 1. The highest grain protein content was obtained from plants receiving 92 kg ha⁻¹N Figure 2b. This treatment's value significantly differed from the one with no N application. N rates increased from zero to 92 kg ha⁻¹, raised GPC. The positive relationship between GPC and N rate is logical as N is the major component of Grain protein (Boulelouah et al., 2022). Our findings agree with another study which reported improved protein content in Tef grains due to applying N fertilizer (Tadele, 2019). The planting method also significantly (P<0.05) altered the grain protein content Figure 2a. While transplanted plants gave grain with the highest protein content, broadcasted plants resulted in grain with the lowest protein content. High grain protein content from transplanted plants might be due to improved N uptake as transplanted plants had little competition for plant nutrients and other resources. Higher GPC in transplanted Tef seedlings may be due to the advance to sufficient amount of moisture and nutrients, to reach out the roots to the lower soil layer, which eventually facilitates the uptake of water and minerals better than broadcasting. This finding agrees with (Assefa et al., 2001) that transplanted method performs better in grain yield and nutrient use efficiency than broadcasting and row planting Tef.

Table 2. Response of Heading Date (HD) and to Maturity Date (MD days) as influenced by interaction of planting method and N rates.

			HI	D				MD							
Planting Method			N rates				N rates								
	N1	N2	N3	N4	N5	N6	N1	N2	N3	N4	N5	N6			
Pelleting	61.50g-i	62.25f-h	63.50e-h	64.50d-g	65.50c-f	68.50bc	96.75c-f	99.50b-e	96.75c-f	97.00c-f	100.50b-d	102.50bc			
Broadcasting	53.50k	56.50jk	54.50k	56.54jk	58.50ij	60.50hi	89.75g	94.25d-g	93.50e-g	90.50fg	93.00e-g	94.00d-g			
row planting	63.50e-h	64.00d-h	64.50d-g	66.50c-e	67.50b-d	68.50bc	94.25d-g	93.00 d-g	96.25c-g	95.50d-g	97.50c-e	100.50b-d			
Transplanting	61.50g-i	62.25f-h	63.50e-h	68.50bc	70.50ab	73.50a	98.75b-e	99.50b-e	105.00b	114.50a	116.25a	118.50a			
LSD(0.05)	3.74						LSD(0.05)			7.98					
CV	1.6									11.92					

N1=0 kg ha⁻¹, N2=23 kg ha⁻¹, N3=46 kg ha⁻¹, N4=69 kg ha⁻¹, N5=92 kg ha⁻¹, N6=115 kg ha⁻¹ nitrogen, CV (%) = coefficient of variation

Table 3. Response of PH and NPT as impacted by interaction effect of planting method and N rates.

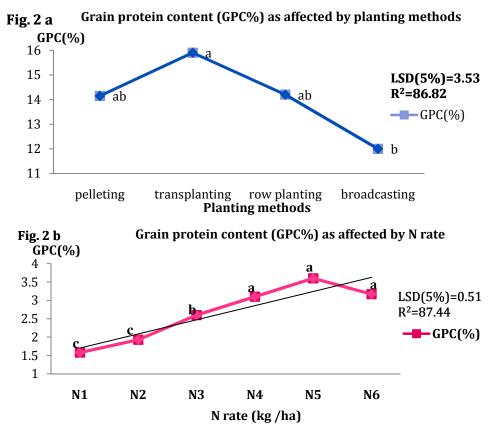
	PH (cm)					NPT (numbers) N rates						
Planting Methods	N rates											
	N1	N2	N3	N4	N5	N6	N1	N2	N3	N4	N5	N6
Pelleting	90.50i	92.5g-i	92.50f-i	94.60e-i	98.50de	111.70bc	9.70h-j	15.10fg	12.50gh	15.3fg	18.00ef	19.30e
Broadcasting	68.70n	75.60lm	71.50mn	77.20kl	81.20jk	85.30j	1.30l	2.10l	1.50l	1.80l	2.00l	2.50l
row planting	91.40g-i	91.2hi	93.5f-i	95.70e-h	98.50de	101.4d	1.20l	2.30l	4.50kl	6.50jk	8.50ij	11.5g-i
Transplanting	97.5d-f	96.20e-g	101.50d	107.40c	112.50b	117.50a	29.20d	26.50d	35.0c	40.30b	43.8ab	44.50a
LSD 0.05	4.1						LSD (0.05)	3.92				
CV	2.98						CV	18.82				

PH (Plant Height in cm), NP (Number of Productive Tillers), and N1 (0), N2 (23), N3 (46), N4 (69), N5 (92), N6 (115) kg ha⁻¹ nitrogen, CV (Coefficient of Variation in percent) under vertisols of Ethiopia.

Table 4. Response of Above Ground Biomass Yield (AGBY) and Grain Yield (GY) as influenced by interaction effect of planting method and N rates

		h h a 1)			dift	in lates.	$CV(0+h_{c})$							
	AGBY (Q	t na ¹)					GY (Qt ha ⁻¹)							
Planting methods	N rates							N rates						
	N1	N2	N3	N4	N5	N6	N1	N2	N3	N4	N5	N6		
Pelleting	30.20ij	40.30h	54.10g	60.20d-f	66.10d	60.50 de	10.21ij	13.62h	18.29g	20.34d-f	22.29d	20.45de		
Broadcasting	23.97k	27.50jk	39.40h	42.47h	45.22h	39.50h	8.09k	9.28jk	13.31h	14.34h	15.28h	13.33h		
row planting	25.10jk	33.40i	42.40h	54.68e-g	60.40de	54.45fg	8.49h	11.29i	14.33h	18.47eg	20.40de	18.40fg		
Transplanting	40.60h	45.23h	60.03ef	77.90c	101.50a	86.10b	13.74jk	15.29h	20.29ef	26.31c	34.30a	29.10b		
LSD (0.05)	5.89						LSD(0.05)	1.99						
CV	8.29						CV	8.30						

N1 (0), N2 (23), N3 (46), N4 (69), N5 (92), N6 (115) kg ha⁻¹ nitrogen, CV(Coefficient of Variation in percent), and Means with the same letter are not significantly different at 5% probability level, and LSD (least significance difference at 5%).





N1 (0), N2 (23), N3 (46), N4 (69), N5 (92), N6 (115) kg ha⁻¹ nitrogen, CV (Coefficient of Variation in percent), and Means with the same letter are not significantly different at 5% probability level, R² (coefficient of determination), LSD (least significance difference at 5%).

CONCLUSION

To enhance the productivity of Tef under an irrigation system, determining the appropriate agronomic practice is very basic. To determine the appropriate combination of planting method with N rate, an experiment was conducted at the Mokoni Agricultural Research center (MARC) in the northern part of Ethiopia. The research result revealed that all agronomic traits except grain protein content were influenced by the interaction effect of the planting method and N rates. In this study, higher yield traits were obtained from the planting method of transplanting and N rate of 92 kg ha⁻¹. Plants receiving this method and rate were taken longer time to head and to mature, tall, better productive tillers, and higher above ground biomass yield shoot biomass yield and grain yield as compare to broadcasting and no N rates. Consequently, the higher grain yield associated with higher grain nitrogen content resulted in higher grain protein content than broadcasting and no N rates. Compared to Tef production in rainfed, nitrogen rate optimizes the shoot biomass yield and grain yield, higher grain protein content which eventually enhances grain quality better under irrigation. Considering the most important traits, the recommendation given for the experimental site is the combination of "transplanting and 92 kg ha-1 N". In this study, information on the cost-benefit of the practice or the system is missing; it is, therefore, important to include a cost-benefit analysis using a partial budget method for variable costs, whether the recommended practices provide a higher return in both productivity and monetary terms by smallholder Tef farmers under irrigation.

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AUTHOR CONTRIBUTIONS

Conceptualization data collection and analysis; Kidu Gebremeskel, Yonas Gebremariam, Welegerima Gebrelibanos, kidist Tolosa, Yazachew Genet, Gebregergis Berhe, methodology; Kidu Gebremeskel, Mitiku Haile, Zerihun Tadele, Emiru Birhane, Solomon Haftu, Kbebew Assefa, Solomon Chanyalew, writing; Kidu Gebremeskel, Mitiku Haile, Zerihun Tadele, Emiru Birhane, review and editing; Zerihun Tadele, Emiru Birhane, supervision, Mitiku Haile, Zerihun Tadele, Emiru Birhane.

COMPETING INTERESTS

The authors declare they have no conflict of interest. The manuscript has not been submitted for publication in other journal.

ETHICS APPROVAL

Not applicable

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